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Investigating the impact of dipeptidyl peptidase-1 inhibition in humans using multi-omics

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Background: Dipeptidyl peptidase-1 (DPP-1), also known as cathepsin C, processes and activates neutrophil serine proteases. Brensocatib (an oral, reversible, competitive DPP-1 inhibitor) is a novel therapy for bronchiectasis previously shown to reduce sputum protease activity and prevent exacerbations. Broader effects of DPP-1 inhibition on the immune response have not been investigated.

Objective: We sought to profile effects of DPP-1 inhibition using a secondary analysis of the STOP-COVID19 trial.

Methods: The STOP-COVID19 trial was a randomized placebo-controlled trial of brensocatib 25 mg in patients hospitalized for severe coronavirus disease 2019 (COVID-19) in the United Kingdom. In the primary analysis, brensocatib did not improve clinical outcomes at day 29. A prespecified substudy was performed at 2 UK hospitals to explore the effects of DPP-1 inhibition on the immune response. Blood samples were obtained at baseline and days 8, 15, and 29. Analyses included peripheral blood neutrophil mass spectrometry, neutrophil functional testing, serum cytokine analysis, whole blood mRNA sequencing, and measurement of circulating neutrophil-associated markers.

Results: Between June 2020 and January 2021, 161 patients were enrolled (brensocatib: n = 80; placebo: n = 81). Neutrophil proteomics showed significant alterations in 15 proteins (false discovery rate-adjusted $P < .01$) including reductions in cathepsin G and the pseudoenzyme azurocidin-1 (Azu-1) (false discovery rate-adjusted $P < .0001$) by day 29. In serum, Azu-1 levels, but not total elastase or proteinase 3, were significantly reduced ($P < .0001$). Neutrophil surface expression of protease-cleavable C5aR1/CD88 was significantly increased by day 29 ($P < .05$). There were no differences in neutrophil extracellular traps, phagocytosis, circulating immune cell proportions, or gene expression between treatment groups.

Conclusions: Brensocatib treatment in COVID-19 altered multiple neutrophil proteins including profound effects on Azu-1, identifying this as a key DPP-1 target and potentially highly sensitive biomarker of treatment efficacy. (J Allergy Clin Immunol 2025;■■■:■■■-■■■.)

Key words: *Dipeptidyl peptidase-1, DPP-1, cathepsin C, azurocidin-1, heparin binding protein, brensocatib, neutrophil, proteomics, multi-omics, inflammation*

Neutrophil serine proteases (NSPs) such as neutrophil elastase (NE), proteinase-3 (PR3), and cathepsin G (CatG) contribute to the pathogenesis of inflammatory lung diseases, including bronchiectasis, chronic obstructive pulmonary disease, cystic fibrosis, and coronavirus disease 2019 (COVID-19).[1-3] NSPs are released upon neutrophil activation and subsequent neutrophil degranulation or neutrophil extracellular trap (NET) formation, promoting tissue damage and extracellular matrix destruction.[4] NETosis is associated with bacterial persistence, cleavage of cell surface molecules, mucus hypersecretion, and impaired mucociliary clearance.[5] In bronchiectasis—a chronic lung disease characterized by permanent dilation of the airways associated with excessive neutrophil accumulation and dysfunction—high sputum NSP levels are associated with worse lung function and risk of exacerbations.[1,6,7] Further, in individuals with high NE activity levels, sputum NSP activity is persistent and minimally responsive to common clinical interventions, with levels remaining high during subsequent exacerbations and even after antibiotic treatment.[1,8]

NSPs are synthesized within neutrophil progenitor cells, stored mainly in the primary (azurophilic) granules, and activated by N-terminal peptide cleavage by dipeptidyl peptidase-1 (DPP-1), also known as cathepsin C, before release of mature cells into the bloodstream.[9] Patients with the autosomal recessive condition Papillon-Lefèvre syndrome (PLS) lack active DPP-1 and subsequently also lack active NSPs.[10,11] Studies of neutrophils from patients with PLS show an inability to form NETs, but neutrophil functions, including phagocytosis and killing of bacteria, appear to be preserved.[10,11] Patients with PLS do not appear to be at increased risk of serious infections.[12]

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Abbreviations used
Azu-1: Azurocidin-1
CatG: Cathepsin G
COVID-19: Coronavirus disease 2019
DPP-1: Dipeptidyl peptidase-1
FDR: False discovery rate
FITC: Fluorescein isothiocyanate
LC-MS: Liquid chromatography–mass spectrometry
Log2FC: Log2 fold change
MPO: Myeloperoxidase
NE: Neutrophil elastase
NET: Neutrophil extracellular trap
NSP: Neutrophil serine protease
PLS: Papillon-Lefèvre syndrome
PMA: Phorbol 12-myristate 13-acetate
PR3: Proteinase-3

In a phase II randomized, double-blind, placebo-controlled trial of the oral reversible competitive DPP-1 inhibitor brensocatib in bronchiectasis, treatment significantly reduced NE, PR3, and CatG activity in sputum and prolonged time to first exacerbation.[13] The recently completed large phase III trial demonstrated a significant reduction in annual exacerbation rates as well as a slowing of lung function decline with the 25-mg dose.[14] DPP-1 inhibition is therefore considered a promising therapy in development for inflammatory lung conditions including bronchiectasis and cystic fibrosis.[15,16] As a novel therapy, the effects of DPP-1 inhibition on the immune response, beyond NSP inhibition, have not been fully characterized. DPP-1 has been shown to have activity beyond serine protease activation and, for example, has been indicated as an activator of lymphocyte and natural killer cell granzymes important for clearance of infected cells.[17] It is therefore likely that DPP-1 inhibition has both direct and indirect effects on the immune response that have not yet been characterized.

NSPs and hyperinflammation have also been implicated in severe COVID-19.[18] The STOP-COVID19 trial was a double-blind, randomized, placebo-controlled trial of brensocatib 25 mg per day for 28 days in patients hospitalized with COVID-19, conducted during the first and second waves of the COVID-19 pandemic in the United Kingdom.[19] Although brensocatib did not demonstrate clinical benefits in this trial, successful inhibition of NE activity over 29 days was demonstrated in zymosan-stimulated blood, and comprehensive biological sampling was performed.[19] In the present study, we report data from a prespecified substudy performed within the STOP-COVID19 trial designed to elucidate the effects of DPP-1 and NSP inhibition on the systemic immune response and inflammation. The aim of this study was to inform further research and clinical application of DPP-1 inhibitors such as brensocatib in inflammatory lung diseases, with particular emphasis on investigating effects on neutrophil activation and function.

METHODS

Study design and participants

STOP-COVID19 was a double-blind, randomized, parallel-group, placebo-controlled trial conducted at 14 sites in the United Kingdom between June 5, 2020, and January 25, 2021. The study was conducted to test the hypothesis that reducing NSP activity with brensocatib would improve clinical status in patients hospitalized with COVID-19. The trial randomized 406 participants to brensocatib 25 mg once daily or placebo for 28 days and the results of the primary and secondary end points have been previously reported.[19]

Inclusion and exclusion criteria are detailed in the Methods section in this article's Online Repository available at www.jacionline.org. The study was approved by the South of Scotland Research Ethics Committee (20/SS/0057); all patients or legal representatives provided written informed consent. The study was prospectively registered (clinical trial registration number ISRCTN30564012).[19]

Mechanistic substudy

The present substudy was designed to investigate the mechanism of action of brensocatib in patients with COVID-19. This study was prespecified in the protocol and conducted at 2 centers, Dundee and Sheffield, United Kingdom. Participants in the substudy were asked to provide blood samples at baseline, day 8 and day 15 (if still in hospital), and day 29. Participants who had been discharged by day 29 were asked to return to the research center for sampling and data collection.

Neutrophil isolation, liquid chromatography–mass spectrometry, and functional testing

Neutrophils were isolated from venous blood using STEMCELL Technologies (Vancouver, British Columbia, Canada) direct immunomagnetic negative selection kit and processed for liquid chromatography–mass spectrometry (LC-MS) as previously described.[20] To determine surface protein expression, isolated neutrophils were labeled with fluorescently-conjugated antibodies for CD63, CXCR2, CD11b, CD88, and CD66b. To determine phagocytic capacity, neutrophils were incubated with opsonized fluorescein isothiocyanate (FITC)-labeled heat-killed *Escherichia coli* or vehicle control. Washed and fixed cells were analyzed by flow cytometry. Gating strategy and experimental design are shown in Fig E1 (in the Online Repository at www.jacionline.org).

To investigate *in vitro* NETosis, neutrophils were incubated with no treatment (spontaneous NETosis control), LPS (5 μg/mL, Merck KGaA, Darmstadt, Germany), or phorbol 12-myristate 13-acetate (PMA, 100nM, Merck KGaA). SYTOX green nucleic acid stain (Thermo Fisher Scientific, Eugene, Ore) was added to detect extracellular DNA, and all wells were read after 4 hours of total incubation time.

