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Drug Discovery and Delivery

Plasma Protein Analysis for Biomarker Discovery in Lung Cancer Treated With Atezolizumab Combination Therapy in J-TAIL-2

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ABSTRACT

J-TAIL-2 evaluated the efficacy and safety of atezolizumab, an anti–programmed death-ligand 1 (PD-L1), plus chemotherapy in patients with non-small cell lung cancer (NSCLC) and extensive-stage small cell lung cancer (ES-SCLC) in Japanese clinical practice, demonstrating comparable outcomes to those in the corresponding Phase 3 trials. Although PD-L1 expression is predictive of atezolizumab efficacy in some settings, additional minimally invasive, blood-based biomarkers are needed. This exploratory biomarker study included 359 J-TAIL-2 patients. The NSCLC cohort received atezolizumab combined with carboplatin and nab-paclitaxel (CnP, $n = 42$), cisplatin/carboplatin and pemetrexed ($n = 72$), or bevacizumab plus carboplatin and paclitaxel (bev + CP, $n = 135$). The ES-SCLC cohort received atezolizumab plus carboplatin and etoposide ($n = 100$). Approximately 560 cancer- or immune-related proteins were evaluated using the Proximity Extension Assay from blood plasma collected at three timepoints: baseline, before the second atezolizumab dose, and at the onset of immune-related

Abbreviations: AE, adverse event; Atezo + bev + CP, atezolizumab plus bevacizumab plus carboplatin and paclitaxel; Atezo + CE, atezolizumab plus carboplatin and etoposide; Atezo + CnP, atezolizumab plus carboplatin and nab-paclitaxel; Atezo + PP, atezolizumab plus carboplatin or cisplatin plus pemetrexed; CI, confidence interval; CnP, carboplatin and nab-paclitaxel; ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*mt, *EGFR*-mutant; ES-SCLC, extensive-stage small-cell lung cancer; irAE, immune-related adverse event; mOS, median overall survival; mPFS, median progression-free survival; NSCLC, non-small cell lung cancer; OS, overall survival; PD-L1, programmed death-ligand 1; PEA, Proximity Extension Assay; PFS, progression-free survival; PP, carboplatin or cisplatin plus pemetrexed.

adverse events (irAEs). Protein-wise comparisons were made to evaluate significant changes ($P < 0.05$ and > 0.5 log2 fold change) and linked to clinical outcomes reported in J-TAIL-2. IL-6, MUC-16, and KRT-19 were associated with shorter progression-free survival across multiple regimens. Granzyme A and B, immune activation markers, were elevated in responders who received atezolizumab + CnP and atezolizumab + bev + CP. High levels of baseline immune stimulation proteins were associated with irAEs across regimens. Protein expression changes were varied and regimen-dependent in subgroup analyses including older patients and those with *EGFR*-mutant tumors. These data warrant further investigation across different cancer types and atezolizumab-containing regimens to determine their relevance to efficacy and irAE occurrence of atezolizumab combination therapy.

Trial Registration: ClinicalTrials.gov ID, NCT04501497 (J-TAIL-2) and NCT04818983 (biomarker study)

1 | Introduction

Globally, lung cancer is the leading cause of cancer-related death and commonly diagnosed at advanced stages [1]. Non-small cell lung cancer (NSCLC) represents 85% of lung cancer cases and has a poor prognosis, indicated by a 5-year survival rate of less than 10% [2, 3]. Extensive-stage small-cell lung cancer (ES-SCLC) accounts for approximately 10% of lung cancer cases and is characterized by high metastatic potential, rapid progression, and poor prognosis [4–6].

Recent advances in combination immune checkpoint inhibitor (ICI) therapy, including atezolizumab (anti–programmed death-ligand 1 [PD-L1]) with chemotherapy, have significantly improved survival, resulting in these regimens becoming a standard treatment for both ES-SCLC and advanced NSCLC [7, 8]. Approvals of first-line atezolizumab plus chemotherapy for advanced or recurrent nonsquamous NSCLC treatment were based on the global Phase 3 trials IMpower130 [9], IMpower132 [10], and IMpower150 [11], and for ES-SCLC treatment following the IMpower133 [12] trial. J-TAIL-2 (NCT04501497) evaluated the efficacy and safety of atezolizumab plus chemotherapy combinations in real-world patients with NSCLC or ES-SCLC in Japanese clinical practice. Previously, the primary analyses from the J-TAIL-2 NSCLC and ES-SCLC cohorts reported comparable efficacy and safety of atezolizumab combination therapy to those in corresponding IMpower studies [13, 14].

Although PD-L1 expression is a routine marker predictive of the efficacy of atezolizumab in many settings, intra-tumor heterogeneity and changes of expression over time can make PD-L1 an insufficient biomarker on its own. The identification of additional biomarkers is needed [15, 16]. ICI efficacy may be associated with baseline levels and changes in immune-related proteins found in the blood plasma shortly after treatment [17]. These protein changes may also predict the occurrence of serious immune-related adverse events (irAEs) [18]. There is a clinical need to identify new biomarkers that predict both efficacy and safety of atezolizumab.

To investigate this clinical gap, an exploratory biomarker study (NCT04818983) was conducted in a subpopulation of J-TAIL-2. We conducted plasma proteomics analyses to evaluate potential predictive biomarkers of efficacy and safety for four atezolizumab-containing regimens in real-world patients with lung cancer from J-TAIL-2.

2 | Methods

2.1 | Study Design and Patient Population

The main J-TAIL-2 study was an observational, prospective study that enrolled patients with NSCLC or ES-SCLC scheduled to receive atezolizumab combination therapy in clinical practice in Japan (based on the latest guidelines for optimal use and prescribing information) across 150 study sites. In the NSCLC cohort, patients received atezolizumab combined with chemotherapy (carboplatin and nab-paclitaxel [CnP], carboplatin or cisplatin plus pemetrexed [PP], or bevacizumab plus carboplatin and paclitaxel [bev + CP]) in clinical practice. These atezolizumab combination regimens were investigated in the global, randomized, Phase 3 IMpower130, IMpower132, and IMpower150 trials, respectively [9–11]. Patients with ES-SCLC received atezolizumab plus carboplatin and etoposide (CE), which was evaluated in the IMpower133 trial [12].

The J-TAIL-2 subset for this exploratory biomarker study enrolled patients from 97 study sites with methodology previously described [14, 19]. Evaluable patients in this analysis had received ≥1 dose of atezolizumab and had provided samples for biomarker measurements as described below. Patients determined to be ineligible on study and those missing efficacy assessments were not included (Figure S1).

J-TAIL-2 was conducted in accordance with the Declaration of Helsinki (Japan Medical Association translation), the Act on the Protection of Personal Information, and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. Enrolled patients received a full explanation of the contents of this clinical research and provided written consent. The Ethics Review Committee of each study site approved the study protocol and informed consent form before the site could participate in the study. Additional study details were previously reported [14, 19].

2.2 | Assessments

Collected plasma was used to measure expression levels of approximately 560 proteins using the Proximity Extension Assay (PEA; Olink Proteomics). The detailed protocol of the PEA has been previously published [20]. PEA analysis was performed at three timepoints: baseline (≤7 days before first dose of atezolizumab), before the second dose of atezolizumab (≤3 days before the second dose), and onset of irAEs. Peripheral blood was

collected at the same time as a blood test performed during routine care: 1 mL before the first dose of atezolizumab, 1 mL before the second dose of atezolizumab, and 1 mL at the onset of irAE. The following standard Olink panels were used in all PEA analyses: cell regulation, immune response, immuno-oncology, inflammation, metabolism, oncology II, and organ damage. There are some proteins across multiple Olink panels (e.g., immune response and inflammation panels), but these proteins are captured by the same pair of antibodies. Panel-specific optimization can result in differences in upper and lower limits of quantification and dynamic range across panels (Figure S2).

