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Systemic effects of cystic fibrosis transmembrane conductance regulator modulators on the plasma and serum proteome

Highlights

- Resource for systemic treatment effects of CFTR modulators LUM/IVA and ELX/TEZ/IVA
- Highlights greater improvement in inflammatory markers with ELX/TEZ/IVA treatment
- Compares local (lung) and systemic (serum) effects of ELX/TEZ/IVA treatment
- Suggests SFTPB as a potential marker for lung injury

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In brief

CFTR modulator drugs improve the function of mutant CFTR and significantly improve clinical outcomes in people with cystic fibrosis (pwCF). This study identifies specific and common signatures associated with inflammation and metabolic processes in the plasma and serum proteomes of pwCF treated with the CFTR modulator combinations lumacaftor/ivacaftor or elexacaftor/tezacaftor/ivacaftor.

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Systemic effects of cystic fibrosis transmembrane conductance regulator modulators on the plasma and serum proteome

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SUMMARY

Cystic fibrosis (CF) is caused by dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel in epithelial organs leading to a complex multi-organ disease. CFTR modulator drugs improve mutant CFTR function and markedly improve clinical outcomes in people with CF (pwCF), but their broader molecular and systemic effects remain to be fully elucidated. We employed mass-spectrometry-based proteomics to compare the plasma and serum proteomes of pwCF treated with the dual combination CFTR modulator lumacaftor/ivacaftor (LUM/IVA) or the triple combination of elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA). We found specific and common signatures associated with inflammation and metabolic processes. ELX/TEZ/IVA showed more consistent effects that were directed toward profiles in healthy individuals. Our findings indicate that ELX/TEZ/IVA leads to broader improvements in protein dysregulation than LUM/IVA in pwCF. Residual proteome changes may inform future therapeutic strategies for CF.
A record of this paper's transparent peer review process is included in the supplemental information.

INTRODUCTION

Cystic fibrosis (CF) is a severe, autosomal recessive disease that significantly impacts the life expectancy and overall well-being of affected individuals. The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. The CFTR gene encodes a chloride and bicarbonate channel on the apical membrane of various epithelial tissues. The most common mutation, *F508del*, affects 85% of all people with CF[1,2] and leads to disruptions in ion homeostasis, dehydration of epithelial surfaces, mucus thickening, chronic airway inflammation, and recurrent infections.[1] Together, these factors contribute to the challenges faced by individuals with CF. However, it is still unknown whether the systemic inflammation in CF is caused by CFTR dysfunction itself or a consequence of the overwhelming local inflammation in the lungs.[3–6]

The most significant cause of disability and mortality in CF today is the mucus obstruction in the lungs, which is accompanied by a progressive deterioration of lung function.[3] Beyond that, CF also exerts systemic effects by impairing the pancreatic, liver, and intestinal function, as well as affecting bone health.[1]

In the last decade, CFTR modulators have been developed to pharmacologically restore CFTR function.[2,7] Among these, lumacaftor/ivacaftor (LUM/IVA) and the more recent triple combination elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA) have shown promise. Lumacaftor, elexacaftor, and tezacaftor act as CFTR correctors, enhancing the folding and trafficking of mutant CFTR, and are used in combination with ivacaftor, a potentiator of CFTR function (Figure S1A).

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The dual combination therapy LUM/IVA was approved in 2015 for people with CF with two copies of the *F508del* mutation (*F508del*/*F508del*), which constitutes approximately 50% of the CF population. This combination has shown modest improvements in ion homeostasis, with reduced sweat chloride concentration by about 10%–20%.[8–10] Although a reduction in pulmonary exacerbations of about 30% was observed, there were only subtle effects on lung function (FEV_1% predicted).[11–13] The triple combination therapy ELX/TEZ/IVA has recently been approved for people with CF with at least one copy of the *F508del* mutation, making CFTR modulator therapy accessible to a majority of people with CF. The therapy has demonstrated significant positive outcomes, including a 40%–50% reduction in sweat chloride concentration, a 10%–20% improvement in FEV_1% predicted, and a notable 60% decrease in pulmonary exacerbations.[14–16] These benefits extend to various clinical parameters. People with CF receiving ELX/TEZ/IVA therapy exhibit better lung ventilation and reduction in mucus plugging, detected by magnetic resonance imaging (MRI).[17–19] Analysis of sputum from people with CF treated with ELX/TEZ/IVA showed that these clinical findings are associated with positive changes in parameters such as rheology, microbiome, and proteome.[20–22] These findings are also reflected in an increased life expectancy and quality of life.[5,23,24]

In recent years, proteomics has emerged as a powerful tool for investigating complex disease mechanisms. Efficient protein extraction conditions employed in shotgun proteomics workflows and the untargeted detection of proteins by mass spectrometry make this technology, in particular, suitable for systems medicine studies.[25,26] Multiple groups have used proteomics to investigate the CF proteome in cell lines, primary cells, as well as plasma, serum, and sputum samples.[20,21,27–31] Several studies have investigated changes in the plasma or serum proteome longitudinally, before and after pulmonary exacerbations (PEx), both with and without antibiotic treatment.[32–35] These studies have highlighted the importance of inflammatory proteins, such as those involved in the complement pathway, C-reactive protein (CRP), alpha-1-antitrypsin (SERPINA1), and matrix metalloproteases,[35–37] which have also been described in other biomarker studies, alongside calprotectin (S100A8/9), serum amyloid A 1/2 protein (SAA1/2), and CD14.[38–41] Additionally, proteins related to lipid and vitamin transport and metabolism, such as apolipoproteins (apolipoprotein A-I [APOA1], apolipoprotein B-100 [APOB]), vitamin-D-binding protein (GC), and retinol-binding protein 4 (RBP4), were shown to be altered in CF.[35,36,39,42] In the context of CF, plasma or serum proteomics is especially valuable, as it provides an almost non-invasive means of assessing disease progression and treatment efficacy. This is particularly important as collecting sputum samples to understand local lung changes becomes increasingly challenging, especially in young children who may be unable to produce sputum.

The primary objective of this study was to identify and compare changes in the plasma and serum proteome composition resulting from LUM/IVA and ELX/TEZ/IVA therapies. These proteomics alterations were systematically correlated with the clinical efficacy of the treatments on key endpoints, such as CFTR function (sweat chloride concentration) and lung function (FEV_1% predicted). Further, the study aimed to differentiate between effects emerging from the lung by including the corresponding sputum proteome from our previous research[20] and systemic effects observed in the serum. Our integrated analysis provides a valuable resource for understanding the pharmacodynamics of CFTR modulators, offering insights into the systemic and local protein regulation in CF (Figure 1).

RESULTS

Study population characteristics and clinical effects of LUM/IVA and ELX/TEZ/IVA

A total of 86 people with CF and 11 healthy controls were analyzed in this study (Figure 1). In the LUM/IVA cohort, all 32 people with CF were homozygous for the *F508del* mutation and were modulator-naive at baseline. The ELX/TEZ/IVA cohort included 54 people with CF, of whom 30 (55.6%) were homozygous for the *F508del* mutation, and 24 (44.4%) were heterozygous for the *F508del* mutation and a minimal function mutation. In this cohort, 29 (53.7%) were modulator-naive and 25 (46.3%) had received dual CFTR modulator therapy (LUM/IVA or TEZ/IVA) at baseline (Table 1). Consistent with previous studies,[8–13] the LUM/IVA cohort did not exhibit a significant improvement in FEV_1% predicted or a change in BMI following initiation of this therapy (Figure S1B). In contrast, the ELX/TEZ/IVA cohort showed a significant increase in both parameters. Both cohorts exhibited a decrease in sweat chloride concentration, with the ELX/TEZ/IVA group showing a more pronounced effect (Figure S1B). Notably, 23 people with CF who received ELX/TEZ/IVA were also included in a previously described sputum cohort.[20]

LUM/IVA therapy results in moderate and variable changes in the plasma proteome

To investigate the systemic effects of LUM/IVA therapy, we performed a label-free, shotgun mass-spectrometry analysis in data-independent acquisition mode (DIA) on plasma samples from 32 people with CF at baseline and 3 months after initiation of LUM/IVA therapy. Out of the 572 quantified proteins, 78 (13.6%) showed significant differences (Figure 2A; Table S1). Immunoglobulins were highly enriched in the group of downregulated proteins (cluster 1), while hydrolases, such as pantetheinase (VNN1) and mannan-binding lectin serine protease 2 (MASP2), were found among the upregulated proteins upon treatment (cluster 2; Figure 2B).

Although significant shifts in the plasma proteome were observed from baseline to treatment in individual people with CF, there was no clear separation of the two groups on a global level, as shown by principal-component analysis (PCA) (Figure 2C). The first two principal components (PC1 and PC2) suggested a heterogeneous response, with only partial co-directional changes, particularly along PC1. Single-sample gene set enrichment analysis (ssGSEA) using UniProt terms of the eigenvectors from PC1 and PC2 showed that changes in immune responses, including adaptive and innate immunity, as well as changes in extracellular matrix (ECM) proteins (ECM, cell adhesion, and EGF-like domain), contributed to the variance in PC1 and PC2 (Figure 2C; Table S2).

To relate the proteome changes to clinical characteristics, we performed correlation analysis of significantly changed proteins with the clinical parameters sweat chloride concentration,

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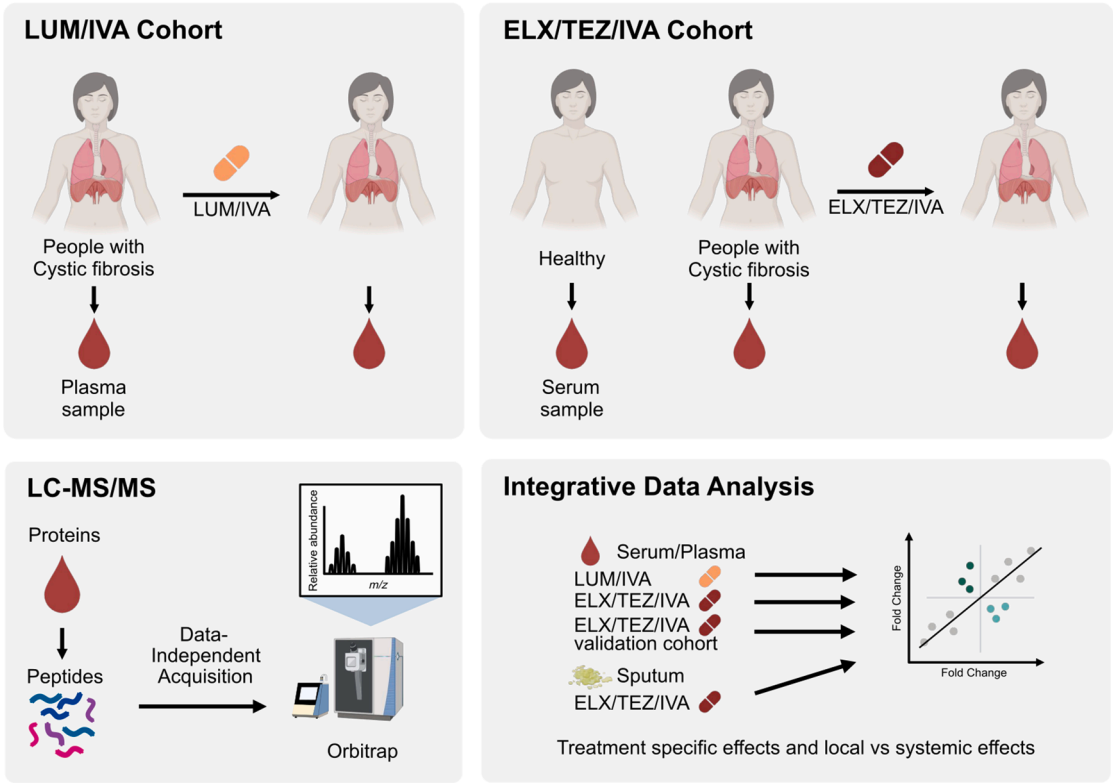

Figure 1. Study overview

Overview of the two cohorts treated with LUM/IVA ($n = 32$) or ELX/TEZ/IVA ($n = 54$) with proteomics workflow. The analysis included a comparison of both cohorts and an integrative analysis of overlapping people with CF from the ELX/TEZ/IVA cohort ($n = 23$), comparing serum data with sputum data from Schaupp et al. (2023).[20] Key findings were validated in an independent cohort ($n = 32$) analyzed on an Astral mass spectrometry platform.

a biomarker of CFTR function, and FEV_1% predicted, which serves as a marker of lung function. A full matrix of proteins correlating with either the sweat chloride level or the FEV_1% predicted is provided in (Figure S2B). Interestingly, SFTPB is negatively correlating with the lung function (Figure 2D; $\rho = -0.64$).

ELX/TEZ/IVA induces substantial proteomic shifts toward a healthier serum profile

To understand the systemic effects of ELX/TEZ/IVA therapy, we analyzed serum samples from 54 people with CF before and after 3 months of treatment and compared them with serum samples from 11 healthy individuals. Of the 559 quantified proteins, 196 (35.1%) exhibited significant differences between people with CF at baseline and healthy individuals, while 68 proteins (12.2%) showed significant changes between baseline and 3 months after initiation of ELX/TEZ/IVA (Figure 3A; Table S1). Despite treatment, 101 proteins (18.1%) remained significantly different from healthy controls in the ELX/TEZ/IVA cohort, and 170 proteins (30.4%) were significantly different across the three groups (Figure 3B; Table S1).