Plasma and serum immunoassays

NE activity was measured in plasma from whole blood after stimulation of degranulation responses using zymosan as described previously.[19] Total NE was measured in the same plasma samples by ELISA (AssayPro LLC, St Charles, Mo). ELISAs were used to measure serum levels of azurocidin-1 (Azu-1) (Axis-Shield Diagnostics Ltd, Dundee, UK), PR3 (Bio-Techne Corportation, Minneapolis, Minn), CitH3 (Cayman Chemical Company, Ann Arbor, Mich), and calprotectin (Bio-Techne). Myeloperoxidase (MPO)-DNA complex ELISA was performed as previously described.[21] Serum Olink analysis was performed using the Target48 panel (Olink Proteomics AB, Uppsala, Sweden), as described previously.[20]

mRNA sequencing and mass cytometry

mRNA sequencing was performed in venous blood drawn into PAXgene tubes as described elsewhere.[22] For mass cytometry analysis, whole blood was processed for the Maxpar Direct Immune Profiling Assay (Standard BioTools, San Francisco, Calif) according to the manufacturer's instructions with minor modifications of centrifugation speed (final recovery at 800*g*). Cryopreserved samples were processed for analysis on the Helios Mass Cytometer (Cell Sorting and Mass Cytometry facility, University of Liverpool, Liverpool, UK).

Microbiota analysis

To investigate effects of DPP-1 on secondary bacterial infection, a further substudy was performed at the Dundee site only. Participants had nasopharyngeal swabs performed at baseline, day 15 (if still in hospital), and day 29. DNA from swabs was extracted by DNeasy PowerSoil Pro Kit (QIAGEN GmbH, Hilden, Germany). Full-length 16S ribosomal RNA sequencing was performed (Loopseq) allowing taxonomic resolution to species level with processing of data through DADA2 and Phyloseq in R.[23]

Statistical analysis

Serum/plasma protein immunoassays and flow cytometry data were log10-transformed as appropriate. Marker levels were compared between the treatment arms with a restricted maximum likelihood–based mixed model for repeated measures approach. For markers with any values above the upper limit of quantification, levels were instead compared between randomized arms using censored longitudinal models, estimated by maximum likelihood. A likelihood ratio test comparing the full marker model (described above) with a reduced model (removing the arm and arm-by-time interaction terms) was used to test for any differences between the arms over time. Adjustment for multiple comparisons was performed by controlling the false discovery rate (FDR). Full methods for all assays and analyses are detailed in the Methods section in the Online Repository at www.jacionline.org.

RESULTS

A total of 404 participants were enrolled in the STOP-COVID19 trial across all centers, and 161 participants were enrolled in the mechanistic substudy and are included in the subsequent analyses. Table I shows the baseline characteristics of patients randomized to brensocatib versus placebo. There were no significant differences in age, comorbidities, or severity of disease between groups.

TABLE I. Baseline characteristics of patients included in the mechanistic cohort of the STOP-COVID19 trial

Characteristics	Brensocatib group	Placebo group
No. of patients	80	81
Age, mean (SD)	63.9 (12.4)	65.8 (13.1)
Sex at birth, no. (%)		
Male	48 (60)	49 (60.5)
Female	32 (40)	32 (39.5)
Comorbidities, no. (%)		
Cardiac disease	21 (26.3)	17 (21.0)
COPD	14 (17.5)	11 (13.6)
Asthma	9 (11.3)	9 (11.1)
Obesity	22 (27.5)	19 (23.5)
Diabetes	12 (15)	19 (23.5)
Smoking, no. (%)		
Never	40 (50)	37 (45.7)
Ex-smoker	29 (36.3)	33 (40.7)
Current smoker	6 (7.5)	6 (7.4)
Unknown	5 (6.3)	5 (6.2)
Disease severity at baseline, no. (%)		
Hospitalized and not requiring supplemental oxygen	16 (20)	21 (25.9)
Hospitalized and requiring supplemental oxygen	56 (70)	49 (60.5)
Hospitalized and on noninvasive ventilation or high flow oxygen devices	8 (10)	11 (13.6)

COPD, Chronic obstructive pulmonary disease.

Specific changes within the neutrophil proteome were observed with brensocatib treatment

The primary site of action of DPP-1 inhibition is believed to be the bone marrow, where DPP-1 acts on zymogens during neutrophil maturation.[9] Previous data examining NSP activity following treatment with brensocatib show a treatment effect evident by day 15, once circulating neutrophils have been replaced by inhibited cells released from the bone marrow, with maximal effects by day 29.[24]

Neutrophil proteomics showed that 15 proteins were significantly altered with an FDR-adjusted P value < .01 (55 proteins FDR P < .05) (Tables E1 and E2 in the Online Repository at www.jacionline.org) in participants receiving brensocatib compared with placebo by day 29 (Fig 1, *A-C*). Change in protein abundance from day 1 to day 29 was determined for each participant (log ratio of day 1 to day 29) and then compared between the 2 treatment groups. With the largest decrease of all proteins significantly altered in the brensocatib group by day 29, the pseudoenzyme Azu-1 (AZU1) (log2 fold change [Log2FC]: log2 (I29I1)B − log2(I29I1)P – 3.31, referred to as Log2FC herein) was identified as a major, novel target of DPP-1 inhibition (FDR P < .00001). Other expected targets, including the NSP CatG (CTSG) (Log2FC −2.42, FDR P < .00001), were also reduced in neutrophils from patients receiving brensocatib, though to a lesser magnitude compared with Azu-1. Further novel changes were observed, including increased expression of the antimicrobial peptide neutrophil defensin A3 (DEFA3) (Log2FC 2.38, FDR P < .0001) and the serine carboxypeptidase (CPVL) (Log2FC 1.72, FDR P < .01) (Fig 1). A heatmap is shown in Fig E2 (in the Online Repository at www.jacionline.org). DPP-1 levels in neutrophils were not significantly altered (Log2FC 0.350, FDR P = .21) (Fig E3 in the Online Repository at www.jacionline.org).

Serine protease 57/NSP4 (PRSS57), a known target of DPP-1 activation,[25] was significantly reduced by day 29 in the brensocatib group (Log2FC −0.76, FDR P < .01). Isoform A of serine/threonine-protein kinase 24 (STK24) was also significantly lower (Log2FC −2.81, FDR P <.00001), whereas glia maturation factor gamma (GMFG) (Log2FC 0.54), tubulin beta chain (TUBB) (Log2FC 0.57) and tubulin beta-3 chain (TUBB3) (Log2FC 0.71) were significantly higher (all FDR P < 0.01). Fig 1, *C*, shows