2.3 | Statistical Analysis

At each evaluated timepoint (baseline, before the second dose, and for the change between before the second dose and baseline), protein-wise comparisons were performed between two sample groups using the Wilcoxon test, with nominal *P*-values <0.05 considered significant. *P*-values were not adjusted for multiplicity in this exploratory analysis. Sample group comparisons for all regimens included those with shorter versus longer ($<$ vs. $\geq$ median) progression-free survival (PFS) or overall survival (OS) based on the main J-TAIL-2 study data, older vs. younger patients (patients with NSCLC ≥ 75 vs. <75 years, and patients with ES-SCLC ≥ 70 vs. <70 years), and those who achieved complete or partial responses vs. progressive disease per Response Evaluation Criteria in Solid Tumors (patients with stable disease or those not evaluable were excluded). If no differential proteins were identified between sample groups, a comparison analysis could not be performed and those results are not described or shown. Protein expression of patients who did vs. did not experience an irAE were compared by regimen; the atezolizumab + CnP and atezolizumab + PP regimens were combined for this analysis due to the small subset with post-irAE samples. For patients given atezolizumab + bev + CP, *EGFR*-mutant (*EGFR*mt) vs. non-mutant status was compared. OS analysis was performed in patients with *EGFR* mutations. Volcano plot evaluation of protein changes focused on those considered significant and with ≥ 0.5 log2 fold change between groups.

3 | Results

3.1 | Patients and Baseline Characteristics

In total, 359 patients who were scheduled to receive treatment in the J-TAIL-2 study between April 2021 and February 2022 were included in the intention-to-treat population of this biomarker study. The analysis population ($n=349$) included patients who had received ≥ 1 dose of atezolizumab and had provided samples for biomarker measurements; 10 patients were excluded from the analysis due to not having received study treatment ($n=9$) or other exclusions ($n=1$) (Figure S1). The NSCLC cohort consisted of 42 patients treated with atezolizumab + CnP (regimen from IMpower130), 72 patients treated with atezolizumab + PP (regimen from IMpower132), and 135 patients treated with atezolizumab + bev + CP (regimen from IMpower150). The ES-SCLC cohort included 100 patients treated with atezolizumab + CE (regimen from IMpower133). Baseline patient characteristics in the biomarker study were generally representative of the main J-TAIL-2 population (Table 1) [14, 19].

3.2 | PFS

As previously reported for the main J-TAIL-2 study, the median PFS (95% confidence interval [CI]) for patients with NSCLC was 5.6 months (5.1, 6.9) with atezolizumab + CnP, 7.0 months (5.5, 8.3) with atezolizumab + PP, and 6.3 months (5.7, 6.7) with atezolizumab + bev + CP [14]. The median PFS for patients with ES-SCLC was 5.1 months (4.7, 5.3) with atezolizumab + CE [19]. At baseline, proteins significantly associated with longer or shorter ($\geq$ or $<$ median) PFS were identified in common across all regimens at baseline. Although longer PFS was associated with higher baseline expression of several immune activation factors, these were regimen specific: IL-12 and IL-12B in patients given atezolizumab + CnP and CD40-L in those given atezolizumab + CE (Figure 1). By contrast, shorter PFS was significantly associated with expression of MUC-16, IL-6, and KRT19 in multiple regimens (Figure 1 and Figure S2). MUC-16 and KRT19 expression was also non-significantly associated with short PFS for the atezolizumab + bev + CP regimen. No proteins were found to be significantly associated with shorter or longer PFS for patients given atezolizumab + bev + CP.

3.3 | OS

In the main J-TAIL-2 study, the median OS (95% CI) was 19.7 (16.1, 25.9), 23.5 (17.7, not estimable), 17.3 (15.6, 19.9), and 16.5 (14.9, 18.2) months in patients receiving atezolizumab + CnP, atezolizumab + PP, atezolizumab + bev + CP, and atezolizumab + CE, respectively [14, 19]. Protein expression analysis of OS at baseline is shown in Figure S3. For the atezolizumab + CnP regimen, no significant correlates of longer OS were seen, but shorter OS was associated with markers including elevated N-cadherin and NECTIN-2 (CD112) (Figure S3A), markers which are related to epithelial-to-mesenchymal and TIGIT ligand, respectively, and potentially associated with poor prognosis. For the atezolizumab + bev + CP regimen, several proteins were significantly elevated in patients with long OS including OMG and LHB, although relevance to cancer immunology is poorly defined; proteins associated with short OS included syndecan-1, CXCL9, and EN-RAGE (S100A12) among others (Figure S3B). MUC-16 and IL-6 had high baseline protein expression in the shorter OS group, but this was not statistically significant. For the atezolizumab + CE regimen, long OS was associated with TRANCE (RANK-L) and shorter OS was linked to proteins including KRT-19, MUC-16, IL-8, and CCL-20 (Figure S3C).

3.4 | Treatment Response

Protein expression analysis at baseline and before the second dose are shown in Figure 2 and Figure S4, respectively. At baseline, patients given atezolizumab + CnP who achieved a response on study had significantly higher expression of CXCL10 and granzyme B compared with those with progressive disease (Figure 2A). Before dose 2, response in this group was also associated with granzyme B and other immune activation markers, including granzyme A, FASLG, PDCD1 (PD-1), TNF, TLR3, CD28, and CX3CL1 (Figure S4A). Before dose 2, responders given atezolizumab + bev + CP also had high granzyme A and B, and CD8 had similar high expression in post-treatment (Figure S4C).

TABLE 1 | Baseline patient characteristics in the ITT population.

Patient characteristics	NSCLC cohort				ES-SCLC cohort
	Overall ($n=259$)	Atezo + CnP ($n=42$)	Atezo + PP ($n=77$)	Atezo + Bev + CP ($n=140$)	Atezo + CE ($n=100$)
Age, *n* (%)					
< 70 years	126 (48.6)	14 (33.3)	23 (29.9)	89 (63.6)	38 (38.0)
≥ 70 to < 75 years	75 (29.0)	12 (28.6)	30 (39.0)	33 (23.6)	35 (35.0)
≥ 75 years	58 (22.4)	16 (38.1)	24 (31.2)	18 (12.9)	27 (27.0)
Sex, *n* (%)					
Male	176 (68.0)	36 (85.7)	55 (71.4)	85 (60.7)	81 (81.0)
ECOG PS, *n* (%)					
0	93 (35.9)	14 (33.3)	28 (36.4)	51 (36.4)	31 (31.0)
1	142 (54.8)	24 (57.1)	40 (51.9)	78 (55.7)	54 (54.0)
2	21 (8.1)	4 (9.5)	8 (10.4)	9 (6.4)	13 (13.0)
3	2 (0.8)	0	1 (1.3)	1 (0.7)	2 (2.0)
4	1 (0.4)	0	0	1 (0.7)	0
Histology, *n* (%)					
Adenocarcinoma	221 (85.3)	26 (61.9)	75 (97.4)	120 (85.7)	0
NOS	22 (8.5)	10 (23.8)	0	12 (8.6)	0
Large cell carcinoma	5 (1.9)	2 (4.8)	1 (1.3)	2 (1.4)	0
LCNEC	3 (1.2)	1 (2.4)	0	2 (1.4)	98 (98.0)
Small cell carcinoma	NA	NA	NA	NA	2 (2.0)
Staging, *n* (%)					
IIIA/B/C	11 (4.2)	1 (2.4)	4 (5.2)	6 (4.3)	5 (5.0)
IVA/B	207 (79.9)	33 (78.6)	66 (85.7)	108 (77.1)	86 (86.0)
Recurrence after operation/ chemotherapy/ radiotherapy	41 (15.8)	8 (19.0)	7 (9.1)	26 (18.6)	9 (9.0)
EGFR mutation status, *n* (%)					
Negative	155 (59.8)	39 (92.9)	53 (68.8)	63 (45.0)	NA
Positive	82 (31.7)	1 (2.4)	13 (16.9)	68 (48.6)	NA