These proteins can be divided into two clusters. Cluster 1, representing proteins that were more abundant in people with CF at baseline in comparison with healthy individuals, was enriched in immunoglobulins and proteins involved in the complement pathway. On the other hand, cluster 2, which contained proteins less abundant in CF at baseline, was enriched in transport proteins such as transthyretin (TTR), vitamin-D-binding protein (GC), and retinol-binding protein 4 (RBP4) (Figure 3B).

Remarkably, serum proteomes from most people with CF evolved upon treatment in the same direction, which was toward healthy proteomes (Figure 3C). The primary contributors to the variance on PC1 were lipoproteins, proteins involved in lipid transport, as well as proteins involved in host immune defense.

Multiple proteins were found to correlate with sweat chloride concentration and with FEV_1% predicted (Figure S2B). However, SFTPB was the only protein that not only correlated with lung function but also had changes in SFTPB that correlated with the change in FEV_1% predicted (Figure 3D).

Stronger and distinctive response in ELX/TEZ/IVA- compared with LUM/IVA-treated people with CF

Since ELX/TEZ/IVA elicits a more robust response and improves clinical parameters and serum proteome of people with CF, we sought to determine whether the differences were due to a larger effect size, exceeding a critical threshold to induce a clinical response, or whether the response pattern was fundamentally distinct between both therapies.

Comparison of significantly different proteins between baseline and treated conditions in the two cohorts revealed that the

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Table 1. Baseline characteristics of the study population at baseline

	PwCF LUM/IVA (plasma)	Healthy controls (serum)	PwCF ELX/TEZ/IVA (serum)	Healthy controls (sputum)	PwCF ELX/TEZ/IVA (sputum)	PwCF ELX/TEZ/IVA (serum) *
Clinical characteristic	mean (± SD) or *n* (%)					
Number of pwCF (n)	32	11	54	7	23	32
Age (years)	19.4 (± 8.7)	34.2 (± 6.5)	32.4 (± 12.4)	34.0 (± 6.7)	30.1 (± 10)	27.9 (± 9.4)
Female participants	14 (43.8%)	7 (63.6%)	30 (55.6%)	4 (57.1%)	15 (65.2%)	15 (46.9%)
Sweat chloride (mmol/L)	88.3 (±8.1)	—	94.3 (±16.4)	—	96.8 (±14)	85.1 (±21.8)
FEV_1% predicted	65.3 (±18.3)	—	39.6 (±16.6)	—	35.3 (±14.3)	58 (±19.2)
BMI (kg/m^2)	19.1 (±3.0)	—	19.6 (±2.9)	—	19.1 (±2.6)	20.6 (±2.4)
Genotype						
F508del/F508del	32 (100%)	—	30 (55.6%)	—	10 (43.5%)	17 (53.1%)
F508del/MF	0 (0%)	—	24 (44.4%)	—	13 (56.5%)	15 (46.9%)
Prior CFTR modulator therapy						
No prior CFTR modulator	32 (100%)	—	29 (53.7%)	—	14 (60.9%)	17 (53.1%)
TEZ/IVA	—	—	19 (35.2%)	—	6 (26.1%)	6 (18.8%)
LUM/IVA	—	—	6 (11.1%)	—	3 (13%)	7 (21.9%)
IVA	—	—	—	—	—	2 (6.2%)

People with CF (pwCF) in the ELX/TEZ/IVA sputum cohort are a subset of people with CF in the ELX/TEZ/IVA serum cohort.
*Validation cohort.

LUM/IVA cohort therapy led to a higher number of significantly different proteins (Figure 4A), despite the larger effect size in ELX/TEZ/IVA-treated people with CF (Figure 4B). Interestingly, the differences between the two cohorts extended beyond the fold change. The majority of significantly different proteins did not overlap (Figure 4A), and some of these proteins were regulated in the opposite direction (Figures 4A and 4B).

Among the 471 proteins identified in both cohorts, 66 (14.0%) showed significant differences between the two groups (Figure S3E). While some proteins changed in the same direction (light green), many others showed opposite effects (dark green and dark blue) or only in one sample (light blue) (Figure 4B). These differences were further reflected in the normalized enrichment scores (NES) of UniProt terms (Figure 4C). Although most terms, such as "immunity," "adaptive immunity," "lipoproteins," and "cell adhesion," changed in the same direction in both therapies, many terms related to innate immunity, such as "complement pathway," "complement alternate pathway," and "inflammatory response," were reduced after ELX/TEZ/IVA treatment but not after LUM/IVA treatment. These differences seemed not to be dependent on differences in mutations as a comparison of fold changes in people with CF homozygous for the *F508del* mutation showed similar trends (Figure S3F).

Next, we compared the changes of proteins representative of lung damage (SFTPB) and vitamin transport (GC, RBP4, and TTR), which are key pathological pathways in CF, between the two treatment cohorts. Interestingly, none of the proteins, except for TTR, showed significant changes in the LUM/IVA cohort. Proteins related to vitamin transport (RBP4 and TTR), which were higher in healthy individuals compared with people with CF at baseline, exhibited significant increases with ELX/TEZ/IVA treatment but not with LUM/IVA treatment (Figure 4D).

Differential responses in sputum and serum proteome after ELX/TEZ/IVA treatment

For a subgroup of the ELX/TEZ/IVA cohort, previously published proteome data of matching sputum samples[20] were available, allowing us to determine the relationship between local treatment response in the lung (sputum) with systemic changes (serum). This comparison is especially interesting, considering the recent improvements in treatment efficacy that will make sputum collection more challenging, and for children, who are often unable to produce sputum. Additionally, we compared sputum and serum proteome data of the cohort, including all people with CF (Figure S4).

Out of the 2,541 proteins identified in the sputum samples and 558 proteins in the serum samples, 275 proteins overlapped (Figure S4A). Notably, ELX/TEZ/IVA treatment induced more significant changes in protein abundance in serum than in sputum samples (Figure 5A; Figure S4B).

Several inflammatory proteins, including CRP, SERPINA1, and alpha-1-antichymotrypsin (SERPINA3), showed significant differences and co-directional changes in both sputum and serum samples (Figures 5A and 5B). In contrast, other proteins, including CD14, SFTPB, complement factor H (CFH), polymeric immunoglobulin receptor (PIGR), intercellular adhesion molecule 1 (ICAM1), and cystatin-F (CST7), displayed significant differences with opposite directionality (Figures 5A and 5B). Independent of the significance level, we compared the relation of effect size and found 89 proteins (32.4%) had a difference in absolute log2(fold change) of >0.25 between the drug responses observed in sputum and serum datasets (highlighted proteins in Figure 5B).

It is worth noting that in particular some inflammatory proteins, such as CRP and SERPINA3, were more abundant in the sputum and people with CF at baseline compared

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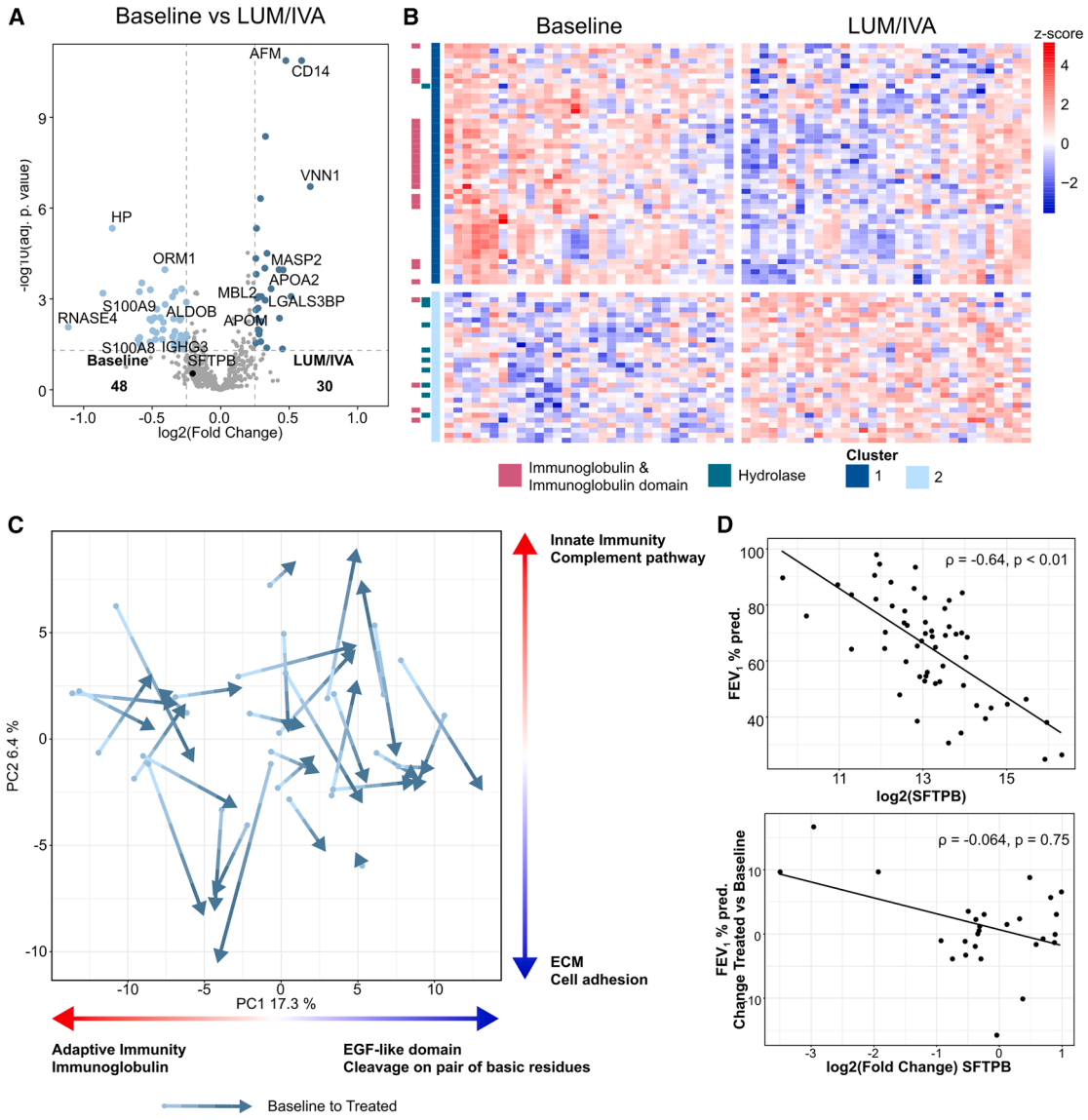

Figure 2. Moderate response in plasma proteome after 3 months of LUM/IVA treatment

(A) Volcano plot of proteins showing significantly increased or decreased (moderated *t* test, Benjamini-Hochberg adjusted $p < 0.05$, ±0.25-log2(fold change)) protein abundances before and after 3 months of LUM/IVA treatment ($n = 32$).

(B) Heatmap of the 64 differential proteins (Figure 2A). Hierarchical clustering was performed within the groups. Protein clusters are described by chosen UniProt terms enriched in one of the clusters. Color gradient indicates *Z* scored protein intensities.

(C) Induced plasma proteome changes of people with CF from baseline to treated visualized in PCA. PCs are additionally described by 2 (top (red) and bottom (blue)) of the most significantly enriched UniProt terms calculated by ssGSEA of eigenvectors. Arrows point from baseline (light blue) to treated (mid blue) time points for the same people with CF.

(D) Spearman correlations between SFTPB and FEV_1% predicted (FEV_1% pred.) (top) and between log2(fold change) (treated vs. baseline) of SFTPB and change in FEV_1% predicted (bottom).

with healthy individuals and decreased upon ELX/TEZ/IVA treatment in both compartments. Others, such as CFH and SFTPB, were more abundant in healthy sputum but less abundant in serum and developed in opposite directions upon treatment (Figure 5C). This development is particularly pronounced for SFTPB (Figures 5B and 5C), which showed a significant correlation with lung function in the ELX/TEZ/IVA cohort (Figure 3D). These results illustrate the diverse impacts of ELX/TEZ/IVA therapy on local and systemic sites, as well as the dissimilarities in baseline protein levels in distinct tissues (Figure 5C).