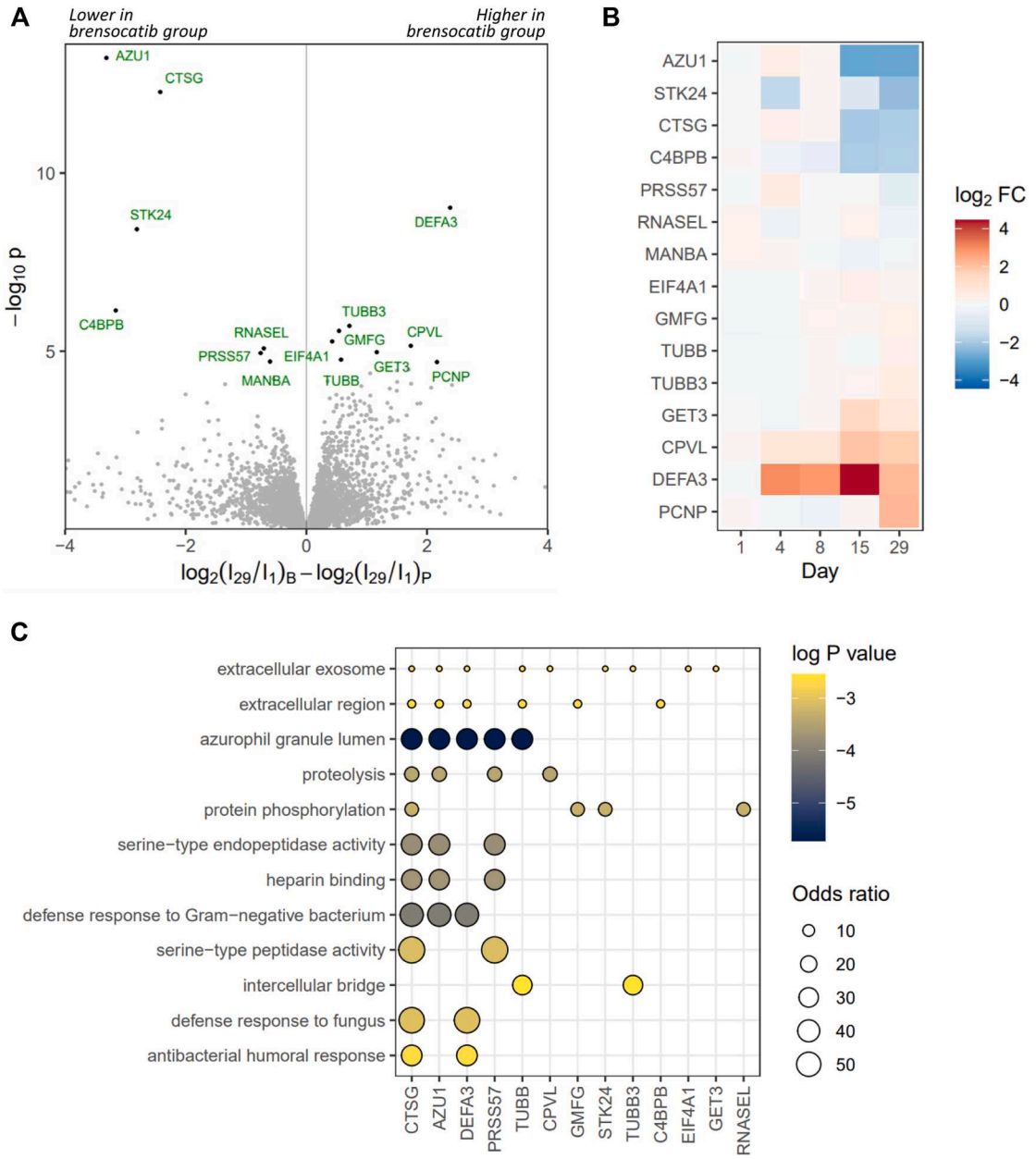

FIG 1. Significant effects of DPP-1 inhibition with brensocatib on the peripheral blood neutrophil proteome. Label-free LC-MS was used to profile the human neutrophil proteome in cells isolated from whole blood of participants in the STOP-COVID19 trial of once daily oral-dose brensocatib (25 mg) for 28 days. **(A)** Differential expression analysis at day 29 (end of trial) between the brensocatib and placebo groups. **(B)** Neutrophils were isolated for analysis at day 1, day 4 (participant subset), day 8, day 15, and day 29, and log2FC was plotted for selected significantly differentially expressed proteins between the drug and placebo group. Protein abundance per individual at the indicated time points was calculated as a log-ratio of intensity at day 29 to day 1 for each participant and each protein to determine changes in each participant and to identify differentially expressed proteins with brensocatib treatment. **(C)** Pathways in which altered proteins between the drug and placebo group are significantly involved. For all plots, proteins included are significantly (FDR $P <$ .01) differentially abundant between brensocatib and placebo based on paired log-ratio of day 1 and 29. Total samples included at each time point: day 1 = 124 (brensocatib 61, placebo 63), day 4 = 6 (brensocatib 4, placebo 2), day 8 = 42 (brensocatib 19, placebo 23), day 15 = 16 (brensocatib 10, placebo 6), day 29 = 91 (brensocatib 48, placebo 43). Total with paired day 1 and 29 samples: placebo = 33, brensocatib = 29. *AZU1,* Azurocidin-1; *STK24,* serine/threonine-protein kinase 24; *C4BPB,* C4b-binding protein; *CTSG,* cathepsin G; *RNASEL,* ribonuclease L; *PRSS57,* serine protease 57; *MANBA,* mannosidase beta; *EIF4A1,* eukaryotic translation initiation factor 4A1; *TUBB,* tubulin beta class I; *GMFG,* glial maturation factor γ; *TUBB3,* tubulin beta 3 class III; *GET3,* guided entry of tail-anchored proteins factor 3; *CPVL,* carboxypeptidase vitellogenic like; *PCNP,* PEST proteolytic signal containing nuclear protein; *DEFA3,* defensin A3.

the pathways in which the top 15 differentially expressed proteins are implicated. Of the top 21 differentially abundant proteins by day 29 in the brensocatib group, several microtubule components (TUBB, TUBA1B, TUBB3) were increased in abundance, in addition to alterations in proteins associated with cell migration (STK24, LSP1, GMFG), cell cycle (EVI2B, TUBA1B, PCNP), and apoptosis (ANO6, GSDMD, STK24) (Table II; Fig E4 in the Online Repository at www.jacionline.org).

Consistent with the pharmacology of brensocatib, Azu-1 levels, ELANE (NE), PRTN3 (PR3), and CTSG (CatG) were not altered at day 8, but appeared to be lowered from day 15 (Fig 2, *A*), although statistically significant differences in any proteins were seen only at day 29. Of note, ELANE and PRTN3 were numerically lower at day 29, but this did not reach statistical significance (Log2FC −0.74 and −0.359, FDR $P = .149$ and .583, respectively). In contrast, DEFA3 seemed to increase by day 8, whereas TUBB levels seemed to diverge only at day 29 (Fig 2, *A*), highlighting potential differences in effect kinetics.

DPP-1 inhibition with brensocatib resulted in significant reductions in circulating Azu-1 levels

Neutrophil proteomics highlighted Azu-1 as the most altered protein within these cells following brensocatib treatment. Azu-1 levels were measured in serum to determine whether this translated into a reduction in circulating protein levels. Azu-1 levels were similar in both the brensocatib and placebo arms at baseline (22.30 ± 2.24 μg/mL and 21.81 ± 2.23 μg/mL in brensocatib- and placebo-treated participants, respectively), but by day 15 significantly lower levels were observed in the brensocatib group, whereas levels were higher than baseline in the placebo group in individuals still hospitalized at this time point (3.74 ± 4.45 μg/mL vs 31.97 ± 4.57 μg/mL) (Fig 3, *A*). A sustained reduction in serum Azu-1 levels was shown up to day 29 in the brensocatib group (4.90 ± 1.45 μg/mL vs 17.79 ± 1.49 μg/mL, $P < .0001$ across all time points) (Fig 3, *A*). It should be noted that across many of these analyses we see higher levels at day 8 and/or 15 in the placebo group because this represents a subset of patients still hospitalized at those time points and therefore enrichment for patients with more severe illness.

Whereas neutrophil proteomics indicated changes in several serine proteases, including NE and PR3, within neutrophils with brensocatib treatment and significant reductions in plasma NE activity in the STOP-COVID19 trial were demonstrated in this trial,[19] when total NE levels were measured in the same plasma samples, there were no differences between the drug- and placebo-treated groups at any study sampling time points (Fig 3, *B*). Similar results were demonstrated for total PR3 levels measured in serum (Fig 3, *C*). To explore overall effects on release of primary/azurophilic granule content and overall neutrophil activation, serum MPO (primary granule component) (Fig 3, *D*) and calprotectin (cytoplasmic protein) (Fig 3, *E*) were also measured; both of these proteins showed no differences between treatment groups over time indicating no alterations in degranulation responses with brensocatib.

Neutrophil extracellular trap generation was unaltered by brensocatib treatment

Humans with genetic deficiency of DPP-1 in PLS are unable to form NETs.[10,11] Although elastase-independent mechanisms have recently been proposed, intracellular NE activity is widely reported to be essential for NETosis, and therefore the mechanism for inhibited NETosis in PLS is believed to be reduced NE activity.[26] Whether the magnitude of reduction in NE activity seen with brensocatib treatment is sufficient to inhibit NETosis is unknown. We therefore measured NET formation *in vitro* and *in vivo* using multiple methods. *In vivo* markers of NETs in serum, CitH3 (Fig 4, *A*) and DNA-MPO complexes (Fig 4, *B*), were unaltered by brensocatib treatment.

Isolated peripheral blood neutrophils stimulated with PMA (Fig 4, *C*) or LPS (Fig 4, *D*) for 4 hours *in vitro* showed no reductions in NETosis measured by SYTOX green nucleic acid staining. No differences in spontaneous NETosis were observed between the treatment groups at any time point (Fig 4, *E*).

Peripheral blood neutrophil surface expression of C5aR1 and CXCR2 was increased by day 29 in brensocatib-treated participants

In a panel of 5 inflammation-associated neutrophil surface proteins, expression of the elastase-cleavable C5a receptor (C5aR1/CD88)[4] (Fig 5, *A* and *B*) and chemokine receptor CXCR2 (Fig 5, *C* and *D*) was significantly higher in brensocatib-treated participants. No differences in abundance of these proteins in the neutrophil proteome were observed (Fig E3), potentially indicating differences exclusively in cell surface expression. Markers of primary granule release (CD63) (Fig 5, *E*) and cell activation (CD66b and CD11b) (Fig 5, *F* and *G*) were unchanged.

Although expression of CD88 has been associated with phagocytic capacity,[27] neutrophil phagocytosis of FITC-labeled *E coli* was unaffected by treatment, and no differences were observed between the drug and placebo-treated groups at day 29 (Fig 5, *H*).

Brensocatib had no detectable effect on peripheral blood leukocyte transcriptome, circulating immune cell proportions, or inflammatory cytokines

Despite significant changes in several neutrophil proteins identified in the treatment group by day 29, no significant differences in peripheral blood leukocyte gene expression were detected (Fig E5 in the Online Repository at www.jacionline.org), and no differences were identified in cell numbers or proportions using either clinically obtained white blood cell counts (Fig E6 in the Online Repository at www.jacionline.org) or mass cytometry in peripheral blood in a subset of participants at day 29 (Fig E7 in the Online Repository at www.jacionline.org), indicating that DPP-1 inhibition with brensocatib results in specific posttranslational changes with minimal impact on transcriptome and, importantly, that brensocatib did not affect granulopoiesis or production and release of other cell types over 29 days of treatment.