Abbreviations: Bev, bevacizumab; CE, carboplatin + etoposide; CnP, carboplatin + nab-paclitaxel; CP, carboplatin + paclitaxel; ECOG PS, Eastern Cooperative Oncology Group performance status; ES-SCLC, extensive-stage small cell lung cancer; ITT, intention-to-treat; LCNEC, large cell neuroendocrine carcinoma; NA, not applicable; NSCLC, non-small cell lung cancer; PP, carboplatin or cisplatin + pemetrexed.

3.5 | Older Patient Subgroup Analysis

At baseline, protein comparisons between older and younger patients (patients aged ≥ 75 or < 75 years with NSCLC and patients aged ≥ 70 vs. < 70 years with ES-SCLC) were performed by regimen (Figure 3). The number of older vs. younger patients was 16 vs. 26 in the atezolizumab + CnP group, 24 vs. 53 in the atezolizumab + PP group, 18 vs. 122 in the atezolizumab + bev + CP group, and 62 vs. 38 in the atezolizumab + CE group (Table 1). For patients given atezolizumab + CnP, inflammatory proteins including IL-6, IL-8, and IL-10 were significantly associated with older age (Figure 3A). For those given atezolizumab + PP, prognostic markers like MUC-16 and KRT-19 were associated with older age (Figure 3B). No proteins were identified to be significantly and highly expressed in older patients given atezolizumab + bev + CP and atezolizumab + CE (Figure 3C,D). In older patients, protein expression was associated with OS only in patients who received atezolizumab + CE (Figure S5). Of

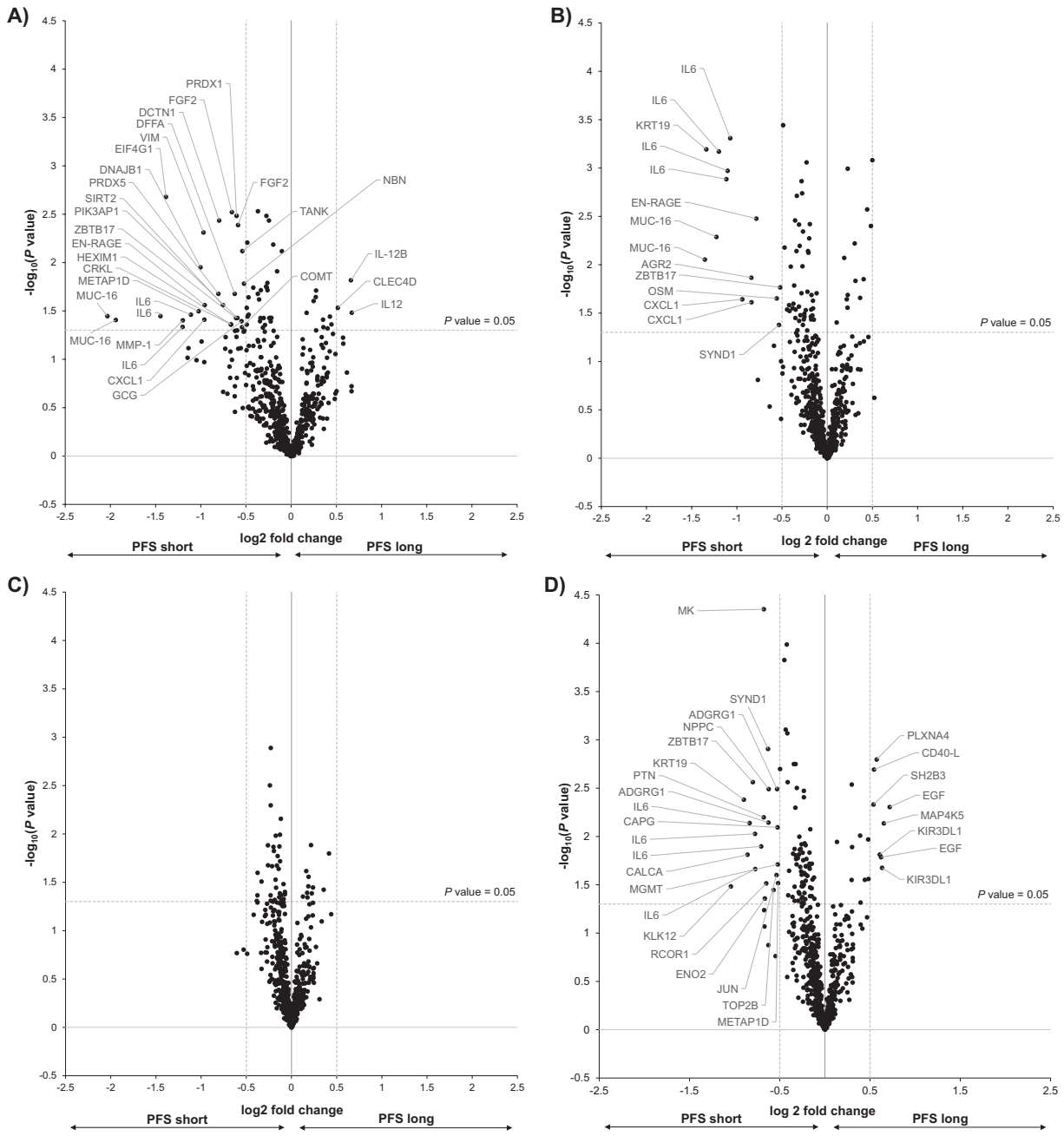

FIGURE 1 | Baseline protein expression analysis of PFS by regimen. (A) Atezolizumab + CnP (NSCLC, $n = 42$), (B) atezolizumab + PP (NSCLC, $n = 72$), (C) atezolizumab + bev + CP (NSCLC, $n = 135$), (D) atezolizumab + CE (ES-SCLC, $n = 100$). Bev, bevacizumab; CE, carboplatin + etoposide; CnP, carboplatin + nab-paclitaxel; CP, carboplatin + paclitaxel; ES-SCLC, extensive-stage small cell lung cancer; NSCLC, non-small cell lung cancer; PFS, progression-free survival; PP, carboplatin or cisplatin + pemetrexed.

note, short OS was associated with elevated expression of IL-17C, MMP12, CCL20, SYND1, and IL-8, proteins regardless of age (Figure S3C).