Validation of ELX/TEZ/IVA-induced proteome alterations with an independent cohort

To validate the key findings of the ELX/TEZ/IVA cohort, serum samples were analyzed from an independent group of 32 people

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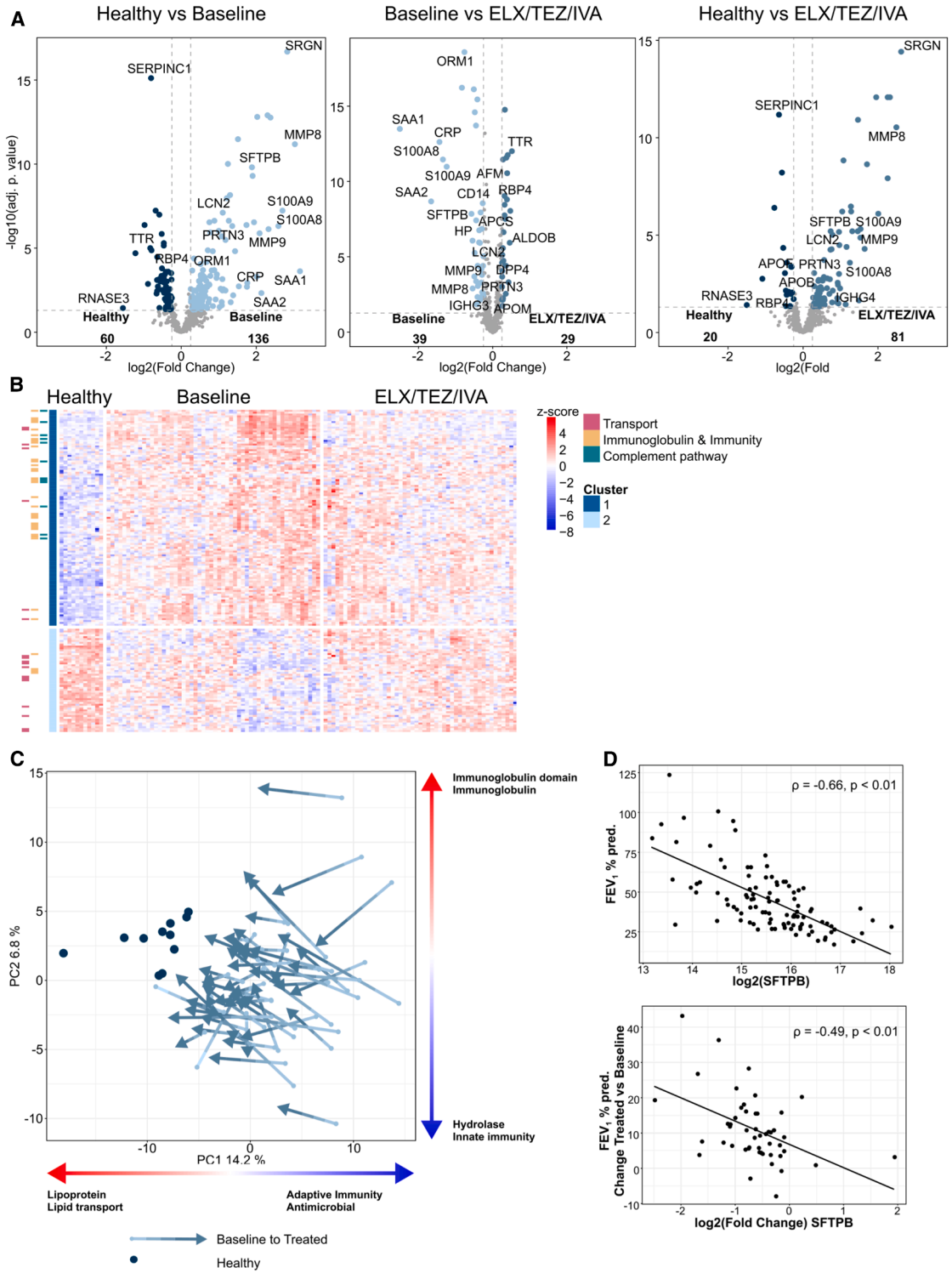

Figure 3. Strong and uniform response in serum proteomes after 3 months of ELX/TEZ/IVA treatment

(A) Volcano plots of proteins with significantly increased or decreased (moderated *t* test, Benjamini-Hochberg-adjusted $p < 0.05$, ±0.25-log2(fold change)) protein abundances before treatment compared to healthy (left panel), before treatment compared with after 3 months of ELX/TEZ/IVA treatment (middle panel), and 3 months of ELX/TEZ/IVA treatment compared with healthy (right panel) (people with CF: $n = 54$, healthy: $n = 11$).

(B) Heatmap of 170 significantly differential proteins between healthy, baseline, and treated (moderated F-test, Benjamini-Hochberg-adjusted $p < 0.05$). Hierarchical clustering was performed within the groups. Protein clusters are described by chosen UniProt terms enriched in one of the clusters. Color gradient indicates *Z* scored protein intensities.

(legend continued on next page)

with CF before and 3 months after initiation of ELX/TEZ/IVA therapy (Figure 6), with similar clinical outcomes as in the original cohort (Figure S5A). 17 (53.1%) of the individuals were homozygous for the F580del mutation, and 15 (46.9%) were treated with a CFTR modulator (IVA, LUM/IVA, or TEZ/IVA) prior to ELX/TEZ/IVA treatment initiation (Table 1). Of the 998 quantified proteins, 46 (4.6%) showed significant changes following treatment initiation (Figure 6A; Table S1). A total of 488 proteins overlapped between the validation and original cohorts based on gene name and UniProt identifier (Figure S5B). Proteins that reached statistical significance in both cohorts demonstrated consistent directionality and similar effect sizes (Figure 6B).

Importantly, our analysis of the validation cohort reproduced the strong negative correlation between SFTPB levels and lung function (FEV1% predicted) we observed in the original cohort (Figures 6C–6D; $\rho = -0.72$ and -0.77; Figure 3D; $\rho = -0.66$). The inverse relationship between SFTPB fold changes and improvements in lung function was also validated (Figures 6E–F; $\rho = -0.45$ and -0.51; Figure 3D; $\rho = -0.49$).

To evaluate technical reproducibility across instruments, peptides from the original cohort were remeasured using the Astral mass spectrometry platform (Figures S6A and S6B). Additionally, samples from the original cohort were prepared using the Bravo liquid handling system and analyzed on the Astral platform to evaluate the reproducibility of the entire workflow, including both library-based and direct DIA approaches (Figures S6C and S6D). These comparisons revealed a strong correlation between protein intensities and fold changes for significantly different proteins across measurement platforms (Figures S6A–S6D; $\rho = 0.85–0.94$).

DISCUSSION

Here, we applied a robust and highly scalable MS-based proteomics workflow to analyze serum and plasma samples from people with CF who were either treated with CFTR modulator dual (LUM/IVA) or triple (ELX/TEZ/IVA) combination therapy. Previous CF research has primarily focused on analyzing specific serum and plasma proteins or conducting 2D-GE, MS, or MRM-MS, mostly comparing people with CF with healthy controls or pulmonary exacerbation (PEx).[35,36,39,43–45] To our knowledge, this is the first study assessing the serum and plasma proteome in people with CF treated with LUM/IVA and ELX/TEZ/IVA.

We observed moderate effects in the LUM/IVA-treated cohort (Figure 2) and demonstrated that the proteome of people with CF in the ELX/TEZ/IVA cohort changes more uniformly in its composition toward a healthier state upon treatment (Figure 3). While the proportions of significantly altered proteins were similar between treatments (12.2% for ELX/TEZ/IVA versus 13.6% for LUM/IVA), the key distinction lies in the pathways and protein classes that respond, as well as the effect size of the responses. For example, proteins involved in innate immunity, including the complement pathway, only decreased with ELX/TEZ/IVA (Figure 4C), while vitamin transport proteins (RBP4 and TTR) only increased with ELX/TEZ/IVA (Figure 4D). These divergent pathway responses likely reflect the substantially greater CFTR functional restoration achieved by ELX/TEZ/IVA (~40%–50% restoration of CFTR function[46]) versus LUM/IVA (~10%–20% restoration of CFTR function[9]). This suggests that only with the triple combination therapy ELX/TEZ/IVA, but not with the double therapy LUM/IVA, a certain threshold can be reached that is sufficient to decrease systemic inflammatory and metabolic abnormalities. Furthermore, potential off-target effects of the structurally distinct corrector molecules (lumacaftor versus elexacaftor/tezacaftor) may also contribute to these differences.

In addition, combining quantitative proteome analyses of serum and sputum samples allowed the comparison of local (lung) and systemic (serum) changes upon treatment with ELX/TEZ/IVA (Figure 5). Taken together, this integrated analysis provides a valuable resource for understanding the pharmacodynamics of different CFTR modulators and offers insights into the systemic and local protein regulation in CF.

Effects of CFTR modulator therapy on inflammation

A central challenge for people with CF is the persistent inflammation and infection of the lungs. Biomarkers such as CRP, S100A8, and S100A9 are widely discussed as indicators of CF severity. Particularly, calprotectin (S100A8/A9) has been shown in multiple studies to be potentially useful for assessing the risk of PEx and monitoring disease progression.[35,39,43,44,47–49]

Despite the reduction in some immune related proteins with LUM/IVA, especially in immunoglobulins, the overall impact on the proteome and clinical parameters remains limited. This is likely due to a sustained innate immune response and continuous activation of the complement pathway, alongside a lack of significant reduction in CRP levels.

However, our data show decreased levels of the inflammation marker S100A8/9 after LUM/IVA and ELX/TEZ/IVA treatment, which has been previously linked to a decreased risk of PEx,[50] suggesting a protective function against further decline of lung function. In contrast, treatment with ELX/TEZ/IVA reduces proteins involved in both innate and adaptive immunity, which is also represented in serum proteome biomarkers such as CRP.[51]

Another protein of interest is LTA4H, an inflammatory enzyme involved in the production of leukotriene B4 (LTB4), which in turn is part of neutrophil signaling.[52] Although its levels did not change significantly with modulator treatment, LTA4H correlated with sweat chloride concentration in the ELX/TEZ/IVA cohort. LTA4H inhibitors have been tested in phase 1[52] and phase 2[53] trials, resulting in moderate improvements in the risk for PEx in people with CF with better lung function or modulator-treated people with CF. These moderate improvements may be due to its additional capacity to limit chronic neutrophilic airway inflammation.[54]

(C) Proteome regulation of people with CF from baseline to treated in comparison to healthy individuals visualized in PCA PCs are additionally described by 2 (top (red) and bottom (blue)) of the most significantly enriched UniProt terms calculated by ssGSEA of eigenvectors. Arrows point from baseline (light blue) to treated (mid blue) time points for the same people with CF, dark blue dots indicate healthy individuals.
(D) Spearman correlations between SFTPB and FEV_1% predicted (FEV_1% pred.) (top) and between log2(fold change) (treated vs. baseline) of SFTPB and change in FEV_1% predicted (bottom).

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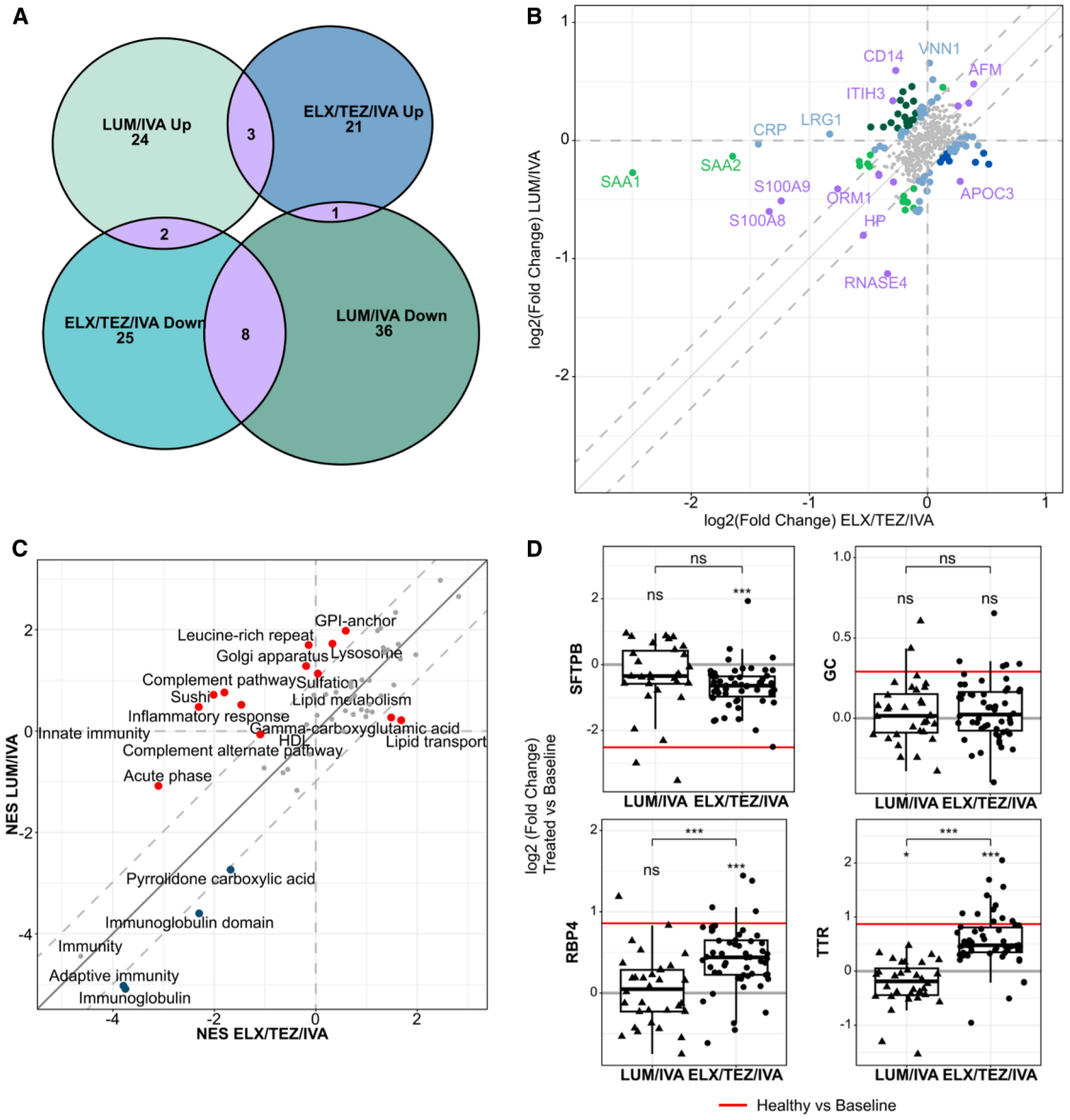

Figure 4. Innate immunity and vitamin transport are differentially affected by LUM/IVA and ELX/TEZ/IVA treatment

(A) Venn diagram comparing proteins significantly up and down between 3 months of treatment and baseline in LUM/IVA ($n = 32$) and ELX/TEZ/IVA ($n = 54$), after correcting for differences in age (moderated t test, Benjamini-Hochberg-adjusted $p < 0.05$, ±0.25-log2 (fold change)).