Functional cell surface markers were included in the mass cytometry analysis, and no significant differences were observed for any of these markers (of note, CD88 and CXCR2 were not part of this panel); there was a nominal increase in eosinophil HLADR (low expression detected) and CD66b, but these changes were not significant after adjustment for multiple comparisons (Fig E7, *F*).

Using the Olink Target 48 panel to measure 45 serum cytokines, we observed significant changes in FLT3LG, TNF, and CCL19 at day 15 only and IL-4 at day 29 only (FDR $P < .05$);

TABLE II. Top differentially abundant proteins in patients receiving brensocatib versus controls by day 29

Gene symbol	Protein name	Log2FC*	Averaged expression	*P* value	FDR	Protein description/ function	Cellular localization and cell types
AZU1	Azurocidin-1	−3.31426	−1.12888	5.85×10^{-14}	3.08×10^{-10}	Pseudoenzyme; similar structure to NSPs, roles in monocyte chemotaxis, endothelial permeability, antimicrobial responses	Neutrophil primary/ azurophilic granules, other cell types including monocytes
CTSG	Cathepsin G	−2.42068	−1.0575	5.31×10^{-13}	1.40×10^{-9}	NSP; antimicrobial activity, roles in tissue remodeling	Neutrophil primary/ azurophilic granules, other cell types including monocytes, DCs, mast cells
DEFA3	Defensin A3 (a.k.a. HNP-3)	2.383271	1.351047	9.44×10^{-10}	1.66×10^{-6}	Antimicrobial peptide; broad antimicrobial activity	Primary/azurophilic granules, B cells, nonimmune cells
STK24	Serine/threonine kinase 24	−2.81013	−1.57401	3.78×10^{-9}	4.97×10^{-6}	Kinase; function upstream of MAPK signaling, roles in apoptosis and cell migration	Nucleoli, cytosol, broad expression
C4BPB	Complement component 4 binding protein beta	−3.16008	−1.76515	7.17×10^{-7}	7.54×10^{-4}	Beta chain of the C4b-binding protein; regulation of classic complement cascade activation	Golgi, RNA expression in immune cells (not neutrophils) and in nonimmune cells, particularly hepatocytes
TUBB3	Tubulin beta 3 class III	0.711587	−0.08049	1.95×10^{-6}	0.001712	Part of a heterodimer with α-tubulin; structural component of microtubules	Microtubules; broad expression
GMFG	Glia maturation factor gamma	0.539624	0.684855	2.68×10^{-6}	0.002016	ADF/cofilin superfamily protein; role in actin reorganization, neutrophil chemotaxis, and polarity (localized to leading edge)	Granules, nucleoplasm, cytosol; broad cell expression, enriched in neutrophils
EIF4A1	Eukaryotic translation initiation factor 4A1	0.426552	−0.1166	5.29×10^{-6}	0.003477	RNA helicase; facilitates mRNA binding to ribosomes	Cytosol; broad expression
CPVL	Carboxypeptidase vitellogenic like	1.727668	1.732844	7.06×10^{-6}	0.004125	Carboxypeptidase; unknown function, hypothesized digestion within lysosome, role in antigen processing for presentation	Endoplasmic reticulum; neutrophil, monocyte, macrophage, DC
RNASEL	Ribonuclease L	−0.70515	−0.5341	8.30×10^{-6}	0.004364	Endoribonuclease; role in antiviral interferon responses	Cytosol; broad expression
GET3	Guided entry of tail-anchored proteins factor 3	1.164497	−0.12239	1.06×10^{-5}	0.004946	ATPase; delivery of tail-anchored proteins to the endoplasmic reticulum	Nucleoli, nucleoplasm; broad expression
PRSS57	Serine protease 57	−0.75763	−0.20187	1.13×10^{-5}	0.004946	NSP; antimicrobial activity	Primary/azurophilic granules; broad expression, enriched in neutrophils
TUBB	Tubulin beta class I	0.573507	0.037794	1.72×10^{-5}	0.006961	Part of a heterodimer with α-tubulin; structural component of microtubules	Microtubules; broad expression
MANBA	Mannosidase beta	−0.60179	−0.02051	1.95×10^{-5}	0.007015	N-linked glycoprotein oligosaccharide catabolism	Vesicles (lipid droplets, endosomes, lysosomes); broad expression, enriched in neutrophils and monocytes

(Continued)

TABLE II. *(Continued)*

Gene symbol	Protein name	Log2FC*	Averaged expression	*P* value	FDR	Protein description/ function	Cellular localization and cell types
PCNP	PEST proteolytic signal containing nuclear protein	2.16532	0.693218	2.00×10^{-5}	0.007015	Protein ubiquitination and processing, possible role in cell cycle regulation	Nucleoplasm, nuclear bodies; broad expression
PPP1R11	Protein phosphatase 1 regulatory inhibitor subunit 11	1.693883	−0.02379	3.25×10^{-5}	0.010672	Inhibitor of protein phosphatase-1, E3 ubiquitin ligase acing on TLR2, roles in cytokine and antibacterial responses	Vesicles (lipid droplets, endosomes, lysosomes); broad expression
LSP1	Lymphocyte specific protein 1	1.497357	1.425726	3.48×10^{-5}	0.010772	F-actin binding protein, potential role in neutrophil migration and adhesion	Undefined intracellular localization; broad expression, enriched in neutrophil
GSDMD	Gasdermin D	1.056811	−0.07265	4.15×10^{-5}	0.012132	Pore-forming protein; roles in inflammatory signaling, inflammasome activity, and apoptosis	Nucleoplasm; broad expression
ANO6	Anoctamin 6	−0.54393	0.064896	5.99×10^{-5}	0.016571	Transmembrane protein; facilitates cell surface phosphatidyl serine exposure	Cell membrane and cytosol; broad expression
TUBA1B	Tubulin alpha 1b	0.352102	−0.01269	6.93×10^{-5}	0.018221	Microtubule component; role in cellular organization, implicated in cell cycle regulation and response to IL-4	Microtubules; broad expression, enriched in monocytes
EVI2B	Ecotropic viral integration site 2B	1.502531	0.778449	7.29×10^{-5}	0.018244	Transmembrane glycoprotein; hematopoietic cell cycle regulation, promotion of granulocyte differentiation	Plasma membrane; expression across immune cell types, enriched in neutrophils

Protein description/function from GeneCards, The Human Gene Database; cellular localization and cell type from The Human Protein Atlas.
ADF, Actin-depolymerizing factor; *a.k.a.,* also known as; *DC,* dendritic cell; *MAPK,* mitogen-activated protein kinase; *TLR2,* toll-like receptor 2.
*log2(I29I1)B − log2(I29I1)P.

all of these proteins were increased in the brensocatib-treated group. Nominal changes in FLT3LG, EGF, CCL13, and CCL19 were observed during the total study period (unadjusted $P < .05$; increased in brensocatib group), but all 45 markers were not statistically significant between the brensocatib and placebo groups after FDR adjustment for treatment-by-time interaction overall (Fig E8 in the Online Repository at www.jacionline.org).

Nasopharyngeal microbiota analysis

There were no significant differences over the course of the study in any alpha diversity metric (Shannon, Chao1, or Simpson index) between brensocatib and placebo groups. Beta diversity was not significantly different between groups at day 29 (PERMANOVA; $P = .8$) (Fig E9 in the Online Repository at www.jacionline.org) suggesting no effect of brensocatib treatment on the nasopharyngeal microbiota.

DISCUSSION

DPP-1 inhibition is a promising novel therapy for people with bronchiectasis and other chronic inflammatory lung diseases.[15,24,28] Excessive neutrophil accumulation and dysfunctional activation in the airways is a common feature of a number of diseases, including acute respiratory infections, chronic obstructive pulmonary disease, bronchiectasis, and cystic fibrosis. Airway NSP activity is significantly associated with disease pathology and severity in patients with bronchiectasis, and brensocatib treatment successfully lowered sputum NE, PR3, and CatG activity and improved clinical outcomes in the phase II WILLOW trial, with significantly reduced annual exacerbation rates also announced in the large phase III ASPEN trial.[13,14,28,29] A phase II trial in cystic fibrosis has also been completed.[30]

Given the potential for clinical use and further development of DPP-1 inhibition, a greater understanding of the impact of DPP-1 inhibition and subsequent serine protease inhibition on inflammation and immune responses is vital. In the STOP-COVID19 trial of brensocatib in patients hospitalized with COVID-19, comprehensive immunoprofiling in participant samples was performed within a preplanned mechanistic substudy. Although the STOP-COVID19 trial did not demonstrate benefit of DPP-1 inhibition in COVID-19, successful and significant inhibition of NE activity was achieved with brensocatib treatment, and therefore this study represents a unique resource to better