3.6 | *EGFR*mt Subgroup

J-TAIL-2 enrolled patients with *EGFR* mutations NSCLC after tyrosine kinase inhibitor failure. Most of these patients received atezolizumab + bev + CP ($n = 68$); therefore, only patients in this regimen are the focus of the PEA subgroup analysis reported here. When comparing *EGFR*mt vs. non-mutant samples at baseline (Figure 4), many proteins related to poor prognosis or efficacy, including IL-6, KRT19, MUC-16, IL-8, and CCL20, were significantly elevated in the *EGFR*mt group (Figure 4A). However, almost all significantly elevated proteins associated with the *EGFR*mt group at baseline were no longer significant in the samples taken before the second dose of atezolizumab (Figure 4B). Of note, changes in the significance of IL-6 and CCL20 expression based on *EGFR* status became apparent when evaluating expression between the timepoints (Figure S6). In patients with *EGFR*mt tumors at baseline, OMG was found to be significantly associated with longer OS (Figure 5A). Proteins ZBTB17, KIM1, SYND1, LAMP3, and CALCA were significantly expressed in patients with longer OS independent of *EGFR*mt status (Figure S3B). In patients with *EGFR*mt tumors, before

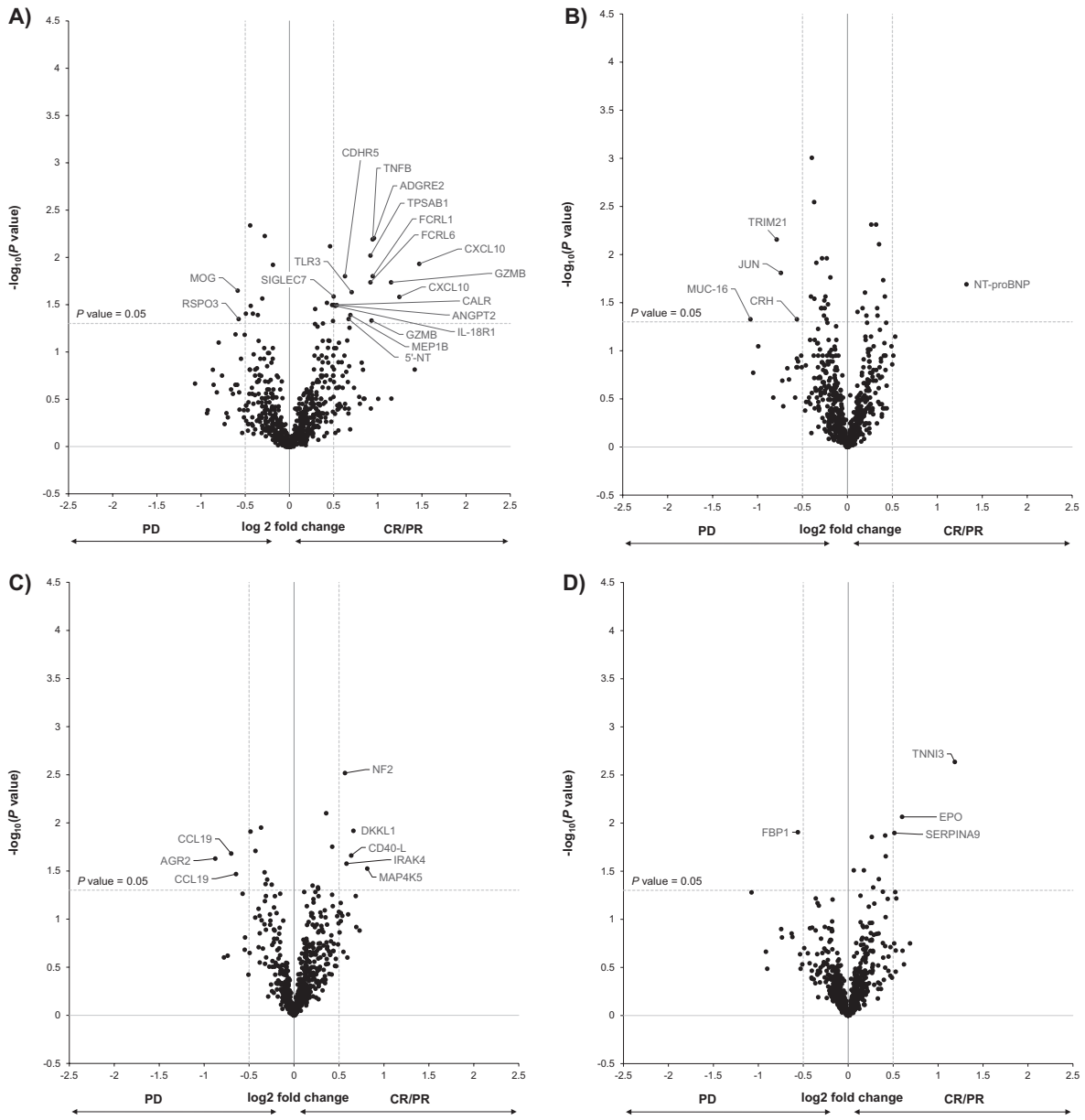

FIGURE 2 | Baseline protein expression analysis of CR/PR vs PD by regimen. (A) Atezolizumab + CnP (NSCLC, $n = 42$), (B) atezolizumab + PP (NSCLC, $n = 72$), (C) atezolizumab + bev + CP (NSCLC, $n = 135$), (D) atezolizumab + CE (ES-SCLC, $n = 100$). Bev, bevacizumab; CE, carboplatin + etoposide; CnP, carboplatin + nab-paclitaxel; CP, carboplatin + paclitaxel; CR, complete response; ES-SCLC, extensive-stage small cell lung cancer; NSCLC, non-small cell lung cancer; PD, progressive disease; PP, carboplatin or cisplatin + pemetrexed; PR, partial response.

dose 2, proteins associated with immune activation, including CXCL10, PD-L1, FASLG, and IL-18, were elevated in patients with longer OS, whereas VEGFA and IL-6 were elevated and associated with shorter OS (Figure 5B). This observation was further confirmed when analyzing protein expression differences between the two timepoints, wherein numerous markers of immune stimulation were found significant in patients with *EGFR*mt tumors with longer OS (Figure S7).

3.7 | irAE Analysis

The irAE incidence was relatively balanced between the atezolizumab + CE group ($n = 25$, 25.0%) and the combined atezolizumab + CnP or PP group ($n = 28$, 22.7%), and higher in the atezolizumab + bev + CP group ($n = 46$, 32.9%). At baseline, the incidence of any-grade irAEs was significantly associated with several chemokines for multiple regimens. CCL17 and CXCL9 were elevated in the combined group treated with atezolizumab + CnP or PP, and CXCL5 and CXCL1 were elevated in the atezolizumab + bev + CP regimen. There were no relevant proteins with high expression associated with the occurrence of irAEs in patients who received atezolizumab + CE (Figure 6).