(B) Scatterplot of log2(fold changes) in commonly identified proteins between paired treated and baseline people with CF in the LUM/IVA or ELX/TEZ/IVA cohort, after correcting for age as a confounder. Proteins highlighted have an absolute log2(fold change) difference of at least 0.25. Proteins changing in the same direction are highlighted in light green, proteins only changing in one sample (log2(fold change) in the other sample < 0.1) are highlighted in light blue, proteins that changing counter directional are highlighted in dark green (higher in LUM/IVA) and dark blue (higher in ELX/TEZ/IVA). Significant proteins overlapping in the Venn diagram (Figure 4A) are highlighted in purple.

(C) Scatterplot of NES from ssGSEA analysis with UniProt keywords using paired log2(fold changes) from the LUM/IVA and the ELX/TEZ/IVA cohort. Terms significantly different between the LUM/IVA and the ELX/TEZ/IVA cohort are highlighted in red (moderated t test, Benjamini-Hochberg adjusted $p < 0.05$, ±0.1-log2(fold change)). Terms with a difference in NES below 1 are shown in gray.

(D) Within and between cohort comparison of paired log2(fold changes) in LUM/IVA (triangles) and ELX/TEZ/IVA (dots) people with CF of representative proteins for different CF disease processes including the lung protein SFTPB and vitamin transport related proteins (GC, RPB4, and TTR). Triangles and dots represent the paired log2(fold changes) between baseline and treated people with CF, red lines represent the log2(fold change) between CF baseline and healthy in the ELX/TEZ/IVA cohort. Welch t test or paired t test, Benjamini-Hochberg-adjusted, *** adj.p.val < 0.001, ** adj.p.val < 0.01, * adj.p.val < 0.05.

The observed differences in inflammatory markers between LUM/IVA and ELX/TEZ/IVA may be attributed to ongoing disease progression despite treatment in the LUM/IVA group. This could also account for the correlation between baseline inflammatory proteins and lung function (Figure S2B).

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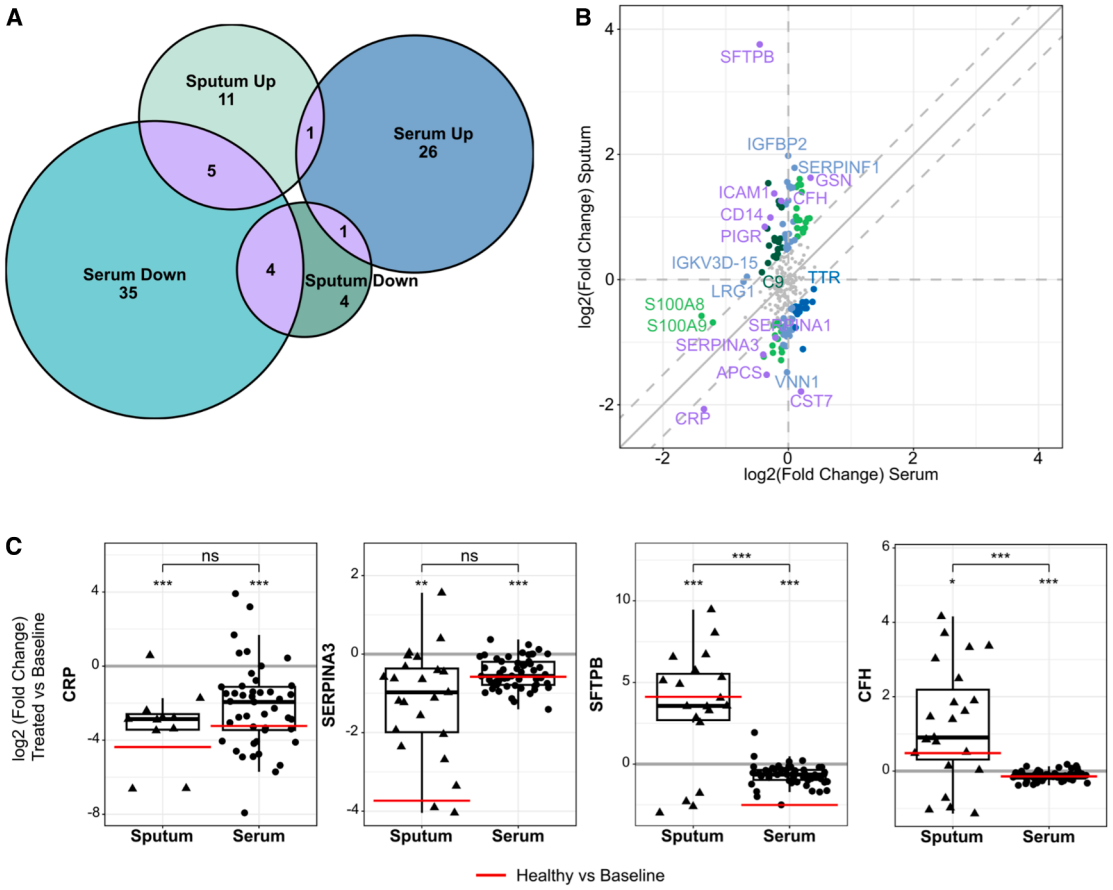

Figure 5. Differences in systemic (serum) and local (sputum) changes after treatment with ELX/TEZ/IVA

Paired sputum and serum samples were analyzed from the same 23 people with CF.

(A) Venn diagram of overlapping proteins that are significantly up or down in the sputum ($n = 23$) and serum ($n = 23$) samples of ELX/TEZ/IVA-treated people with CF (moderated t test, Benjamini-Hochberg-adjusted $p < 0.05$).

(B) Scatterplot of log2(fold changes) between treated and baseline people with CF in commonly identified proteins in serum and sputum samples. Proteins highlighted have an absolute log2(fold change) difference of at least 0.25. Proteins changing in the same direction are highlighted in light green, proteins only changing in one sample (log2(fold change) in the other sample < 0.1) are highlighted in light blue, proteins that changing counter directional are highlighted in dark green (sputum) and dark blue (serum). Significant proteins overlapping in the Venn diagram are highlighted in purple.

(C) Within and between cohort comparison of log2(fold changes) in sputum (triangles) and serum (dots) of representative proteins developing in the same direction (inflammation markers (CRP, SERPINA3)) and opposite direction (lung protein SFTPB and complement regulator CFH). Triangles and dots represent the paired log2(fold changes) between treated and baseline people with CF, red lines represent the log2(fold change) between healthy and baseline in the sputum or serum cohort. Welch t test, Benjamini-Hochberg adjusted, *** adj.p.val < 0.001, ** adj.p.val < 0.01, * adj.p.val < 0.05.

CFTR modulator therapy restores protein levels implicated in malabsorption and vitamin transport

CFTR dysfunction also affects mucus properties and clearance in the gastrointestinal tract and leads to abnormal pancreatic secretions in people with CF. This leads to malabsorption of nutrients due to decreased secretion of digestive enzymes from the pancreas in pancreatic insufficient people with CF.[55] Furthermore, inadequate synthesis of lipid-processing proteins, such as APOA1 and APOB, in the intestinal tissue has been proposed as a contributing factor to insufficient lipid absorption. This may explain why pancreatic enzyme replacement therapy does not completely restore lipid absorption in people with CF.[56]

Consistent with previous research,[36,56] we observed a reduction in APOA1 and APOB levels in people with CF compared with healthy controls, which was partially restored in people with CF treated with ELX/TEZ/IVA, but not in those treated with LUM/IVA. The significant difference in enrichment of the "lipid transport" UniProt term, including other apolipoproteins and phospholipid transfer protein (PLTP), further emphasizes the contrast between the two treatments. However, these significant changes in APOA1 and APOB could not be confirmed in the smaller, independent validation cohort (Figure S6E).

In addition to fat resorption and dyslipidemia, transporters of fat-soluble vitamins, such as vitamin-D-binding protein (GC), retinol-binding protein 4 (RBP4), and transthyretin (TTR), are reduced in serum of people with CF.[36] Although RBP4 is expressed in various tissues, studies suggested that circulating RBP4 is mostly derived from the liver, and its level is highly regulated but dependent on liver retinol (vitamin A) levels.[57–59] The

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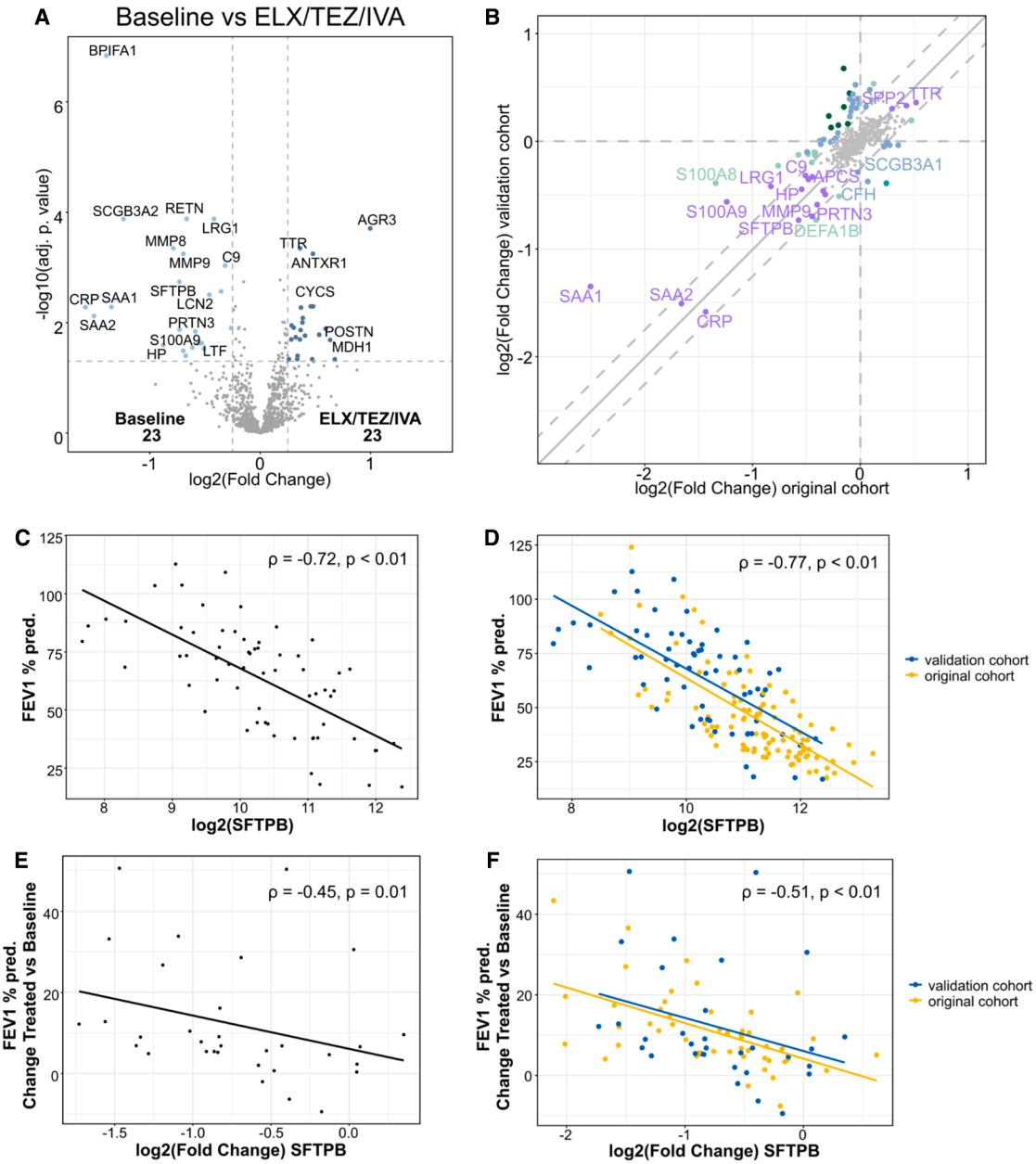

Figure 6. Validation cohort for ELX/TEZ/IVA treatment

(A) Volcano plot of proteins showing significantly increased or decreased (moderated *t* test, Benjamini-Hochberg-adjusted $p < 0.05$, ±0.25-log2(fold change)) protein abundances before and after 3 months of ELX/TEZ/IVA treatment in the validation cohort ($n = 32$).

(B) Scatterplot of log2(fold changes) between treated and baseline people with CF in commonly identified proteins (Figure S5F) in the original cohort ($n = 54$, Figure 3) and the validation cohort ($n = 32$). Proteins highlighted have an absolute log2(fold change) difference of at least 0.25. Proteins changing in the same direction are highlighted in light green, proteins only changing in one sample (log2(fold change) in the other sample < 0.1) are highlighted in light blue, proteins that changing counter directional are highlighted in dark green (validation cohort) and dark blue (original cohort). Proteins that are significantly different in both cohorts (Figure S5G) are highlighted in purple.