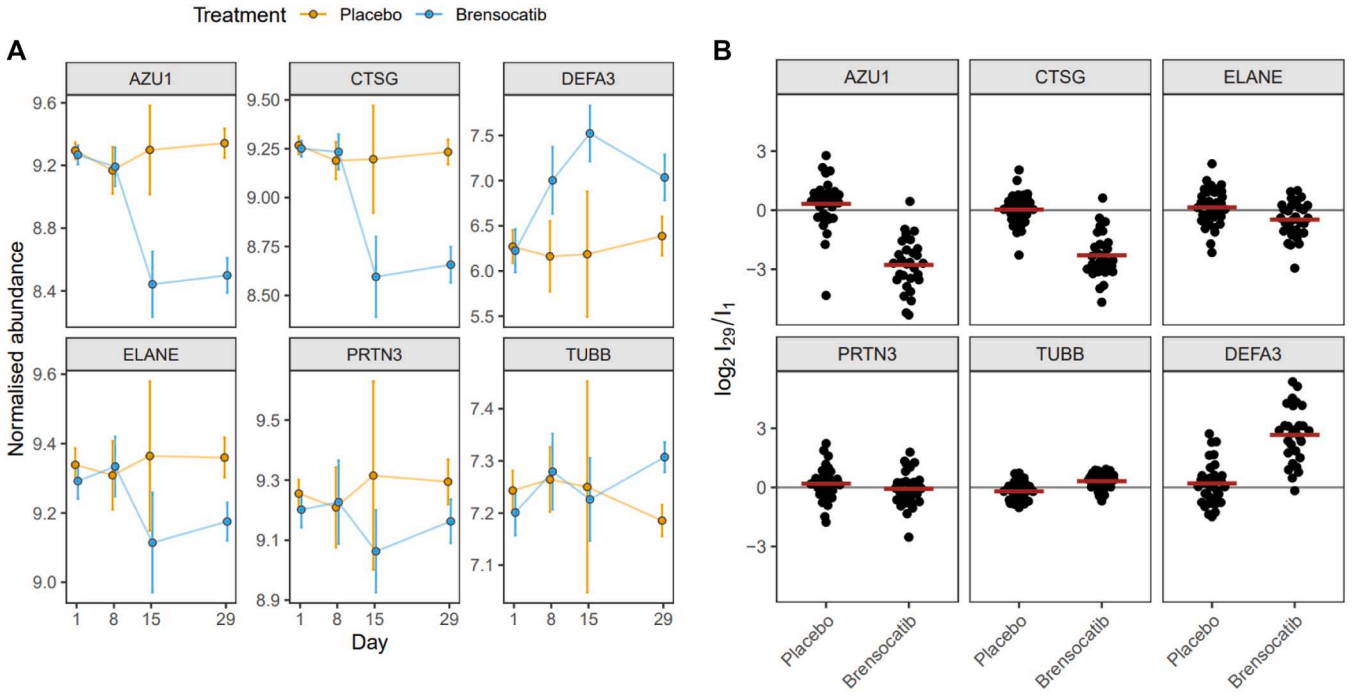

FIG 2. Effects of DPP-1 inhibition with brensocatib on key neutrophil proteins. LC-MS was used to profile the human neutrophil proteome in cells isolated from whole blood of participants in the STOP-COVID19 trial. **(A)** Protein abundance after quantile normalization, over 29 days of the trial for key proteins of interest. Data points are plotted for the group mean, and error bars indicate 95% CI. **(B)** Protein abundance per individual was calculated as a log-ratio of intensity at day 29 to day 1 for each participant and each protein. *Red line* indicates the mean. Total samples included at each time point: day 1 = 124 (brensocatib 61, placebo 63); day 8 = 42 (brensocatib 19, placebo 23); day 15 = 16 (brensocatib 10, placebo 6); day 29 = 91 (brensocatib 48, placebo 43). Total with paired day 1 and day 29 samples: placebo = 33, brensocatib = 29. *AZU1,* Azurocidin-1; *CTSG,* cathepsin G; *DEFA3,* defensin α3; *ELANE,* elastase; *PRTN3,* proteinase 3; *TUBB,* tubulin beta class I.

understand drug mechanisms of action when NSP inhibition is achieved.[19]

In the present work, we report a number of key findings. Most notably, we identify novel effects of DPP-1 inhibition on the pseudoenzyme Azu-1, also known as heparin-binding protein, with a significant and large reduction in Azu-1 levels within the neutrophil proteome, and consequently in serum, following brensocatib treatment. In addition to reducing the activity of NSPs as previously shown, total protein levels of CatG were greatly reduced in neutrophils in our study at the end of the trial, while total protein levels of NE and PR3 were largely preserved, as confirmed by both LC-MS and ELISA. Importantly, the lack of reduced levels of DPP-1, NE, and PR3 protein should not be misinterpreted to mean that DPP-1 inhibitors do not reduce the activity of these enzymes as proteomics and ELISA assays do not measure enzyme activity.

Brensocatib treatment significantly changed the expression levels of 55 neutrophil proteins with diverse functions (FDR $P < .05$). Interestingly, key neutrophil functions were unaffected by brensocatib, including phagocytosis, NET formation, and adhesion molecule expression. We observed an increase in CD88 (C5aR) expression—which has previously been shown to be cleaved from the surface of neutrophils by NE[4]—and CXCR2 expression on the surface of neutrophils at day 29. mRNA sequencing found no differences in peripheral leukocyte gene expression between the 2 treatment groups. Taken together, our data suggest a highly specific and focused impact of brensocatib on select NSPs and related proteins, in addition to highlighting novel potential substrates requiring further research attention as potential mechanisms of action of DPP-1 inhibition.

Azu-1 shows a high degree of structural homology to NE and is part of class 6 of the trypsin superfamily, which includes the serine proteases as well as other DPP-1 targets such as chymase-1 and granzyme A and B.[31] DPP-1 removes the N-terminal dipeptide of NSPs before packaging into granules during neutrophil maturation.[9] The NSP CatG was significantly and consistently reduced within neutrophils in our study and in PLS patient neutrophil granules, indicating that when the N-terminal peptide is not removed by DPP-1 these serine proteases are subsequently targeted for degradation.[10] However, why some NSPs were less affected in terms of protein abundance requires further research investigation. Azu-1 is unique among the serine proteases in that it has no known proteolytic activity and is classified as a pseudoenzyme. Studies of the pathophysiologic role of Azu-1 are limited by the fact that there is no homologue in mice. There are, however, extensive data implicating Azu-1 in various pathologies in humans. In sepsis and acute lung injury, Azu-1 has been shown to increase vascular permeability, enhance inflammatory cytokine release, and promote monocyte chemotaxis and endothelial adhesion,[32-34] and Azu-1 has consequently been identified as an early biomarker of circulatory failure in sepsis.[35] Exposure