Given the limited number of proteins identified, an exploratory approach was adopted to investigate the association of irAEs and efficacy, including all proteins with *P*-values < 0.05. Table 2 summarizes proteins with significant differences identified in this exploratory analysis that were associated with irAEs and

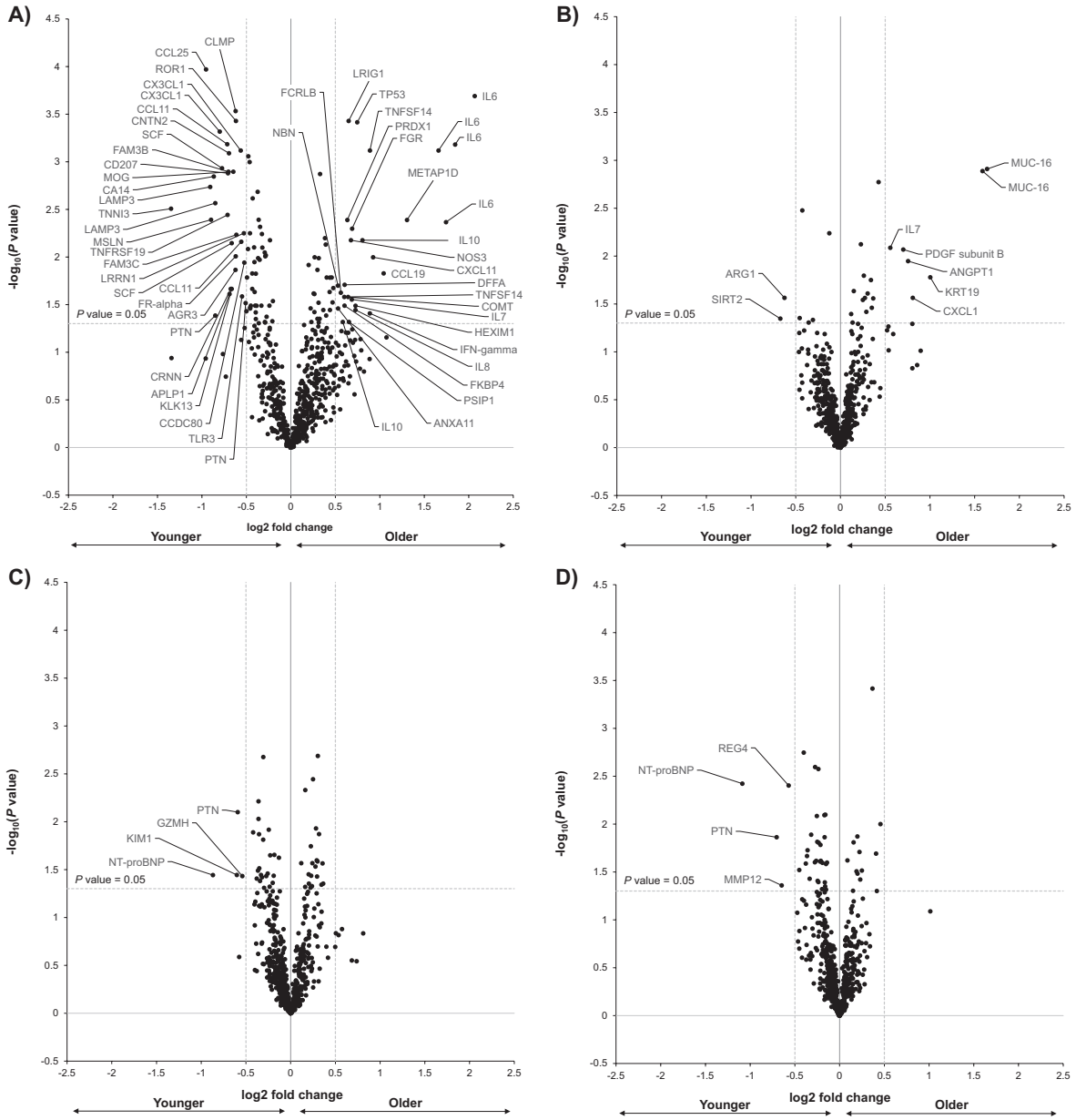

FIGURE 3 | Baseline protein expression analysis of older vs younger patients by regimen. (A) Atezolizumab + CnP (NSCLC, $n = 42$), (B) atezolizumab + PP (NSCLC, $n = 72$), (C) atezolizumab + bev + CP (NSCLC, $n = 135$), (D) atezolizumab + CE (ES-SCLC, $n = 100$). Bev, bevacizumab; CE, carboplatin + etoposide; CnP, carboplatin + nab-paclitaxel; CP, carboplatin + paclitaxel; ES-SCLC, extensive-stage small cell lung cancer; NSCLC, non-small cell lung cancer; PP, carboplatin or cisplatin + pemetrexed.

PFS at baseline. Although no significantly elevated proteins were common among all regimens, proteins with elevated expression that could be potential markers of immune activation were seen across regimens.

4 | Discussion

There is a clinical need for more minimally invasive, blood-based biomarkers that are predictive of immunotherapy efficacy and safety in lung cancer. In this exploratory PEA analysis of a J-TAIL-2 subpopulation, we identified several candidate biomarkers in patient plasma with predictive value on clinical outcomes across multiple atezolizumab combination regimens.

A comprehensive protein assay was used to examine more than 500 proteins related to cancer biology, immune responses, and inflammation, and several proteins were associated with PFS and OS. Most notably, IL-6, MUC-16, and KRT-19 had significant associations with shorter PFS in patients treated across multiple regimens. Many reports have demonstrated an association between high IL-6 expression and poor prognoses across cancer types, including a recent bioinformatic study in lung cancer [21, 22]. IL-6 has also been associated with resistance to ICIs through various immune-related mechanisms including direct suppression of CD8+ T cells and enhanced production of immunosuppressive adenosine [23, 24]. A well-established tumor marker in ovarian cancer, MUC-16, also known as CA125, has been recently identified as a potential predictor of immunotherapy in lung cancer

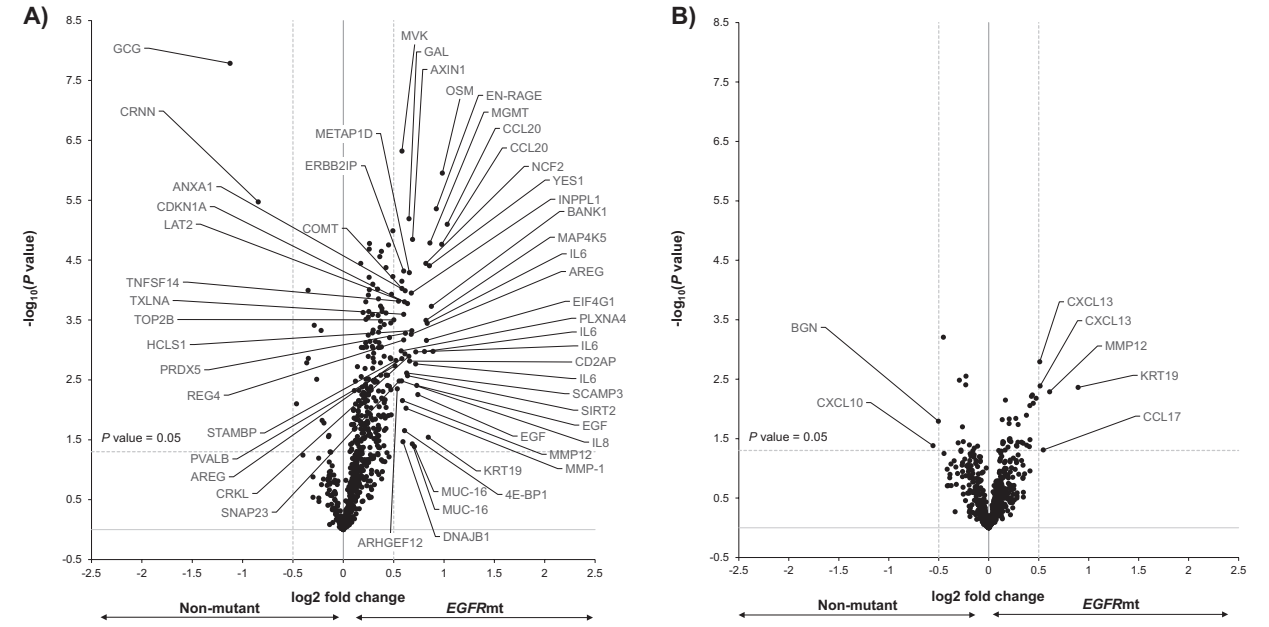

FIGURE 4 | Protein expression analysis of patients with *EGFR*mt vs non-mutant tumors who received atezolizumab + bev + CP (NSCLC, $n = 135$) at (A) baseline and (B) before the second dose of atezolizumab. Bev, bevacizumab; CP, carboplatin + paclitaxel; mt, mutant; NSCLC, non-small cell lung cancer.