(C and D) Scatter plots with Spearman correlations of log2(Intensities) of SFTPB and FEV_1% predicted (FEV_1% pred.) in the validation cohort treated with ELX/TEZ/IVA, measured on the Astral and (D) Spearman correlation in the original (yellow) and validation cohort (blue) together.

(E) Scatterplots with Spearman correlations of log2(Fold changes) in SFTPB intensities and changes in FEV_1% predicted (FEV_1% pred.) between ELX/TEZ/IVA treated and baseline people with CF in the validation cohort, measured on the Astral and (F) correlation in the original (yellow) and validation cohort (blue) together.

levels of circulating retinol and RBP4 in complex with TTR depend on liver function and retinol absorption from food.[58,60] Interestingly, RBP4 has been proposed as a predictive marker for the severity of pulmonary exacerbations in chronic obstructive pulmonary disease (COPD) due to its association with the nutritional status of patients, affecting patient survival.[61] In

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line with previous studies, RBP4 and TTR showed reduced abundance in individuals with CF at baseline compared with healthy individuals[36] and increased upon treatment with ELX/TEZ/IVA, but not LUM/IVA. The changes in RBP4 and TTR levels are most likely due to improved nutritional status after ELX/TEZ/IVA treatment, which is also reflected in increased lipoproteins and BMI after 3 months of treatment. However, we did not observe a significant increase in vitamin-D-binding protein levels post-treatment, suggesting the need for continuous supplementation and regular monitoring of vitamin D levels in people with CF.

Local vs. systemic changes of inflammation markers upon ELX/TEZ/IVA treatment

The people with CF in this ELX/TEZ/IVA cohort partially overlap with those in a previously published cohort that described changes in sputum of people with CF after ELX/TEZ/IVA treatment.[20] To distinguish between systemic effects and local changes in the lung, we compared the changes in proteins in overlapping people with CF. Proteins that were regulated in the same direction in both sputum and serum samples mainly belonged to inflammatory and immune pathways, such as CRP and SERPINA3, AGT, AHSG, GSN, and SERPINF1. However, we observed that many inflammatory proteins exhibited changes that differed in magnitude and even in opposite directions between serum and sputum. This suggests that inflammation markers in the serum are at least partially independent of lung inflammation. Many of the serum inflammation markers observed here are not only spill-over effects from the lung but also indicative of the distinct systemic CF effects.

The protein CFH, which is known for inhibiting C3b, showed a reduction in serum post-treatment. In contrast to our results, CFH has been shown to be increased in lungs[62] and reduced in plasma[63] of people with idiopathic pulmonary fibrosis. This reduction in CFH may be due to reduced systemic inflammation, also highlighted by the decrease in complement pathway enrichment in people with CF treated with ELX/TEZ/IVA, which contrasts with its potential role in lung inflammation modulation. This comparison highlights that, although lung inflammation may contribute to systemic inflammation and serum markers, many serum inflammation markers change independently of lung inflammation. This provides a potential explanation for the challenge in finding reproducible plasma or serum biomarkers for lung infection.

Signs of lung repair

Pro-SFTPB has been discussed as a serum or plasma marker for lung damage, particularly in the context of lung cancer.[64–66] One potential explanation for this association is the increased lung permeability resulting from various forms of lung injury.[67–69] In COPD, SFTPB levels in sputum or bronchoalveolar lavage (BAL) samples correlate with lung function but do not show short-term changes upon treatment.[70]

In our study, SFTPB emerged as a particularly robust biomarker candidate. SFTPB was one of only two proteins (SFTPB and LCN2) that correlated with lung function across multiple comparisons in both treatment cohorts. Notably, SFTPB abundance alterations strongly correlated with improvements in lung function in the ELX/TEZ/IVA cohort, a finding that was independently validated in a cohort with higher baseline lung function (mean FEV1% predicted: 39.6% vs. 58%). Furthermore, SFTPB exhibited counter-directional changes when comparing serum and sputum proteomes, decreasing in serum while increasing in sputum following ELX/TEZ/IVA treatment.

These counter-directional changes, together with the concurrent improvement in lung function, suggest that a decline in serum SFTPB levels may indicate reduced lung permeability. This interpretation is further supported by the absence of these changes in the LUM/IVA cohort, which showed limited clinical improvement in lung function and SFTPB levels. Taken together, the evidence from three independent cohorts, collected at different clinical centers, two sample matrices (serum and plasma), two treatment regimens, and two distinct LC-MS/MS workflows and platforms (Orbitrap HF-X and Astral) supports SFTPB as a promising biomarker of lung permeability and treatment-induced repair in CF. Yet, prospective validation studies with larger cohorts and longer follow-up periods are needed to fully establish its clinical utility.

This study demonstrates that the superior efficacy of ELX/TEZ/IVA over LUM/IVA treatment is clearly reflected in the serum proteome, even though 30 (46%) of the people with CF in the ELX/TEZ/IVA cohort received modulator treatment at baseline. The findings highlighted that ELX/TEZ/IVA resulted in a greater improvement in systemic inflammatory markers than LUM/IVA. Moreover, the study showed that local changes in the lung only partially aligned with systemic changes, emphasizing the challenge of identifying suitable plasma or serum biomarkers for lung diseases. In addition, this study provides a comprehensive proteomic analysis of longitudinally collected serum and plasma samples from people with CF undergoing treatment, offering a valuable reference for future research. It also serves as a resource for understanding how effective treatment may influence the overall health of people with CF, potentially reducing the risk of severe lung damage over time. However, the study also underscores the need for continued development of therapies, as significant differences remain between the proteomes of treated people with CF and healthy individuals, particularly in inflammatory proteins and potential lung injury markers, such as SFTPB.

Limitations of the study

The study also has some limitations: a direct comparison of the LUM/IVA and ELX/TEZ/IVA cohorts was hindered due to differences in age and sample type (serum vs. plasma). However, to address the differences in sample type, we conducted a separate comparison of serum and plasma samples from the same donors. While few proteins were significantly different between the two matrices, the different blood collection protocols did not have a significant impact on differentially regulated proteins between baseline and drug treated people with CF (Figures S3C and S3D). To compare the two cohorts, we additionally used a linear model in limma and corrected for age as a confounding factor.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to the lead contact, Philipp Mertins (philipp.mertins@mdc-berlin.de).

Materials availability

This study did not generate new materials.

Data and code availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[71] partner repository. Data are publicly available as of the date of publication via ProteomeXchange with identifier PRIDE: PXD071549, listed in the key resources table.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, K.F., M.K., M.Z., S.Y.G., M.A.M., and P.M.; formal analysis, K.F., M.K., M.Z., and O.P.; data curation, K.F., M.K., S.N., and S.Y.G.; investigation, K.F., M.K., M.Z., O.P., S.N., J.D., L.S., J.R., S.T., S.H., O.S, M.S., S.Y.G., and M.A.M.; resources, J.D., L.S., J.R., S.T., S.H., O.S., M.S., S.Y.G., M.A.M., and P.M.; supervision, S.Y.G., P.M., and M.A.M.; visualization, K.F.; writing – original draft, K.F., S.Y.G, M.A.M., and P.M.; writing – review & editing, all authors.

DECLARATION OF INTERESTS

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STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

SUPPLEMENTAL INFORMATION

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REFERENCES

1. Mall, M.A., Burgel, P.-R., Castellani, C., Davies, J.C., Salathe, M., and Taylor-Cousar, J.L. (2024). Cystic fibrosis. Nat. Rev. Dis. Primers *10*, 53. https://doi.org/10.1038/s41572-024-00538-6.
2. De Boeck, K., Zolin, A., Cuppens, H., Olesen, H.V., and Viviani, L. (2014). The relative frequency of CFTR mutation classes in European patients with cystic fibrosis. J. Cyst. Fibros. *13*, 403–409. https://doi.org/10.1016/j.jcf.2013.12.003.
3. Bacalhau, M., Camargo, M., Magalhães-Ghiotto, G.A.V., Drumond, S., Castelletti, C.H.M., and Lopes-Pacheco, M. (2023). Elexacaftor-Tezacaftor-Ivacaftor: A Life-Changing Triple Combination of CFTR Modulator Drugs for Cystic Fibrosis. Pharmaceuticals (Basel) *16*, 410. https://doi.org/10.3390/ph16030410.
4. Grasemann, H., and Ratjen, F. (2023). Cystic Fibrosis. N. Engl. J. Med. *389*, 1693–1707. https://doi.org/10.1056/NEJMra2216474.
5. Bell, S.C., Mall, M.A., Gutierrez, H., Macek, M., Madge, S., Davies, J.C., Burgel, P.-R., Tullis, E., Castaños, C., Castellani, C., et al. (2020). The future of cystic fibrosis care: a global perspective. Lancet Respir. Med. *8*, 65–124. https://doi.org/10.1016/S2213-2600(19)30337-6.
6. Ratjen, F., Saiman, L., Mayer-Hamblett, N., Lands, L.C., Kloster, M., Thompson, V., Emmett, P., Marshall, B., Accurso, F., Sagel, S., et al. (2012). Effect of azithromycin on systemic markers of inflammation in patients with cystic fibrosis uninfected with Pseudomonas aeruginosa. Chest *142*, 1259–1266. https://doi.org/10.1378/chest.12-0628.
7. De Boeck, K., Munck, A., Walker, S., Faro, A., Hiatt, P., Gilmartin, G., and Higgins, M. (2014). Efficacy and safety of ivacaftor in patients with cystic fibrosis and a non-G551D gating mutation. J. Cyst. Fibros. *13*, 674–680. https://doi.org/10.1016/j.jcf.2014.09.005.
8. Sagel, S.D., Khan, U., Heltshe, S.L., Clancy, J.P., Borowitz, D., Gelfond, D., Donaldson, S.H., Moran, A., Ratjen, F., VanDalfsen, J.M., et al. (2021). Clinical Effectiveness of Lumacaftor/Ivacaftor in Patients with Cystic Fibrosis Homozygous for F508del-CFTR. A Clinical Trial. Ann. Am. Thorac. Soc. *18*, 75–83. https://doi.org/10.1513/AnnalsATS.202002-144OC.
9. Graeber, S.Y., Dopfer, C., Naehrlich, L., Gyulumyan, L., Scheuermann, H., Hirtz, S., Wege, S., Mairbäurl, H., Dorda, M., Hyde, R., et al. (2018). Effects of Lumacaftor-Ivacaftor Therapy on Cystic Fibrosis Transmembrane Conductance Regulator Function in Phe508del Homozygous Patients

with Cystic Fibrosis. Am. J. Respir. Crit. Care Med. *197*, 1433–1442. https://doi.org/10.1164/rccm.201710-1983OC.