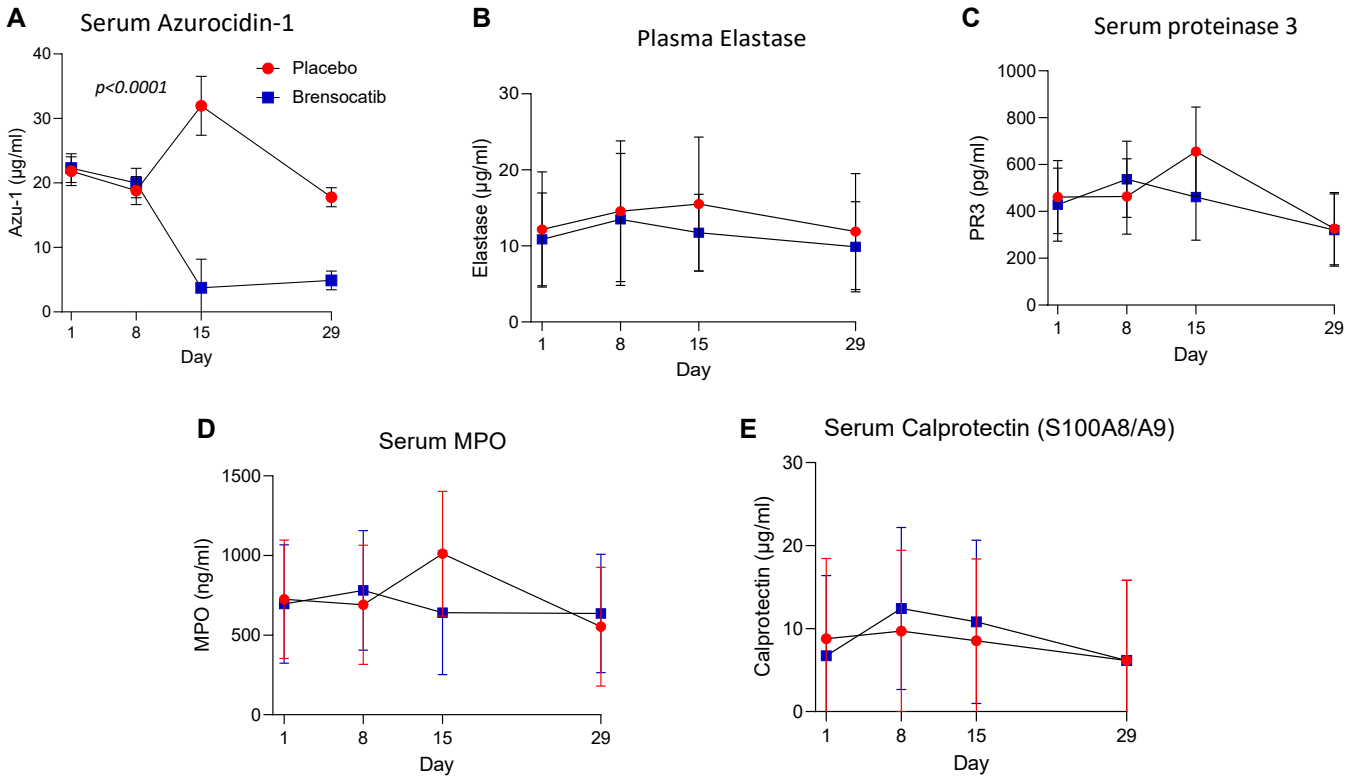

FIG 3. Significant changes in circulating Azu-1 with brensocatib treatment. **(A-E)** Levels of key neutrophil proteins were measured at baseline (day 1), day 8, day 15, and day 29 of treatment to determine the effect of DPP-1 inhibition on circulating neutrophil proteins by ELISA in serum *(A, C-E)* and in plasma *(B)*. Plasma was collected after 30 minutes of stimulation of whole blood with 10 mg/mL zymosan to stimulate neutrophil degranulation. Data were analyzed using a mixed-model repeated measures approach with fixed effects for treatment group and nominal time and treatment-by-time interaction. Azu-1: day 1 brensocatib n = 78, placebo n = 79; day 8 brensocatib n = 27, placebo n = 32; day 15 brensocatib n = 15, placebo n = 13; day 29 brensocatib = 52, placebo n = 49. Elastase: day 1 brensocatib n = 77, placebo n = 77; day 8 brensocatib n = 25, placebo n = 32; day 15 brensocatib n = 16, placebo n = 13; day 29 brensocatib n = 51, placebo n = 47. PR3: day 1 brensocatib n = 68, placebo n = 69; day 8 brensocatib n = 24, placebo n = 29; day 15 brensocatib n = 15, placebo n = 12; day 29 brensocatib n = 41, placebo n = 42. MPO: day 1 brensocatib n = 78, placebo n = 73; day 8 brensocatib n = 26, placebo n = 32; day 15 brensocatib n = 15, placebo n = 13; day 29 brensocatib = 49, placebo n = 48. Calprotectin: day 1 brensocatib n = 79, placebo n = 78; day 8 brensocatib n = 26, placebo n = 32; day 15 brensocatib n = 15, placebo n = 13; day 29 brensocatib n = 52, placebo n = 50.

of endothelial cells to Azu-1 causes increased permeability, implicating Azu-1 directly in the pathophysiology of sepsis and acute lung injury.[34] In bronchiectasis, our group has shown that increased sputum Azu-1 concentration was associated with more severe bronchiectasis, chronic infection with *Pseudomonas aeruginosa*, and frequent exacerbations.[36] Azu-1 was demonstrated to be a ciliotoxin that reduced mucociliary clearance and impaired epithelial function in bronchiectasis.[37] These data suggest a novel potential mechanism through which DPP-1 inhibition may benefit patients with bronchiectasis.

Development of novel DPP-1 inhibitors requires demonstration of target engagement, which has been traditionally achieved using zymosan stimulation of blood and measurement of NE activity.[15,24,28] This is labor-intensive and difficult to standardize across sites in multicenter studies. We show here that measurement of serum Azu-1 is a simple, reproducible, and sensitive measure of DPP-1 target engagement that may be useful in future trials.

A previous study investigated the neutrophil granule proteome in PLS in a 24-year-old woman with aggressive periodontitis.[10] This study showed profound suppression of NE, PR3, and CatG. Whereas Azu-1 was also lower, this difference was of a lesser magnitude than that for all 3 NSPs in PLS. It is important to note, therefore, that brensocatib does not induce PLS as it does not achieve, or attempt to achieve, 100% inhibition of DPP-1 or 100% inhibition of NSPs, as also supported by a lack of effect on NETosis in the present study measured by multiple outputs. Whether higher doses or more potent inhibition of DPP-1 inhibitor would result in complete NSP inhibition and subsequently NET inhibition requires further investigation.

Further significant changes in neutrophil proteins were indicated by day 29, all of which would be of interest for further investigations. The increase in neutrophil defensin A3 (DEFA3) with treatment is particularly interesting in the context of future development of DPP-1 inhibitors. No overall changes in alpha defensin levels were found previously in PLS neutrophils;

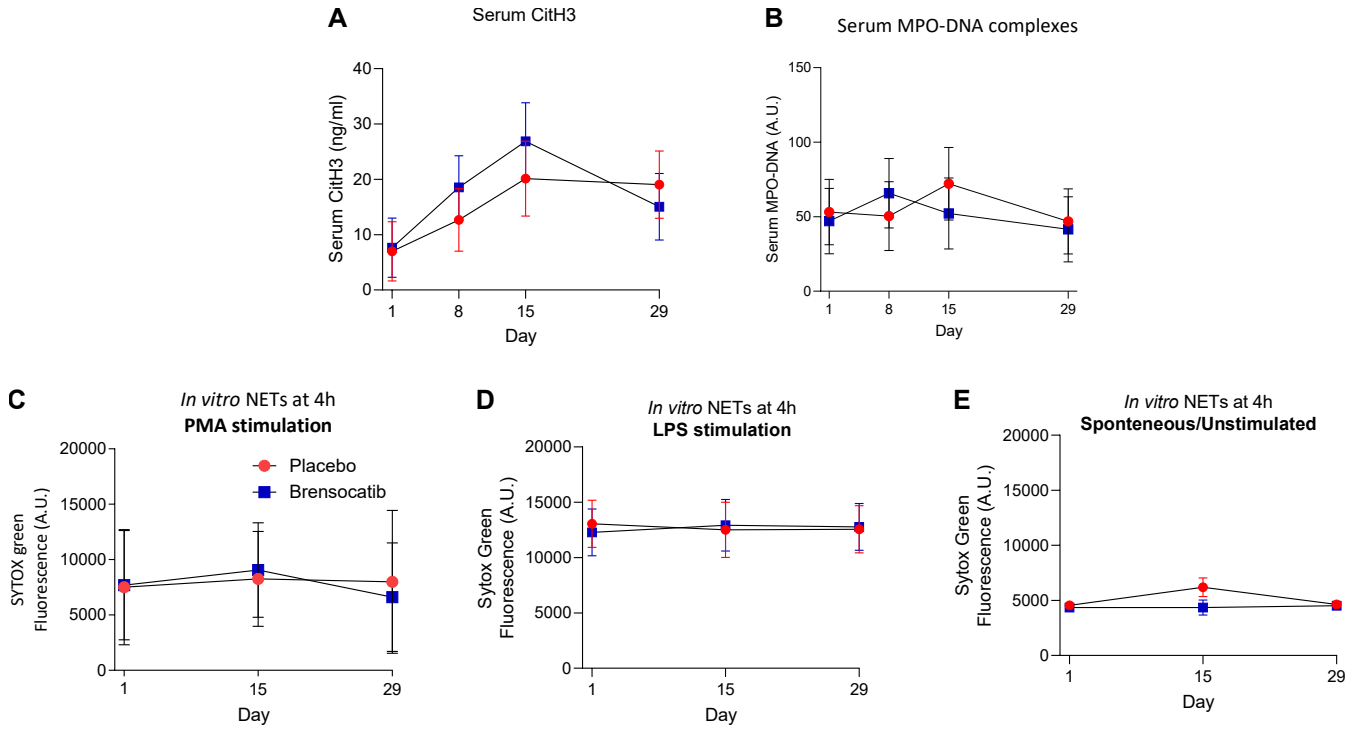

FIG 4. Measurement of *in vivo* and *in vitro* NETs after DPP-1 inhibition. To understand effects of protease inhibition with brensocatib on NETosis, 2 methods were used. **(A** and **B)** NETs were measured *in vivo* at day 1 (baseline), day 8, day 15, and day 29 in serum samples using ELISA for *(A)* CitH3 and *(B)* MPO-DNA complexes. **(C-E)** NET production by isolated peripheral blood neutrophils was measured using a fluorometric in-plate assay with SYTOX green DNA stain after 4 hours of stimulation with *(C)* PMA (100 nM), *(D)* LPS (5 μg/mL final concentration), or *(E)* no additional stimulus (spontaneous NETosis) at day 1 (baseline), day 15, and data 29. All data were analyzed using a mixed-model repeated measures approach with fixed effects for treatment group and nominal time, and treatment-by-time interaction. Serum CitH3 measurement: day 1 brensocatib n = 73, placebo n = 72; day 8 brensocatib n = 26, placebo n = 31; day 15 brensocatib n = 15, placebo n = 13; day 29 brensocatib n = 51, placebo n = 46. Serum DNA-MPO measurement: day 1 brensocatib n = 76, placebo n = 76; day 8 brensocatib n = 27, placebo n = 32; day 15 brensocatib n = 15, placebo n = 13; day 29 brensocatib n = 47, placebo n = 48. *In vitro* NETs: day 1 brensocatib n = 47, placebo n = 42; day 15 brensocatib n = 9, placebo n = 9; day 29 brensocatib n = 39, placebo n = 38.

however, normal expression was suggested as one reason for the lack of significant immunodeficiency in these individuals.[10] Neutrophil alpha defensins have been shown to be antimicrobial, protect neutrophils from microbial virulence factors, reduce inflammation, and enhance the efficacy of antibiotic treatments.[38-40] We have recently extended these findings by showing increased neutrophil DEFA3 in the sputum of patients with bronchiectasis following treatment with brensocatib in a post hoc analysis of the WILLOW trial, demonstrating that our findings presented in the study may be relevant beyond COVID-19.[41] PRSS57 is also known as NSP4 and is a known target of DPP-1 and was significantly lower by day 29.