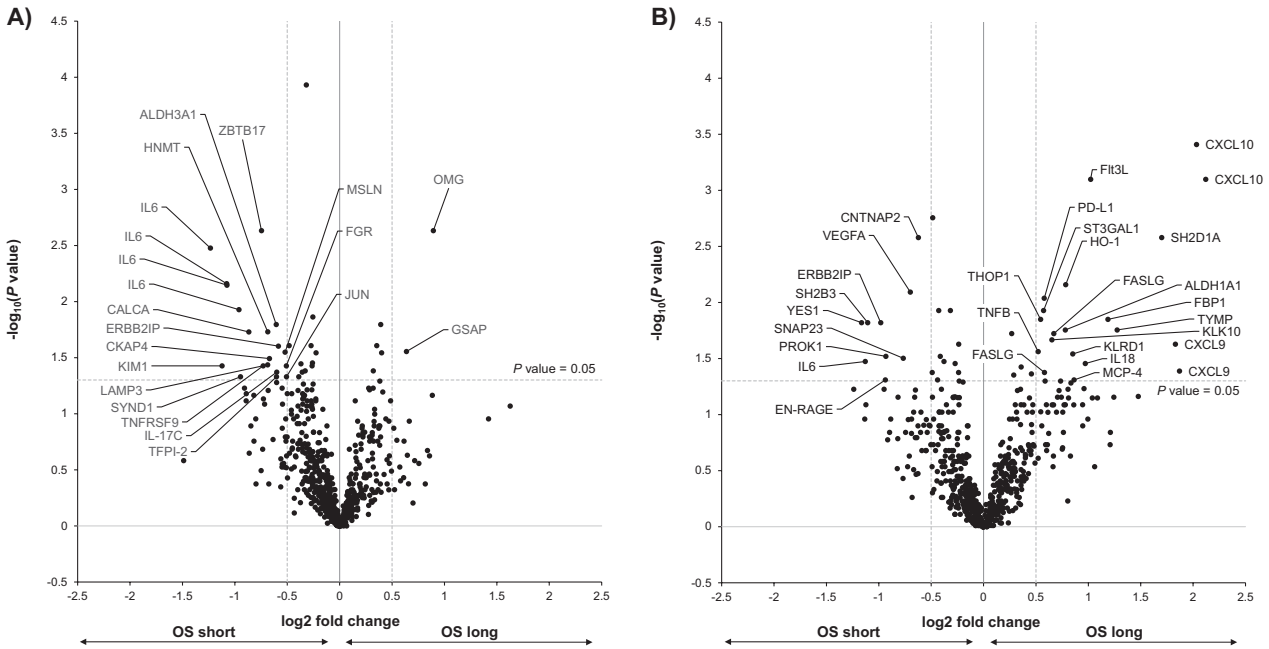

FIGURE 5 | Protein expression analysis of OS in patients with or without *EGFR*mt who received atezolizumab + bev + CP (NSCLC, $n = 135$) at (A) baseline and (B) before the second dose of atezolizumab. Bev, bevacizumab; CP, carboplatin + paclitaxel; mt, mutant; NSCLC, non-small cell lung cancer; OS, overall survival.

[25, 26]. Recent reports have described its potential involvement in evasion of immune surveillance, for instance by binding the inhibitory receptor SIGLEC-9 on T and NK cells to impair their function [27], or promoting Treg differentiation through the production of IL-6 [28]. KRT-19 is associated with epithelial-to-mesenchymal transition and linked to poor prognosis in lung cancer [29]. A pancreatic ductal adenocarcinoma animal model study found that KRT-19+ tumor cells formed CXCL12–KRT-19 cell-surface complexes that reduced the motility of CXCR4+ T cells [30], consequently forming a T-cell–exclusionary tumor microenvironment that can contribute to resistance to anti–PD-1 immunotherapies. Although IL-6, MUC-16, and KRT-19 have been established as poor prognostic markers [21, 31, 32], further study with larger sample sizes and multivariate analyses is needed to confirm their utility as biomarkers of immunotherapy efficacy in lung cancer.