10. Clancy, J.P., Rowe, S.M., Accurso, F.J., Aitken, M.L., Amin, R.S., Ashlock, M.A., Ballmann, M., Boyle, M.P., Bronsveld, I., Campbell, P.W., et al. (2012). Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation. Thorax *67*, 12–18. https://doi.org/10.1136/thoraxjnl-2011-200393.
11. Wainwright, C.E., Elborn, J.S., Ramsey, B.W., Marigowda, G., Huang, X., Cipolli, M., Colombo, C., Davies, J.C., De Boeck, K., Flume, P.A., et al. (2015). Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR. N. Engl. J. Med. *373*, 220–231. https://doi.org/10.1056/NEJMoa1409547.
12. Konstan, M.W., McKone, E.F., Moss, R.B., Marigowda, G., Tian, S., Waltz, D., Huang, X., Lubarsky, B., Rubin, J., Millar, S.J., et al. (2017). Assessment of safety and efficacy of long-term treatment with combination lumacaftor and ivacaftor therapy in patients with cystic fibrosis homozygous for the F508del-CFTR mutation (PROGRESS): a phase 3, extension study. Lancet Respir. Med. *5*, 107–118. https://doi.org/10.1016/S2213-2600(16)30427-1.
13. Rayment, J.H., Asfour, F., Rosenfeld, M., Higgins, M., Liu, L., Mascia, M., Paz-Diaz, H., Tian, S., Zahigian, R., and McColley, S.A. (2022). A Phase 3, Open-Label Study of Lumacaftor/Ivacaftor in Children 1 to Less Than 2 Years of Age with Cystic Fibrosis Homozygous for F508del-CFTR. Am. J. Respir. Crit. Care Med. *206*, 1239–1247. https://doi.org/10.1164/rccm.202204-0734OC.
14. Middleton, P.G., Mall, M.A., Dřevínek, P., Lands, L.C., McKone, E.F., Polineni, D., Ramsey, B.W., Taylor-Cousar, J.L., Tullis, E., Vermeulen, F., et al. (2019). Elexacaftor-Tezacaftor-Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele. N. Engl. J. Med. *381*, 1809–1819. https://doi.org/10.1056/NEJMoa1908639.
15. Heijerman, H.G.M., McKone, E.F., Downey, D.G., van Braeckel, E., Rowe, S.M., Tullis, E., Mall, M.A., Welter, J.J., Ramsey, B.W., McKee, C.M., et al. (2019). Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. Lancet *394*, 1940–1948. https://doi.org/10.1016/S0140-6736(19)32597-8.
16. Griese, M., Costa, S., Linnemann, R.W., Mall, M.A., McKone, E.F., Polineni, D., Quon, B.S., Ringshausen, F.C., Taylor-Cousar, J.L., Withers, N.J., et al. (2021). Safety and Efficacy of Elexacaftor/Tezacaftor/Ivacaftor for 24 Weeks or Longer in People with Cystic Fibrosis and One or More F508del Alleles: Interim Results of an Open-Label Phase 3 Clinical Trial. Am. J. Respir. Crit. Care Med. *203*, 381–385. https://doi.org/10.1164/rccm.202008-3176LE.
17. Graeber, S.Y., Renz, D.M., Stahl, M., Pallenberg, S.T., Sommerburg, O., Naehrlich, L., Berges, J., Dohna, M., Ringshausen, F.C., Doellinger, F., et al. (2022). Effects of Elexacaftor/Tezacaftor/Ivacaftor Therapy on Lung Clearance Index and Magnetic Resonance Imaging in Patients with Cystic Fibrosis and One or Two F508del Alleles. Am. J. Respir. Crit. Care Med. *206*, 311–320. https://doi.org/10.1164/rccm.202201-0219OC.
18. Streibel, C., Willers, C.C., Pusterla, O., Bauman, G., Stranzinger, E., Brabandt, B., Bieri, O., Curdy, M., Bullo, M., Frauchiger, B.S., et al. (2023). Effects of elexacaftor/tezacaftor/ivacaftor therapy in children with cystic fibrosis - a comprehensive assessment using lung clearance index, spirometry, and functional and structural lung MRI. J. Cyst. Fibros. *22*, 615–622. https://doi.org/10.1016/j.jcf.2022.12.012.
19. Stahl, M., Dohna, M., Graeber, S.Y., Sommerburg, O., Renz, D.M., Pallenberg, S.T., Voskrebenzev, A., Schütz, K., Hansen, G., Doellinger, F., et al. (2024). Impact of elexacaftor/tezacaftor/ivacaftor therapy on lung clearance index and magnetic resonance imaging in children with cystic fibrosis and one or two F508del alleles. Eur. Respir. J. *64*, 2400004. https://doi.org/10.1183/13993003.00004-2024.
20. Schaupp, L., Addante, A., Völler, M., Fentker, K., Kuppe, A., Bardua, M., Duerr, J., Piehler, L., Röhmel, J., Thee, S., et al. (2023). Longitudinal Effects of Elexacaftor/Tezacaftor/Ivacaftor on Sputum Viscoelastic Properties, Airway Infection and Inflammation in Patients with Cystic Fibrosis. Eur. Respir. J. *62*, 2202153. https://doi.org/10.1183/13993003.02153-2022.
21. Maher, R.E., Barry, P.J., Emmott, E., Jones, A.M., Lin, L., McNamara, P.S., Smith, J.A., and Lord, R.W. (2023). Influence of highly effective modulator therapy on the sputum proteome in cystic fibrosis. J. Cyst. Fibros. *23*, 269–277. https://doi.org/10.1016/j.jcf.2023.10.019.
22. Casey, M., Gabillard-Lefort, C., McElvaney, O.F., McElvaney, O.J., Carroll, T., Heeney, R.C., Gunaratnam, C., Reeves, E.P., Murphy, M.P., and McElvaney, N.G. (2023). Effect of elexacaftor/tezacaftor/ivacaftor on airway and systemic inflammation in cystic fibrosis. Thorax *78*, 835–839. https://doi.org/10.1136/thorax-2022-219943.
23. Piehler, L., Thalemann, R., Lehmann, C., Thee, S., Röhmel, J., Syunyaeva, Z., Stahl, M., Mall, M.A., and Graeber, S.Y. (2023). Effects of elexacaftor/tezacaftor/ivacaftor therapy on mental health of patients with cystic fibrosis. Front. Pharmacol. *14*, 1179208. https://doi.org/10.3389/fphar.2023.1179208.
24. Graeber, S.Y., and Mall, M.A. (2023). The future of cystic fibrosis treatment: from disease mechanisms to novel therapeutic approaches. Lancet *402*, 1185–1198. https://doi.org/10.1016/S0140-6736(23)01608-2.
25. Geyer, P.E., Kulak, N.A., Pichler, G., Holdt, L.M., Teupser, D., and Mann, M. (2016). Plasma Proteome Profiling to Assess Human Health and Disease. Cell Syst. *2*, 185–195. https://doi.org/10.1016/j.cels.2016.02.015.
26. Messner, C.B., Demichev, V., Wendisch, D., Michalick, L., White, M., Freiwald, A., Textoris-Taube, K., Vernardis, S.I., Egger, A.-S., Kreidl, M., et al. (2020). Ultra-High-Throughput Clinical Proteomics Reveals Classifiers of COVID-19 Infection. Cell Syst. *11*, 11–24.e4. https://doi.org/10.1016/j.cels.2020.05.012.
27. Hoppe, J.E., Wagner, B.D., Kirk Harris, J., Rowe, S.M., Heltshe, S.L., DeBoer, E.M., and Sagel, S.D. (2022). Effects of ivacaftor on systemic inflammation and the plasma proteome in people with CF and G551D. J. Cyst. Fibros. *21*, 950–958. https://doi.org/10.1016/j.jcf.2022.03.012.
28. Braccia, C., Christopher, J.A., Crook, O.M., Breckels, L.M., Queiroz, R.M.L., Liessi, N., Tomati, V., Capurro, V., Bandiera, T., Baldassari, S., et al. (2022). CFTR Rescue by Lumacaftor (VX-809) Induces an Extensive Reorganization of Mitochondria in the Cystic Fibrosis Bronchial Epithelium. Cells *11*, 1938. https://doi.org/10.3390/cells11121938.
29. Trappe, A., Lakkappa, N., Carter, S., Dillon, E., Wynne, K., McKone, E., McNally, P., and Coppinger, J.A. (2023). Investigating serum extracellular vesicles in Cystic Fibrosis. J. Cyst. Fibros. *22*, 674–679. https://doi.org/10.1016/j.jcf.2023.02.005.
30. Pedrazzi, M., Vercellone, S., Barberis, E., Capraro, M., De Tullio, R., Cresta, F., Casciaro, R., Castellani, C., Patrone, M., Marengo, E., et al. (2021). Identification of Potential Leukocyte Biomarkers Related to Drug Recovery of CFTR: Clinical Applications in Cystic Fibrosis. Int. J. Mol. Sci. *22*, 3928. https://doi.org/10.3390/ijms22083928.
31. Hisert, K.B., Birkland, T.P., Schoenfelt, K.Q., Long, M.E., Grogan, B., Carter, S., Liles, W.C., McKone, E.F., Becker, L., Manicone, A.M., et al. (2020). CFTR Modulator Therapy Enhances Peripheral Blood Monocyte Contributions to Immune Responses in People With Cystic Fibrosis. Front. Pharmacol. *11*, 1219. https://doi.org/10.3389/fphar.2020.01219.
32. Quon, B.S., Dai, D.L.Y., Hollander, Z., Ng, R.T., Tebbutt, S.J., Man, S.F.P., Wilcox, P.G., and Sin, D.D. (2016). Discovery of novel plasma protein biomarkers to predict imminent cystic fibrosis pulmonary exacerbations using multiple reaction monitoring mass spectrometry. Thorax *71*, 216–222. https://doi.org/10.1136/thoraxjnl-2014-206710.
33. Roberts, J.M., Dai, D.L.Y., Hollander, Z., Ng, R.T., Tebbutt, S.J., Wilcox, P.G., Sin, D.D., and Quon, B.S. (2018). Multiple reaction monitoring mass spectrometry to identify novel plasma protein biomarkers of treatment response in cystic fibrosis pulmonary exacerbations. J. Cyst. Fibros. *17*, 333–340. https://doi.org/10.1016/j.jcf.2017.10.013.
34. Graham, B.I.M., Harris, J.K., Zemanick, E.T., and Wagner, B.D. (2023). Integrating airway microbiome and blood proteomics data to identify multi-omic networks associated with response to pulmonary infection. Microbe *1*, 100023. https://doi.org/10.1016/j.microb.2023.100023.

35. Dong, K., Moon, K.-M., Chen, V., Ng, R., Foster, L.J., Tebbutt, S.J., and Quon, B.S. (2019). Identification of novel blood biomarkers of treatment response in cystic fibrosis pulmonary exacerbations by label-free quantitative proteomics. Sci. Rep. *9*, 17126. https://doi.org/10.1038/s41598-019-53759-1.

36. Benabdelkamel, H., Alamri, H., Okla, M., Masood, A., Abdel Jabar, M., Alanazi, I.O., Alfadda, A.A., Nizami, I., Dasouki, M., and Abdel Rahman, A.M. (2020). Serum-Based Proteomics Profiling in Adult Patients with Cystic Fibrosis. Int. J. Mol. Sci. *21*, 7415. https://doi.org/10.3390/ijms21197415.

37. DeBoer, E.M., Kroehl, M.E., Wagner, B.D., Accurso, F.J., Harris, J.K., Lynch, D.A., Sagel, S.D., and Deterding, R.R. (2017). Proteomic profiling identifies novel circulating markers associated with bronchiectasis in cystic fibrosis. Proteomics Clin. Appl. *11*. https://doi.org/10.1002/prca.201600147.

38. Hendry, J., Elborn, J.S., Nixon, L., Shale, D.J., and Webb, A.K. (1999). Cystic fibrosis: inflammatory response to infection with Burkholderia cepacia and Pseudomonas aeruginosa. Eur. Respir. J. *14*, 435–438. https://doi.org/10.1034/j.1399-3003.1999.14b32.x.

39. Sagel, S.D., Wagner, B.D., Ziady, A., Kelley, T., Clancy, J.P., Narvaez-Rivas, M., Pilewski, J., Joseloff, E., Sha, W., Zelnick, L., et al. (2020). Utilizing centralized biorepository samples for biomarkers of cystic fibrosis lung disease severity. J. Cyst. Fibros. *19*, 632–640. https://doi.org/10.1016/j.jcf.2019.12.007.

40. Quon, B.S., Ngan, D.A., Wilcox, P.G., Man, S.F.P., and Sin, D.D. (2014). Plasma sCD14 as a biomarker to predict pulmonary exacerbations in cystic fibrosis. PLoS One *9*, e89341. https://doi.org/10.1371/journal.pone.0089341.

41. Bene, Z., Fejes, Z., Macek, M., Amaral, M.D., Balogh, I., and Nagy, B. (2020). Laboratory biomarkers for lung disease severity and progression in cystic fibrosis. Clin. Chim. Acta; Int. J. Clin. Chem. *508*, 277–286. https://doi.org/10.1016/j.cca.2020.05.015.

42. Charro, N., Hood, B.L., Faria, D., Pacheco, P., Azevedo, P., Lopes, C., de Almeida, A.B., Couto, F.M., Conrads, T.P., and Penque, D. (2011). Serum proteomics signature of cystic fibrosis patients: a complementary 2-DE and LC-MS/MS approach. J. Proteom. *74*, 110–126. https://doi.org/10.1016/j.jprot.2010.10.001.

43. Jain, R., Baines, A., Khan, U., Wagner, B.D., and Sagel, S.D. (2021). Evaluation of airway and circulating inflammatory biomarkers for cystic fibrosis drug development. J. Cyst. Fibros. *20*, 50–56. https://doi.org/10.1016/j.jcf.2020.06.017.

44. Gray, R.D., Imrie, M., Boyd, A.C., Porteous, D., Innes, J.A., and Greening, A.P. (2010). Sputum and serum calprotectin are useful biomarkers during CF exacerbation. J. Cyst. Fibros. *9*, 193–198. https://doi.org/10.1016/j.jcf.2010.01.005.

45. Lee, S.J., Jeong, J.H., Heo, M., Ju, S., Yoo, J.-W., Jeong, Y.Y., and Lee, J.D. (2022). Serum Fibrinogen as a Biomarker for Disease Severity and Exacerbation in Patients with Non-Cystic Fibrosis Bronchiectasis. J. Clin. Med. *11*, 3948. https://doi.org/10.3390/jcm11143948.

46. Graeber, S.Y., Vitzthum, C., Pallenberg, S.T., Naehrlich, L., Stahl, M., Rohrbach, A., Drescher, M., Minso, R., Ringshausen, F.C., Rueckes-Nilges, C., et al. (2022). Effects of Elexacaftor/Tezacaftor/Ivacaftor Therapy on CFTR Function in Patients with Cystic Fibrosis and One or Two F508del Alleles. Am. J. Respir. Crit. Care Med. *205*, 540–549. https://doi.org/10.1164/rccm.202110-2249OC.

47. Causer, A.J., Shute, J.K., Cummings, M.H., Shepherd, A.I., Gruet, M., Costello, J.T., Bailey, S., Lindley, M., Pearson, C., Connett, G., et al. (2020). Circulating biomarkers of antioxidant status and oxidative stress in people with cystic fibrosis: A systematic review and meta-analysis. Redox Biol. *32*, 101436. https://doi.org/10.1016/j.redox.2020.101436.

48. Nixon, L.S., Yung, B., Bell, S.C., Elborn, J.S., and Shale, D.J. (1998). Circulating immunoreactive interleukin-6 in cystic fibrosis. Am. J. Respir. Crit. Care Med. *157*, 1764–1769. https://doi.org/10.1164/ajrccm.157.6.9704086.

49. Hanssens, L.S., Cellauro, S., Duchateau, J., and Casimir, G.J. (2021). Immunoglobulin G: A useful outcome marker in the follow-up of cystic fibrosis patients? Immun. Inflam. Dis. *9*, 608–614. https://doi.org/10.1002/iid3.426.

50. Bulloch, M.N., Hanna, C., and Giovane, R. (2017). Lumacaftor/ivacaftor, a novel agent for the treatment of cystic fibrosis patients who are homozygous for the F580del CFTR mutation. Expert Rev. Clin. Pharmacol. *10*, 1055–1072. https://doi.org/10.1080/17512433.2017.1378094.