Brensocatib was shown to prolong time to first exacerbation in patients with bronchiectasis. The proposed mechanism of action is primarily reduction in airway NE activity, but baseline levels of NE activity did not predict the efficacy of treatment.[28] Our data in COVID-19 suggest that the efficacy of DPP-1 inhibitors may go substantially beyond NE inhibition and suggest that measurement of a single DPP-1 target may not identify responders. Reductions in Azu-1 and increases in neutrophil DEFA3 are just 2 potential additional mechanisms with plausible benefits in the context of chronic airway inflammation.

Our study has some important limitations particularly for informing future development in the context of bronchiectasis. Our study used samples from patients with COVID-19, and there may be differences in the inflammatory response and neutrophil behavior between this disease and chronic inflammatory conditions. The present results at this stage can be considered relevant only to COVID-19, but as noted above, we have now validated the observations in relation to Azu-1 and neutrophil DEFA3 directly in patients with bronchiectasis.[37,41] Our studies are limited to 28 days, as 28 days was shown to be a time point when maximal NSP inhibition was achieved in bronchiectasis, but we cannot investigate whether the changes we see may be different over longer periods of follow-up. Limitations of sample availability for some participants as well as on comparison of samples for LC-MS across multiple batches meant that not all analyses included every participant, and further multisite large-scale investigations are warranted.

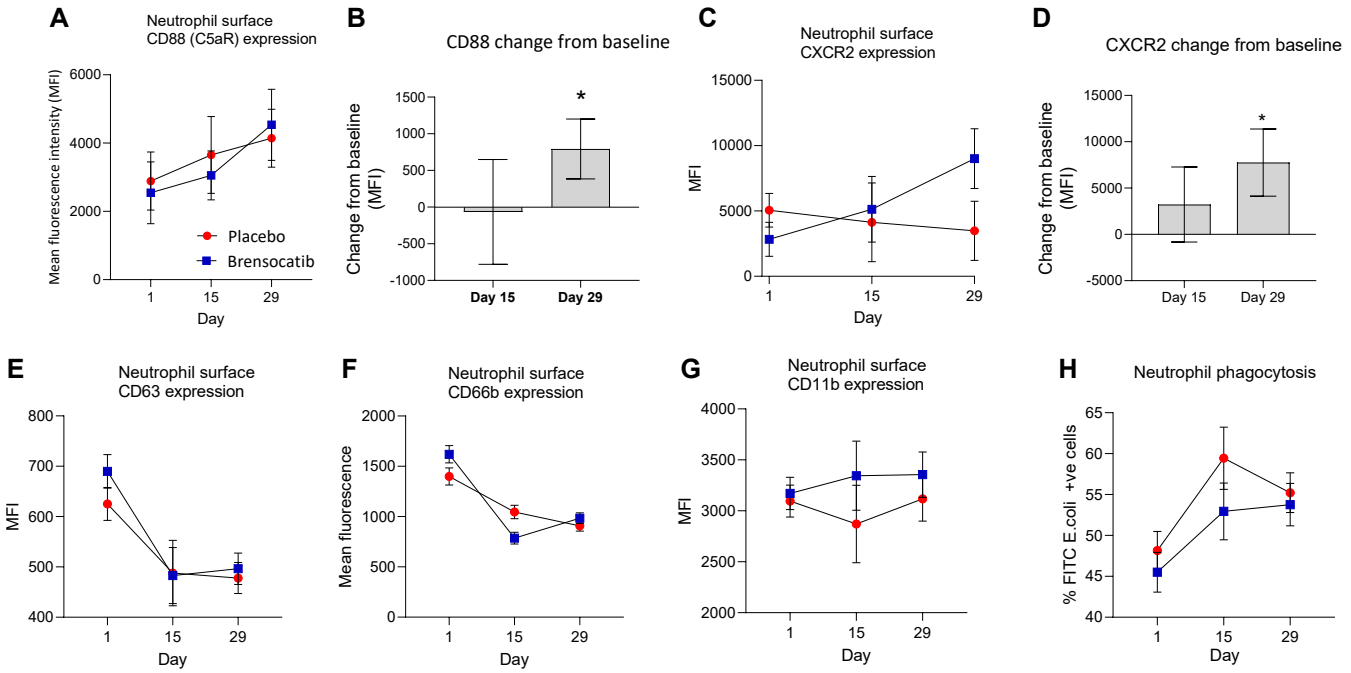

FIG 5. Peripheral blood neutrophil activation status and phagocytic capacity and effects of DPP-1 inhibition. To determine effects of DPP-1 inhibition on neutrophil activation status, peripheral blood neutrophils were isolated at day 1 (baseline), day 15, and day 29 (end of trial) to obtain a highly purified cell population, stained for selected surface markers using fluorescently conjugated antibodies, and analyzed by flow cytometry. Key cell surface markers were **(A** and **B)** CD88 (also known as c5a receptor 1), linked with neutrophil phagocytic capacity and susceptible to elastase-mediated cleavage; **(C** and **D)** CXCR2, a marker of cell activation status and maturity; **(E)** CD63, a marker of primary/azurophilic granule release; **(F)** CD66b, an activation marker, adhesion molecule, and marker of degranulation; and **(G)** CD11b, an activation marker and adhesion molecule required for neutrophil migration affected by inflammation, cell activation status, and protease activity. **(H)** Phagocytic capacity of isolated neutrophils was also investigated at the same time points after 30 minutes of incubation with heat-killed, FITC-labeled, opsonized *E coli*. Data were analyzed using a mixed-model repeated measures approach with fixed effects for treatment group and nominal time and treatment-by-time interaction. Cell surface markers: day 1 brensocatib n = 62, placebo n = 61; day 15 brensocatib n = 6; placebo n = 7; day 29 brensocatib n = 37; placebo n = 38. Phagocytosis: day 1 brensocatib n = 48, placebo n = 49, day 15 brensocatib n = 10, placebo n = 8; day 29 brensocatib n = 36, placebo n = 38.

Conclusion

Neutrophil proteomics identified 55 significantly altered proteins with DPP-1 inhibition in humans over 28 days of treatment. We identify Azu-1 as a novel target of DPP-1 inhibition with relevance to human disease, which may also serve as a useful biomarker of DPP-1 inhibition in the future.

DISCLOSURE STATEMENT

Funded as an investigator-initiated study by Insmed Incorporated; STOP-COVID19 trial (clinical trial registration number ISRCTN30564012).

Disclosure of potential conflict of interest: J. D. Chalmers declares funding from Insmed for the present work and previous receipt of grants, contracts, or consulting fees from AstraZeneca, Genentech, Insmed, Novartis, Gilead Sciences, GlaxoSmithKline, Grifols, Boehringer Ingelheim, Pfizer, Jansen, Chiesi, Antabio, and Zambon. A. J. Dicker declares grants or contracts from AstraZeneca and GlaxoSmithKline. A. A. R. Thompson declares funding from the British Heart Foundation Intermediate fellowship for the present work and previous payment or honoraria from Janssen-Cilag Ltd. A. Singanayagam declares payment or honoraria from AstraZeneca and Insmed. The rest of the authors declare that they have no relevant conflicts of interest.

Key messages

- **DPP-1 inhibitors reduce neutrophil serine protease activity.**
- **A multi-omic substudy of a phase II trial of a DPP-1 inhibitor demonstrated effects on the neutrophil proteome, revealing Azu-1 as a key target.**

REFERENCES

1. Chalmers JD, Moffitt KL, Suarez-Cuartin G, Sibila O, Finch S, Furrie E, et al. Neutrophil elastase activity is associated with exacerbations and lung function decline in bronchiectasis. Am J Respir Crit Care Med 2017;195:1384-93.
2. Gray RD, McCullagh BN, McCray PB. NETs and CF lung disease: current status and future prospects. Antibiotics (Basel) 2015;4:62-75.
3. Dicker AJ, Crichton ML, Pumphrey EG, Cassidy AJ, Suarez-Cuartin G, Sibila O, et al. Neutrophil extracellular traps are associated with disease severity and microbiota diversity in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2018;141:117-27.