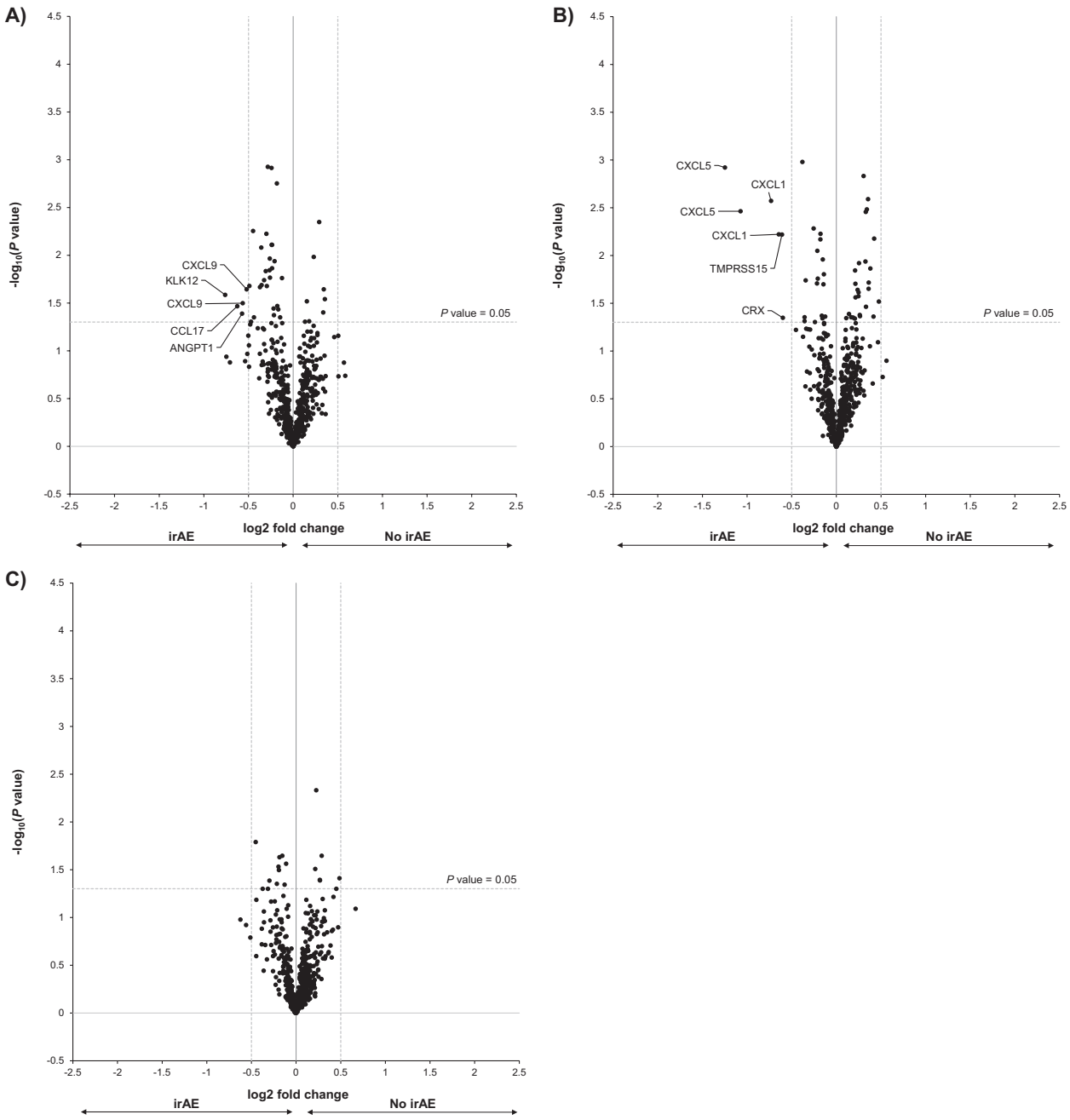

FIGURE 6 | Baseline protein expression analysis of patients with irAE (any grade) vs no irAE. (A) Atezolizumab + CnP (NSCLC, $n = 42$) and atezolizumab + PP (NSCLC, $n = 72$), (B) atezolizumab + bev + CP (NSCLC, $n = 135$), (C) atezolizumab + CE (ES-SCLC, $n = 100$). Bev, bevacizumab; CE, carboplatin + etoposide; CnP, carboplatin + nab-paclitaxel; CP, carboplatin + paclitaxel; ES-SCLC, extensive-stage small cell lung cancer; irAE, immune-related adverse event; NSCLC, non-small cell lung cancer; PP, carboplatin or cisplatin + pemetrexed.

In patients who received atezolizumab + bev + CP, between baseline and dose 2, there was a marked decrease in significantly elevated proteins for patients with *EGFR*mt vs. non-mutant tumors and increased expression of immune stimulation markers in patients with *EGFR*mt tumors with long OS between baseline and before dose 2. Studies have previously reported that VEGF inhibition can induce immune-stimulating mechanisms in many cancer types [33–35] (e.g., by increasing T-cell infiltration into tumors, promoting the maturation of dendritic cells, and reducing Tregs) [36–38]. Whether these effects counteract some functions of the aforementioned resistance proteins (i.e., IL-6, MUC-16, and KRT19), thereby mitigating their impact on drug efficacy, warrants further study. The differences in baseline protein profiles and disparate treatment response between patients with *EGFR*mt and non-mutant tumors may contribute to the difficulty in extracting common factors between regimens. In patients with *EGFR*mt tumors, the clear trend of immune stimulation with longer OS and VEGFA and IL-6 with shorter OS may be attributed to the fact that patients with *EGFR*mt tumors are a relatively homogeneous population compared with the non-mutant population, allowing for pronounced changes to be detected. In the responder group of the all-patient analysis for atezolizumab + bev + CP, many proteins were significantly elevated before the second dose of atezolizumab. When analyzing

TABLE 2 | Proteins associated with irAE incidence and PFS with significant differences at baseline.

	Atezolizumab + CnP (NSCLC, *n* = 42) and atezolizumab + PP (NSCLC, *n* = 72)	Atezolizumab + bev + CP (NSCLC, *n* = 135)	Atezolizumab + CE (ES-SCLC, *n* = 100)
No. of proteins with significant differences between patients with vs. without irAEs	40	49	15
No. of proteins with significant differences between patients with vs. without irAEs and those with long or short PFS	8	5	2
Identified proteins	ICOSLG ENTPD5 CD5 TANK ENO2 S100P CD83 IL12	CD1C FASLG GHRL ADAM8 DSG4	HGF LRRN1

Abbreviations: Bev, bevacizumab; CE, carboplatin + etoposide; CnP, carboplatin + nab-paclitaxel; CP, carboplatin + paclitaxel; ES-SCLC, extensive-stage small cell lung cancer; irAE, immune-related adverse event; NSCLC, non-small cell lung cancer; PFS, progression-free survival; PP, carboplatin or cisplatin + pemetrexed.

the *EGFR*mt subset of this regimen, we observed a substantial increase in proteins associated with anti-tumor activation in the longer OS group, particularly after treatment. These findings suggest that the elevated signal in all-patient population could be due to the strong signal from the *EGFR*mt subset.

Additionally, this study evaluated biomarkers for older patients. At baseline, inflammatory proteins like IL-6, IL-8, and IL-10 were significant in older vs. younger patients who received atezolizumab + CnP. However, other regimens do not share this signature, and further investigation is necessary. This study also evaluated biomarkers associated with irAEs, which represent toxicities commonly seen with immunotherapies [39, 40]. Of the 15 proteins with significant differences between patients with or without irAEs, 14 were significantly associated with longer PFS in patients with irAEs and conversely shorter PFS in patients without irAEs when looking at *P*-values < 0.05 and excluding the log2 fold change. In the population that experienced irAEs, proteins related to immune-stimulation, like ICOSLG, CD5, CD83, IL-12, and FASLG, were present at baseline, suggesting that inflammatory status before treatment may indicate susceptibility to irAEs [41–47]. Collectively, these data warrant further investigation across cancer types and regimens to determine their relevance to efficacy and the occurrence of irAEs.

There were several limitations to this study. As J-TAIL-2 was a real-world observational study, there was no control group. Additionally, the exploratory approach of this study analyzed over 500 plasma proteins using nominal *P*-values (< 0.05) without adjustment for multiple comparisons. As a result, there is an increased risk of false-positive findings due to the potential for type I error. The decision not to apply multiplicity correction was based on the hypothesis-generating nature of the study and the limited sample size, which could reduce statistical power if stringent corrections were used. Therefore, the associations identified in this study should be interpreted with caution and considered preliminary until validated in larger, independent cohorts or through confirmatory analyses. As protein expression analysis is semi-quantitative, specific quantitative assays for selected candidate proteins, such as Multiplex ELISAs, would be necessary to confirm their presence and absolute concentration in plasma. Additional validation of candidate proteins in larger sample sizes and independent cohorts would be helpful to establish feasible systems for diagnosis.

This exploratory biomarker study conducted in Japan identified multiple potential biomarker candidates for predicting the efficacy and irAEs of atezolizumab combination therapies for patients with lung cancer. These findings may contribute to improving personalized medicine by refining patient selection and require further study.

Author Contributions

Yasushi Goto: visualization, conceptualization, methodology, formal analysis, investigation, funding acquisition, writing – original draft, writing – review and editing. **Makoto Nishio:** investigation, supervision, project administration, writing – review and editing. **Kadoaki Ohashi:** investigation, writing – review and editing. **Atsushi Osoegawa:** investigation, writing – review and editing. **Eiki Kikuchi:** investigation, writing – review and editing. **Hideharu Kimura:** investigation, supervision, writing – original draft, writing – review and editing. **Junichi Shimizu:** investigation, writing – review and editing. **Eisaku Miyauchi:** investigation, writing – review and editing. **Hiroshige Yoshioka:** investigation, writing – review and editing. **Ichiro Yoshino:** investigation, writing – review and editing. **Toshihiro Misumi:** formal analysis, investigation, writing – review and editing. **Yasutaka Watanabe:** investigation, writing – review and editing. **Nobuyuki Katakami:** investigation, writing – review and editing. **Akira Kisohara:** investigation,

writing – review and editing. **Masafumi Yamaguchi:** investigation, writing – review and editing. **Hirotaka Kuroki:** writing – review and editing. **Masamichi Sugimoto:** writing – original draft, writing – review and editing. **Hisao Ashimura:** writing – original draft, writing – review and editing. **Misa Tanaka:** writing – original draft, writing – review and editing. **Akihiko Gemma:** project administration, supervision, writing – review and editing.