51. Lepissier, A., Bonnel, A.S., Wizla, N., Weiss, L., Mittaine, M., Bessaci, K., Kerem, E., Houdouin, V., Reix, P., Marguet, C., et al. (2023). Moving the Dial on Airway Inflammation in Response to Trikafta in Adolescents with Cystic Fibrosis. Am. J. Respir. Crit. Care Med. *207*, 792–795. https://doi.org/10.1164/rccm.202210-1938LE.

52. Elborn, J.S., Horsley, A., MacGregor, G., Bilton, D., Grosswald, R., Ahuja, S., and Springman, E.B. (2017). Phase I Studies of Acebilustat: Biomarker Response and Safety in Patients with Cystic Fibrosis. Clin. Transl. Sci. *10*, 28–34. https://doi.org/10.1111/cts.12428.

53. Elborn, J.S., Konstan, M.W., Taylor-Cousar, J.L., Fajac, I., Horsley, A., Sutharsan, S., Aaron, S.D., Daines, C.L., Uluer, A., Downey, D.G., et al. (2021). Empire-CF study: A phase 2 clinical trial of leukotriene A4 hydrolase inhibitor acebilustat in adult subjects with cystic fibrosis. J. Cyst. Fibros. *20*, 1026–1034. https://doi.org/10.1016/j.jcf.2021.08.007.

54. Snelgrove, R.J., Jackson, P.L., Hardison, M.T., Noerager, B.D., Kinloch, A., Gaggar, A., Shastry, S., Rowe, S.M., Shim, Y.M., Hussell, T., et al. (2010). A critical role for LTA4H in limiting chronic pulmonary neutrophilic inflammation. Science *330*, 90–94. https://doi.org/10.1126/science.1190594.

55. Li, L., and Somerset, S. (2014). Digestive system dysfunction in cystic fibrosis: challenges for nutrition therapy. Dig. Liver Dis. *46*, 865–874. https://doi.org/10.1016/j.dld.2014.06.011.

56. Peretti, N., Roy, C.C., Drouin, E., Seidman, E., Brochu, P., Casimir, G., and Levy, E. (2006). Abnormal intracellular lipid processing contributes to fat malabsorption in cystic fibrosis patients. Am. J. Physiol., Gastrointest. Liver Physiol. *290*, G609–G615. https://doi.org/10.1152/ajpgi.00332.2005.

57. Thompson, S.J., Sargsyan, A., Lee, S.-A., Yuen, J.J., Cai, J., Smalling, R., Ghyselinck, N., Mark, M., Blaner, W.S., and Graham, T.E. (2017). Hepatocytes Are the Principal Source of Circulating RBP4 in Mice. Diabetes *66*, 58–63. https://doi.org/10.2337/db16-0286.

58. Fedders, R., Muenzner, M., Weber, P., Sommerfeld, M., Knauer, M., Kedziora, S., Kast, N., Heidenreich, S., Raila, J., Weger, S., et al. (2018). Liver-secreted RBP4 does not impair glucose homeostasis in mice. J. Biol. Chem. *293*, 15269–15276. https://doi.org/10.1074/jbc.RA118.004294.

59. Oh, H.S.-H., Rutledge, J., Nachun, D., Pálovics, R., Abiose, O., Moran-Losada, P., Channappa, D., Urey, D.Y., Kim, K., Sung, Y.J., et al. (2023). Organ aging signatures in the plasma proteome track health and disease. Nature *624*, 164–172. https://doi.org/10.1038/s41586-023-06802-1.

60. Steinhoff, J.S., Lass, A., and Schupp, M. (2021). Biological Functions of RBP4 and Its Relevance for Human Diseases. Front. Physiol. *12*, 659977. https://doi.org/10.3389/fphys.2021.659977.

61. Jin, Q., Chen, Y., Lou, Y., and He, X. (2013). Low Serum retinol-binding protein-4 levels in acute exacerbations of chronic obstructive pulmonary disease at intensive care unit admission is a predictor of mortality in elderly patients. J. Inflamm. (Lond) *10*, 31. https://doi.org/10.1186/1476-9255-10-31.

62. Liu, X., Yang, M., Li, J., Liu, H., Dong, Y., Zheng, J., and Huang, Y. (2024). Identification of CFH and FHL2 as biomarkers for idiopathic pulmonary fibrosis. Front. Med. (Lausanne) *11*, 1363643. https://doi.org/10.3389/fmed.2024.1363643.

63. Saraswat, M., Joenväärä, S., Tohmola, T., Sutinen, E., Vartiainen, V., Koli, K., Myllärniemi, M., and Renkonen, R. (2020). Label-free plasma proteomics identifies haptoglobin-related protein as candidate marker of idiopathic pulmonary fibrosis and dysregulation of complement and oxidative pathways. Label. Sci. Rep. *10*, 7787. https://doi.org/10.1038/s41598-020-64759-x.

64. Taguchi, A., Politi, K., Pitteri, S.J., Lockwood, W.W., Faça, V.M., Kelly-Spratt, K., Wong, C.-H., Zhang, Q., Chin, A., Park, K.-S., et al. (2011). Lung cancer signatures in plasma based on proteome profiling of mouse tumor models. Cancer Cell *20*, 289–299. https://doi.org/10.1016/j.ccr.2011.08.007.

65. Wikoff, W.R., Hanash, S., DeFelice, B., Miyamoto, S., Barnett, M., Zhao, Y., Goodman, G., Feng, Z., Gandara, D., Fiehn, O., et al. (2015). Diacetylspermine Is a Novel Prediagnostic Serum Biomarker for Non-Small-Cell Lung Cancer and Has Additive Performance With Pro-Surfactant Protein B. J. Clin. Oncol. *33*, 3880–3886. https://doi.org/10.1200/JCO.2015.61.7779.

66. Sin, D.D., Tammemagi, C.M., Lam, S., Barnett, M.J., Duan, X., Tam, A., Auman, H., Feng, Z., Goodman, G.E., Hanash, S., et al. (2013). Pro-surfactant protein B as a biomarker for lung cancer prediction. J. Clin. Oncol. *31*, 4536–4543. https://doi.org/10.1200/JCO.2013.50.6105.

67. Doyle, I.R., Bersten, A.D., and Nicholas, T.E. (1997). Surfactant proteins-A and -B are elevated in plasma of patients with acute respiratory failure. Am. J. Respir. Crit. Care Med. *156*, 1217–1229. https://doi.org/10.1164/ajrccm.156.4.9603061.

68. Robin, M., Dong, P., Hermans, C., Bernard, A., Bersten, A.D., and Doyle, I.R. (2002). Serum levels of CC16, SP-A and SP-B reflect tobacco-smoke exposure in asymptomatic subjects. Eur. Respir. J. *20*, 1152–1161. https://doi.org/10.1183/09031936.02.02042001.

69. Probst, C.K., Montesi, S.B., Medoff, B.D., Shea, B.S., and Knipe, R.S. (2020). Vascular permeability in the fibrotic lung. Eur. Respir. J. *56*, 1900100. https://doi.org/10.1183/13993003.00100-2019.

70. Um, S.J., Lam, S., Coxson, H., Man, S.F.P., and Sin, D.D. (2013). Budesonide/formoterol enhances the expression of pro Surfactant Protein-B in lungs of COPD patients. PLoS One *8*, e83881. https://doi.org/10.1371/journal.pone.0083881.

71. Perez-Riverol, Y., Bai, J., Bandla, C., García-Seisdedos, D., Hewapathirana, S., Kamatchinathan, S., Kundu, D.J., Prakash, A., Frericks-Zipper, A., Martin Eisenacher, M., et al. (2022). The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. Nucleic Acids Res *50*, D543–D552. https://doi.org/10.1093/nar/gkab1038.

72. Rappsilber, J., Ishihama, Y., and Mann, M. (2003). Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. Anal. Chem. *75*, 663–670. https://doi.org/10.1021/ac026117i.

73. Pekayvaz, K., Leunig, A., Kaiser, R., Joppich, M., Brambs, S., Janjic, A., Popp, O., Nixdorf, D., Fumagalli, V., Schmidt, N., et al. (2022). Protective immune trajectories in early viral containment of non-pneumonic SARS-CoV-2 infection. Nat. Commun. *13*, 1018. https://doi.org/10.1038/s41467-022-28508-0.

74. Keshishian, H., Burgess, M.W., Gillette, M.A., Mertins, P., Clauser, K.R., Mani, D.R., Kuhn, E.W., Farrell, L.A., Gerszten, R.E., and Carr, S.A. (2015). Multiplexed, Quantitative Workflow for Sensitive Biomarker Discovery in Plasma Yields Novel Candidates for Early Myocardial Injury. Mol. Cell Proteomics *14*, 2375–2393. https://doi.org/10.1074/mcp.M114.046813.

75. Meier, F., Geyer, P.E., Virreira Winter, S., Cox, J., and Mann, M. (2018). BoxCar acquisition method enables single-shot proteomics at a depth of 10,000 proteins in 100 minutes. Nat. Methods *15*, 440–448. https://doi.org/10.1038/s41592-018-0003-5.

76. Frankenfield, A.M., Ni, J., Ahmed, M., and Hao, L. (2022). Protein Contaminants Matter: Building Universal Protein Contaminant Libraries for DDA and DIA Proteomics. J. Proteome Res. *21*, 2104–2113. https://doi.org/10.1021/acs.jproteome.2c00145.

77. Nordmeyer, S., Kraus, M., Ziehm, M., Kirchner, M., Schafstedde, M., Kelm, M., Niquet, S., Stephen, M.M., Baczko, I., Knosalla, C., et al. (2023). Disease- and sex-specific differences in patients with heart valve disease: a proteome study. Life Sci. Alliance *6*, e202201411. https://doi.org/10.26508/lsa.202201411.

78. Tyanova, S., Temu, T., and Cox, J. (2016). The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. Nat. Protoc. *11*, 2301–2319. https://doi.org/10.1038/nprot.2016.136.

79. Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., and Smyth, G.K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. *43*, e47. https://doi.org/10.1093/nar/gkv007.

80. Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., et al. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. USA. *102*, 15545–15550. https://doi.org/10.1073/pnas.0506580102.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Acetonitrile (ACN, LC-MS grade)	VWR	83.640.320
Ammonium bicarbonate (ABC)	Roth	T871.2
Benzonase	Merck	101695
Dithiothreitol (DTT)	Sigma Aldrich	43815–5G
EDTA 0.5M in H_2O	Sigma	E7889-100ML
Formic acid (FA)	Merck-Millipore	1002640100
Chloroacetamide (CAA)	Sigma-Aldrich	C0267-100G
Methanol (MeOH, LC-MS grade)	Th Geyer	1439-2.5L
Modified trypsin, sequencing grade (bulk order)	Promega	V511X
Lysyl Endopeptidase (LysC) (bulk order)	Fujifilm Wako Pure Chemical Corporation	129-02541
Sodium chloride (NaCl)	Sigma-Aldrich	71376-1KG
Sep-Pak tC18, 200 mg	Waters	WAT054925
STAGE tips: 3M™ C18 Empore™ disks	CDS Analytical	2215
Trifluoroacetic acid (TFA)	Merck/Millipore	1082620100
Trizma® hydrochloride solution 1M, pH 8 (Tris)	Sigma	T2694-1L
Sodium deoxycholate (SDC)	Sigma	D6750-25G
IgY14 LC5 column	Sigma-Aldrich	SEP030-1KT
Supermix LC2 column	Sigma-Aldrich	SEP050-1KT
Amicon® Ultra Centrifugal Filter, 3 kDa MWCO	Millipore	UFC900324
HPLC grade water	Th.Geyer	455.2500
Reprosil-pur C18, 1.9 μm beads	Dr. Maisch GmbH	r119.aq.0003
polyimide coated silica capillary ID 75μm OD 375μm	Polymicro	TSP075375
XBridge BEH C18 Column 4.6 mm × 250 mm column, 3.5 μm bead size	Waters	186003943
AssayMAP 5 μL C18 cartridges rack of 96	Agilent Technologies	5190–6532
0.2ml Eppendorf twin.tec® PCR Plate 96	Merck	30128.648
Agilent Reservoir, Seahorse, PP, no walls, pyramid bottoms	Agilent Technologies	201254–100
Agilent pipette tips, 96 V11 LT250 Tip Box (full)		19477-002
96 Deepwell Plate, 0.6 mL	Starlab	E2896-0600
Critical commercial assays		
Pierce™ BCA protein assay kit	Pierce	23225
Deposited data		
Mass spectrometry proteomics data	ProteomeXchange	PRIDE: PXD071549
Software and algorithms		
MaxQuant version 2.0.3.0 and 1.6.0.1	Max Plank Institute of Biochemistry	https://maxquant.org/
R version 4.2.2		https://cran.r-project.org/
RStudio version 2024.9.1.394		http://www.posit.co/
Spectronaut version 18.2	Biognosys	NA
ssGSEA tool	Broad Institute	https://github.com/broadinstitute/ssGSEA2.0
Tune (v2.6 & v2.9)	Thermo Fisher Scientific	
Xcalibur (v3.1.66.10)	Thermo Fisher Scientific	