4. Berg CW, Tambourgi D, Clark HW, Hoong SJ, Spiller OB, McGreal EP. Mechanism of neutrophil dysfunction: neutrophil serine proteases cleave and inactivate the C5a receptor. J Immunol 2014;192:1787-95.
5. Keir HR, Chalmers JD. Neutrophil extracellular traps in chronic lung disease: implications for pathogenesis and therapy. Eur Respir Rev 2022;31:210241.
6. Shoemark A, Cant E, Carreto L, Smith A, Oriano M, Keir H, et al. A point-of-care neutrophil elastase activity assay identifies bronchiectasis severity, airway infection and risk of exacerbation. Eur Respir J 2019;53:1900303.
7. Gramegna A, Aliberti S, Sibila O, Francesco C, Sotgiu G, Perea L, et al. Sputum neutrophil elastase in bronchiectasis: a Southern European cohort study. Eur Respir J 2020;56:2001702.
8. Keir HR, Shoemark A, Dicker AJ, Perea L, Pollock J, Giam YH, et al. Neutrophil extracellular traps, disease severity, and antibiotic response in bronchiectasis: an international, observational, multicohort study. Lancet Respir Med 2021;9:873-84.
9. Chalmers JD, Kettritz R, Korkmaz B. Dipeptidyl peptidase 1 inhibition as a potential therapeutic approach in neutrophil-mediated inflammatory disease. Front Immuno 2023;14:1239151.
10. Sørensen OE, Clemmensen SN, Dahl SL, Østergaard O, Heegaard NH, Glenthøj A, et al. Papillon-Lefèvre syndrome patient reveals species-dependent requirements for neutrophil defenses. J Clin Invest 2014;124:4539-48.
11. Roberts H, White P, Dias I, McKaig S, Veeramachaneni R, Thakker N, et al. Characterization of neutrophil function in Papillon-Lefèvre syndrome. J Leukoc Biol 2016;100:433-44.
12. Pham CT, Ivanovich JL, Raptis SZ, Zehnbauer B, Ley TJ. Papillon-Lefèvre syndrome: correlating the molecular, cellular, and clinical consequences of cathepsin C/dipeptidyl peptidase I deficiency in humans. J Immunol 2004;173:7277-81.
13. Cipolla D, Zhang J, Korkmaz B, Chalmers JD, Basso J, Lasala D, et al. Dipeptidyl peptidase-1 inhibition with brensocatib reduces the activity of all major neutrophil serine proteases in patients with bronchiectasis: results from the WILLOW trial. Respir Res 2023;24:133.
14. Chalmers J, Burgel P, Daley C, De Soyza A, Haworth C, Mauger D, et al. A phase 3, randomized, double-blind, placebo-controlled study to assess the efficacy, safety, and tolerability of brensocatib administered once daily for 52 weeks in patients with non-cystic fibrosis bronchiectasis—the ASPEN Study. Presented at: 7th World Bronchiectasis Conference; July 4-6, 2024; Dundee, Scotland.
15. Chalmers JD, Gupta A, Chotirmall SH, Armstrong A, Eickholz P, Hasegawa N, et al. A phase 2 randomised study to establish efficacy, safety and dosing of a novel oral cathepsin C inhibitor, BI 1291583, in adults with bronchiectasis: Airleaf. ERJ Open Res 2023;9:00633-2022.
16. Chalmers JD, Burgel P-R, Daley CL, Soyza A, Haworth CS, Mauger D, et al. Brensocatib in non–cystic fibrosis bronchiectasis: ASPEN protocol/baseline characteristics. ERJ Open Res 2024;10:00151-2024.
17. Pham CT, Ley TJ. Dipeptidyl peptidase I is required for the processing and activation of granzymes A and B in vivo. Proc Natl Acad Sci U S A 1999;96:8627-32.
18. Guéant JL, Guéant-Rodriguez RM, Fromonot J, Oussalah A, Louis H, Chery C, et al. Elastase and exacerbation of neutrophil innate immunity are involved in multi-visceral manifestations of COVID-19. Allergy 2021;76:1846-58.
19. Keir HR, Long MB, Abo-Leyah H, Giam YH, Vadiveloo T, Pembridge T, et al. Dipeptidyl peptidase-1 inhibition in patients hospitalised with COVID-19: a multicentre, double-blind, randomised, parallel-group, placebo-controlled trial. Lancet Respir Med 2022;10:1119-28.
20. Long MB, Howden AJ, Keir HR, Rollings CM, Giam YH, Pembridge T, et al. Extensive acute and sustained changes to neutrophil proteomes post-SARS-CoV-2 infection. Eur Respir J 2024;63:2300787.
21. Knackstedt SL, Georgiadou A, Apel F, Abu-Abed U, Moxon CA, Cunnington AJ, et al. Neutrophil extracellular traps drive inflammatory pathogenesis in malaria. Sci Immunol 2019;4:eaaw0336.
22. Long MB, Abo-Leyah H, Giam YH, Vadiveloo T, Hull RC, Keir HR, et al. SFX-01 in hospitalised patients with community-acquired pneumonia during the COVID19 pandemic: a double-blind, randomised, placebo-controlled trial. ERJ Open Res 2024;10:00917-2023.
23. Callahan BJ, Grinevich D, Thakur S, Balamotis MA, Yehezkel T. Ultra-accurate microbial amplicon sequencing with synthetic long reads. Microbiome 2021;9:130.
24. Palmer R, Maenpaa J, Jauhiainen A, Larsson B, Mo J, Russell M, et al. Dipeptidyl peptidase 1 inhibitor AZD7986 induces a sustained, exposure-dependent reduction in neutrophil elastase activity in healthy subjects. Clin Pharmacol Ther 2018;104:1155-64.
25. Perera NC, Schilling O, Kittel H, Back W, Kremmer E, Jenne DE. NSP4, an elastase-related protease in human neutrophils with arginine specificity. Proc Natl Acad Sci U S A 2012;109:6229-34.
26. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J Cell Biol 2010;191:677-91.
27. Conway Morris A, Kefala K, Wilkinson TS, Dhaliwal K, Farrell L, Walsh T, et al. C5a mediates peripheral blood neutrophil dysfunction in critically ill patients. Am J Respir Crit Care Med 2009;180:19-28.
28. Chalmers JD, Haworth CS, Metersky ML, Loebinger MR, Blasi F, Sibila O, et al. Phase 2 trial of the DPP-1 inhibitor brensocatib in bronchiectasis. N Engl J Med 2020;383:2127-37.
29. Chalmers JD, Usansky H, Rubino CM, Teper A, Fernandez C, Zou J, et al. Pharmacokinetic/pharmacodynamic evaluation of the dipeptidyl peptidase 1 inhibitor brensocatib for non-cystic fibrosis bronchiectasis. Clin Pharmacokinet 2022;61:1457-69.
30. Konstan M, Flume P, Ramirez C, Li S, Teper A, Vergara M, et al. Pharmacokinetics and safety of brensocatib in patients with cystic fibrosis. Eur Respir J 2023;62(suppl 67)OA1537.
31. Jenne DE. Structure of the azurocidin, proteinase 3, and neutrophil elastase genes. Implications for inflammation and vasculitis. Am J Respir Crit Care Med 1994;150(6 Pt 2):S147-54.
32. Soehnlein O, Lindbom L. Neutrophil-derived azurocidin alarms the immune system. J Leukoc Biol 2009;85:344-51.
33. Edens HA, Parkos CA. Neutrophil transendothelial migration and alteration in vascular permeability: focus on neutrophil-derived azurocidin. Curr Opin Hematol 2003;10:25-30.
34. Gautam N, Olofsson AM, Herwald H, Iversen LF, Lundgren-Akerlund E, Hedqvist P, et al. Heparin-binding protein (HBP/CAP37): a missing link in neutrophil-evoked alteration of vascular permeability. Nat Med 2001;7:1123-7.
35. Lin Q, Shen J, Shen L, Zhang Z, Fu F. Increased plasma levels of heparin-binding protein in patients with acute respiratory distress syndrome. Crit Care 2013;17:R155.
36. Abo-Leyah H, Keir HR, Shoemark A, Finch S, Smith A, Barclay H, et al. M18 heparin-binding protein as a biomarker inflammation, symptoms and severity in bronchiectasis. Thorax 2019;74(suppl 2)A245.
37. Shoemark A, Johnson ED, Shuttleworth M, Schwiening M, Hull R, Stobo J, et al. Azurocidin-1 links bronchiectasis severity and exacerbations through impairment of epithelial defence and is targeted by dipeptidyl peptidase-1 inhibition. Available at SSRN: https://ssrn.com/abstract=5177613. Accessed March 18, 2025.
38. Wang H, Dong B, Lou L. HNP-3 enhanced the antimicrobial activity of CIP by promoting ATP efflux from P. aeruginosa cells. Mol Biol Rep 2011;38:2673-8.
39. Zhao L, Lu W. Defensins in innate immunity. Curr Opin Hematol 2014;21:37-42.
40. Tai KP, Kamdar K, Yamaki J, Le V, Tran D, Tran P, et al. Microbicidal effects of α- and θ-defensins against antibiotic-resistant Staphylococcus aureus and Pseudomonas aeruginosa. Innate Immun 2015;21:17-29.
41. Johnson ED, Long MB, Perea L, Shih VH, Fernandez C, Teper A, et al. Broad immunomodulatory effects of the dipeptidyl-peptidase-1 inhibitor brensocatib in bronchiectasis: data from the phase 2, double-blind, placebo-controlled WILLOW trial. Am J Respir Crit Care Med Published online February 12, 2025.