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Other		
Easy-nLC™ 1200 HPLC system	Thermo Fisher Scientific	NA
Q-Exactive HF-X mass spectrometer	Thermo Fisher Scientific	NA
1290 Infinity II LC System	Agilent Technologies	NA
1260 Infinity II LC System	Agilent Technologies	NA
Agilent Bravo Automated Liquid Handling Platform1260 Infinity II LC System	Agilent Technologies	NA
Thermo Fisher Scientific Orbitrap Astral	Thermo Fisher Scientific	NA
Vanquish Neo UHPLC System	Thermo Fisher Scientific	VN-S10-A-01, VN-C10-A-01, VN-P10-A-01, VN-A10-A-01, 6036.1180

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

All people with CF included in this study participated in two prospective, longitudinal, observational studies followed at the CF Centers at University Hospital Heidelberg, Germany (LUM/IVA cohort, [9]), and at Charité – Universitätsmedizin Berlin, Germany (ELX/TEZ/IVA cohort, [46]). The validation cohort is an independent cohort recruited at Charité – Universitätsmedizin Berlin. The studies were approved by the ethics committees of the University of Heidelberg (S-370/2011) and the Charité – Universitätsmedizin Berlin (EA2/220/18). Written informed consent was obtained from all people with CF, their parents, or legal guardians. People with CF were eligible to take part in the LUM/IVA study if they were homozygous for the *F508del* mutation, had no prior exposure to LUM/IVA, and were willing to remain on a stable medication regimen and administration of LUM/IVA according to the EMA-approved patient label. In the ELX/TEZ/IVA study, people with CF were eligible, if they were compound-heterozygous for *F508del* and a minimal function mutation or homozygous for *F508del*, had no prior exposure to ELX/TEZ/IVA and were willing to remain on a stable medication regimen including ELX/TEZ/IVA according to the patient labeling and the prescribing information for the duration of study participation. Exclusion criteria for both studies were an acute respiratory infection or pulmonary exacerbation at the time of plasma/serum or sputum collection. In both studies, serum or plasma samples were collected at baseline and 3 months after initiation of therapy. Due to limited amounts of samples, different types of blood-derived samples were measured (serum and plasma). Additionally, sweat chloride concentration, lung function, and BMI were assessed at baseline and after 3 months of either LUM/IVA or ELX/TEZ/IVA treatment. Healthy control subjects were age- and sex-matched, non-smoking volunteers without any medical history of respiratory diseases.

Further information on the participant profiles is provided in Table 1.

METHOD DETAILS

Sample Preparation

Serum and plasma samples were processed separately. Samples were diluted 1:10 in HPLC grade water for further processing. 10 µl of the dilution were denatured in SDC buffer (final concentration: 1% SDC, 10 mM DTT, 40 mM CAA, 100 mM Tris (pH 8)) by heating to 95°C for 10 minutes. After cooling the samples, LysC and Trypsin were added with a peptide to enzyme ratio of 50:1, and samples digested overnight at 37°C. The digest was stopped by adding formic acid (FA) to a final concentration of 1%. The samples were diluted in Buffer A (3% Acetonitrile, 0.1% FA) and centrifuged for 10 minutes at 12 000 rpm. The peptide-containing supernatant was collected and cleaned up using Stage Tips.[72]

For the stage tips, two discs of C18 Empore material were excised using a blunt-ended needle and placed into a pipet tip. In preparation of the stage tips, centrifugation was carried out for a minimum duration of the indicated time or until complete liquid flow through the material was achieved. During the conditioning and washing steps, the tips were subjected to centrifugation at 18,000g for 2 minutes and during sample loading, at 885g for 4:30 min. The material was initially conditioned by the addition of 50 µl of methanol to the tips, followed by the introduction of 100 µl of Buffer A. Subsequent to the loading of the sample, the tips were washed on two occasions with 100 µl of Buffer A.

The stage tips were stored at 4°C until the samples were measured.

For the samples in the validation cohort, the preparation was done using an Agilent Bravo automated sample preparation system. The same denaturing and digestion steps were performed, but with an initial volume of 30 µl of 1:10 diluted sample, and the digest was stopped by adding 10% TFA to a final concentration of 1% and diluting the sample with 100 µl 1% FA. For the cleanup, the Peptide Cleanup v3.0 protocol with the Agilent C18 cartridges was used. The priming was performed with 3 times 100 µl (50% ACN, 0.1% TFA), then the cartridges were equilibrated using 100 µl (0.1% TFA) before loading 170 µl of the sample, and then washed 3x using 100 µl of the 0.1% TFA buffer. For the peptide elution, 100 µl of a 50% ACN, 0.1% FA buffer was used. The eluted peptides were dried and stored at -20°C.

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Data-Independent Acquisition Analyses

Peptides were eluted from stage tips using 80 μl Buffer B (90% Acetonitrile, 0.1% FA), dried, and resuspended in 10 μl Buffer A. 2 μl of each sample were injected. Peptides were separated on a 33 min gradient with increasing acetonitrile concentration using an EASY-nLC 1200 System coupled to an Orbitrap HF-X mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) running in data-independent acquisition mode (DIA), as described with some adjustments.[73] The Buffer B concentration increased linearly from 2% to 7% within 1 minute, followed by an increase to 20% in 19 minutes, to 30% within 9 minutes, and to 60% in 3 minutes, at a 0.25 μl/min flow rate. After the linear gradient, a brief washing step at 90% Buffer B for 5 minutes and an equilibration to 50% Buffer B for 5 minutes were used. One MS1 scan at 120k resolution with an AGC target of 3×10^6 and max. injection time of 60 ms in the range of 350 to 1650 m/z was acquired, followed by 40 DIA scans with variable segment widths adjusted to the precursor density. The scan resolution in the Orbitrap was set to 30k with an AGC target of 3×10^6 and max. injection time of 35 ms. The stepped HCD collision energy was set to 25.5, 27, and 30%.

For the measurements on the Astral, the peptides were resuspended in 100 μl Buffer A and then further diluted 1:4 in Buffer A. 2 μl of peptides were injected and separated using a Vanquish Neo UHPLC system coupled to an Orbitrap Astral mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) running in data-independent acquisition mode (DIA).

The gradient increased from 2% Buffer B to 7% Buffer B in 2 minutes, to 20% Buffer B at 9.5 minutes, to 30% Buffer B at 15 minutes, and to 60% Buffer B at 16.5 minutes, at a 0.35 μl/min flow rate. After the linear gradient, the Buffer B concentration was increased to 90% within 1 minute, and the column was washed for 1.5 minutes with 90% Buffer B. The MS1 scan was performed at 240k resolution, at a scan range of 380–980 m/z, and a normalized AGC target of 500%. The maximum injection time was set to 3ms. The MS2 scans were performed with the Astral detector, at a mass range of 150–2000 m/z, with an isolation window of 2 m/z. The normalized AGC target was set to 500%, and the maximum injection time to 7 ms. The HCD collision energy was set to 25%, and loop control to 0.6 seconds.

Plasma Library Preparation

For each cohort, a separate peptide library was measured. 5 μl of each diluted sample within a cohort were pooled (2 mg in total) and digested as described above, cleaned up SepPak C18 columns, and fractionated using high-pH, reversed phase, off-line chromatography (1290 Infinity II, Agilent; XBridge C18, 4.6 mm × 250 mm column [Waters, 3.5 μm bead size]) into 196 fractions, which were concatenated into 52 analytical fractions. The fractions were dried and resuspended in Buffer A.

To desalt peptides using C18 SepPak columns, a vacuum was applied to increase flow through. For conditioning, 3 mL of 100% ACN were added, followed by 3 mL of 50% ACN/0.1% FA buffer. The columns were then equilibrated by adding 3x 3 mL 0.1% TFA. After loading the samples, the columns were washed 3 times with 3 mL 0.1% TFA and once with 3 mL 1% FA. Peptides were eluted by adding twice 1.5 mL 50% ACN/0.1% FA buffer. The samples were then dried and stored at -20°C until measurement.

Depletion of Plasma for Library Generation

In order to increase library coverage, immunoaffinity depletion of 14 most abundant proteins, followed by the next ~50 moderately abundant proteins, using IgY14 LC5, and Supermix LC2 columns (Sigma-Aldrich, St. Louis, MO) was performed as described.[74] Briefly, six rounds of tandem depletion of 400 μl patient plasma, as well as commercial human plasma samples, were performed on an Agilent 1260 Infinity II HPLC (Agilent, Santa Clara, CA) system using Dilution, Stripping, and Neutralization buffers provided by the manufacturer and following manufacturer's instructions (Sigma-Aldrich). Flow throughs of the Supermix column from each round representing depleted plasma were combined, concentrated, and buffer exchanged to 50 mM Ammonium Bicarbonate to the original volume (400 μl) using Amicon 3K concentrators (Millipore, Billerica, MA). The final protein sample was digested and fractionated as described above.

Data-Dependent Acquisition Analyses of Library Samples

1 μg peptide of depleted and non-depleted library peptide fractions was measured on an Orbitrap HF-X mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) running in data-dependent acquisition mode, using the same LC and gradient parameters as described for DIA measurements. The MS parameters were the following: MS1 scan at 60K resolution with AGC target of $3x10^6$ and maximum injection time of 10 ms, followed by twenty MS2 scans at 15K resolution with AGC target of $1x10^5$ and maximum injection time of 22 ms. Dynamic exclusion was set to 20 seconds.

Comparison of Serum and Plasma Samples

Matching serum and plasma samples from three baseline people with CF were digested as described above and measured on an Orbitrap HF-X mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) running in data-dependent acquisition mode, as described in Meier et al. (2018).[75]

The Q Exactive HF-X mass spectrometer was operated with the instrument control software Tune v2.6 (Build 2640, Thermo Fisher Scientific). The 'text method interface' of Xcalibur (v3.1.66.10, Thermo Fisher Scientific) was used to build customized MS acquisition methods and was accessed through 'XmlMode' (provided by Thermo Fisher Scientific). Full scan resolution was set to 120K, AGC target 3e06, maximum injection time 200ms, and mass range was 300–1650 m/z. Boxcar mode was set to 2 scans, 18 boxes, 8K resolution, an AGC target of 1e5, with a maximum injection time of 250 ms.

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Raw data of patient and plasma library fractions were processed using the MaxQuant software package (v1.6.0.1) using the UniProt database including isoforms (downloaded 2019-07, UP000005640, including isoforms, 74 468 entries). The search included variable modifications of oxidation (M), N-terminal acetylation, deamidation (N and Q), and fixed modification of carbamidomethyl cysteine. The FDR was set to 1% for peptide and protein identifications. Unique and razor peptides were considered for quantification. MS2 identifications were transferred from library runs to patient samples with the ''Match Between Runs'' option. The integrated LFQ (label-free) quantitation algorithm was applied. Reverse hits, contaminants, and proteins only identified by site were filtered out. Downstream analysis was done as described above.

QUANTIFICATION AND STATISTICAL ANALYSIS

RAW Data Analyses

A combined spectral library with the two cohorts and a depleted library were created using Spectronaut (version 18.2), using the UniProt database including isoforms (downloaded 2022-03, UP000005640, including isoforms, 101 732 entries) and a universal protein contaminants list (downloaded 08-2022, 381 entries[76]). Modifications were set to Carbamidomethyl (C) as fixed and Acetyl (Protein N-term), Deamidation (NQ), and Oxidation (M) as variable modifications. For the analysis of DIA data, precursor filtering was done using the *Q* value setting.

Raw files from the Astral were analyzed using Spectronaut (version 19.7) in directDIA mode. Other settings, such as the UniProt database including isoforms (downloaded 2022-03, UP000005640, 101 732 entries), contaminants list, and modifications were kept the same as in the library-based analysis of Q-Exactive HF-X files. MaxLFQ was used for quantification.

Proteins were exported with and without background imputation, log2-transformed, and data was filtered to contain at least 50% valid values in patient samples in the non-imputed data frame of each cohort (LUM/IVA, ELX/TEZ/IVA (original and validation)) independently. Remaining missing values were imputed using down-shift imputation by random draw from the Gaussian distribution with 0.3x standard deviation and downshift of 1.8x standard deviation of the observed values per sample.[77]

DDA data from sputum samples were analyzed as described before.[20] In summary, the MaxQuant software package (ver. 2.0.3.0; Max Planck Institute of Biochemistry, Martinsried, Germany)[78] was used to analyze raw data from included patient samples. A UniProt database including isoforms (2023-03, UP000005640, reviewed, 42 405 entries) was included in the search. Modifications were defined as follows: oxidation (M), N-terminal acetylation, and deamidation (N, Q) as variable modifications, and carbamidomethyl cysteine as a fixed modification. Proteins with only one peptide were allowed. The search employed the ''Match Between Runs'' and label-free quantitation (LFQ) algorithm.

Statistical Analysis

Further downstream analysis was performed in R (v. 4.2.2.). MaxQuant output was filtered for ''Reverse'', ''Potential Contaminant'', and ''Only Identified by Site''. Proteins identified with only one peptide were filtered for at least 5 identifications by MS/MS and a minimum Andromeda score of 20. In the Spectronaut output contaminants were removed. For significance calling, moderated t-test (limma package[79]) was used. If Student's or Welch t-test were applied, it was highlighted in the figure legend. Benjamini-Hochberg method was used to adjust *p*-values for multiple comparisons. For the different comparisons, the cut-offs are described in the figure legend. For single-sample gene-set enrichment analysis (ssGSEA[80]), a curated gmt file with UniProt keywords (2021-04) was used. Because of the differences in sample collection between the two cohorts, and the differences in age, we used a linear model in the limma package to adjust for age, and compared log2(fold changes) of proteins identified in both cohorts.