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Ethics Statement

Approval of the research protocol by an Institutional Reviewer Board: J-TAIL-2 was conducted in accordance with the Declaration of Helsinki, the Act on the Protection of Personal Information, and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. The Ethics Review Committee of each study site approved the study protocol and the informed consent form prior to study initiation at the site.

Consent

Enrolled patients received a full explanation of the clinical research and provided written consent to participate.

Conflicts of Interest

All authors declare editorial support from Chugai Pharmaceutical Co. Ltd. All authors declare additional conflicts below. Y.G., is an editorial board member of *Cancer Science*; declares grants (to the institution) from AbbVie, Bristol Myers Squibb, Daiichi Sankyo, Eli Lilly, Kyowa Kirin, Novartis, Ono Pharmaceutical, Pfizer, and Preferred Networks; honoraria from Boehringer Ingelheim, Bristol Myers Squibb, Chugai Pharmaceutical Co. Ltd. Eli Lilly, Merck, MSD, Novartis, Ono Pharmaceutical, Pfizer, Taiho Pharmaceutical, and Thermo Fisher Scientific; advisory board participation for AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Chugai Pharmaceutical Co. Ltd. Daiichi Sankyo, Eli Lilly, Guardant Health Inc., Illumina, MSD, Novartis, Ono Pharmaceutical, Pfizer, and Taiho Pharmaceutical; and leadership or fiduciary roles in Cancer Net Japan and JAMT. M.N., has received payment or honoraria for lectures from AbbVie, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Chugai Pharmaceutical Co. Ltd. Daiichi Sankyo, Janssen, Lilly, Nippon Kayaku, Merck, MSD, Novartis, Ono Pharmaceuticals, Pfizer, Taiho Pharmaceutical, and Takeda. K.O., has received grants or contracts and honoraria from Chugai Pharmaceutical Co. Ltd. A.O., has received grants from Chugai Pharmaceutical Co. Ltd. and Taiho Pharmaceutical; and has received honoraria from Chugai Pharmaceutical Co. Ltd. AstraZeneca, Ono Pharmaceutical, MSD, and Bristol Myers Squibb. E.K., declares honoraria from Chugai Pharmaceutical Co. Ltd. H. Kimura, has received honoraria from Chugai Pharmaceutical Co. Ltd. J.S., has received honoraria for speakers bureaus from Taiho Pharmaceutical, Takeda, Chugai Pharmaceutical Co. Ltd. MSD, AstraZeneca, Novartis, Pfizer, and Amgen. E.M., has received grants from Chugai Pharmaceutical Co. Ltd. has received honoraria from Taiho Pharmaceutical, Daiichi Sankyo KK, Boehringer Ingelheim Japan, Bristol Myers Squibb, Novartis Pharma KK, MSD KK, Kyowa Kirin, Merck Biopharma, Pfizer, Ono Pharmaceutical, Chugai Pharmaceutical Co. Ltd. Amgen, Thermo Fisher Scientific K.K., Nippon Kayaku, Takeda, Eli Lilly Japan KK, Sysmex Co, and AstraZeneca K.K.; and has participated in advisory boards for Chugai Pharmaceutical Co. Ltd. Boehringer Ingelheim Japan, Eli Lilly Japan KK, Merck Biopharma, Daiichi Sankyo KK, and Ono Pharmaceutical. H.Y., received research funding from Daiichi Sankyo, AstraZeneca, Janssen Pharmaceutical, MSD, Novartis Pharma, Delta Fly Pharma, and Boehringer Ingelheim; consulting fees from Delta Fly Pharma; and lecture fees from Eli Lilly, Chugai Pharmaceutical Co. Ltd. MSD, AstraZeneca, Boehringer Ingelheim, Ono Pharmaceutical, Bristol Myers Squibb, Novartis Pharma, Kyowa Kirin, Nippon Kayaku, Otsuka Pharmaceutical, Amgen, Pfizer, Nipro Pharma, Daiichi Sankyo, and Merck Biopharma. I.Y., has received consulting fees from AstraZeneca, Chugai Pharmaceutical Co. Ltd. Medicaroid, Johnson & Johnson, and Covidien Japan; has received honoraria from Chugai Pharmaceutical Co. Ltd. and has received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from AstraZeneca, Johnson & Johnson, Covidien Japan, Daiichi Sankyo, Takeda, and MSD. T.M., has received payment or honoraria for lectures and speakers bureaus, or educational events from Chugai Pharmaceutical Co. Ltd. and has received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from AstraZeneca and Miyarisan. Y.W. declares speaker's fees from Chugai Pharmaceutical Co. Ltd. N.K., has received grants (Institution) and honoraria from Chugai Pharmaceutical Co. Ltd. A.K., declares grants and honoraria from Chugai Pharmaceutical Co. Ltd. M.Y. declares grants from Bristol Myers Squibb, Chugai Pharmaceutical Co. Ltd. Eli Lilly Japan, MSD, Nippon Kayaku, Taiho Pharmaceutical, and Takeda; and honoraria from AstraZeneca, Bristol Myers Squibb, Chugai Pharmaceutical Co. Ltd. Daiichi Sankyo, Eli Lilly Japan, Johnson & Johnson, MSD, Nippon Kayaku, Ono Pharmaceutical, Pfizer Japan, and Taiho Pharmaceutical. H. Kuroki, H.A, M.S., and M.T. are employees of and have received stock/stock options from Chugai Pharmaceutical Co. Ltd. A.G. declares study participation as an investigator for the J-TAIL-2 study; has received honoraria for educational lectures from Nihon Kayaku; and has participated on an ILD board for MSD, AstraZeneca, Daiichi Sankyo, and Chugai Pharmaceutical Co. Ltd.

Data Availability Statement

The data generated/analyzed during the current study are available from the corresponding author on reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Patient disposition. IMpower130 regimen: atezolizumab plus carboplatin and nab-paclitaxel; IMpower132 regimen: atezolizumab plus carboplatin or cisplatin plus pemetrexed; IMpower150 regimen: atezolizumab plus bevacizumab plus carboplatin and paclitaxel; IMpower133 regimen: atezolizumab plus carboplatin and etoposide. **Figure S2:** Temporal dynamics of IL-6, KRT-19, and MUC-16 protein expression at baseline and before the second dose of atezolizumab of PFS by regimen and Olink panel. (A) Atezolizumab + CnP (NSCLC, $n = 42$), (B) atezolizumab + PP (NSCLC, $n = 72$), (C) atezolizumab + CE (ES-SCLC, $n = 100$). CE, carboplatin + etoposide; CnP, carboplatin + nab-paclitaxel; CP, carboplatin + paclitaxel; ES-SCLC, extensive-stage small cell lung cancer; NSCLC, non-small cell lung cancer; PFS, progression-free survival; PP, carboplatin or cisplatin + pemetrexed. **Figure S3:** Baseline protein expression analysis of OS. (A) Atezolizumab + CnP (NSCLC, $n = 42$), (B) atezolizumab + bev + CP (NSCLC, $n = 135$), (C) atezolizumab + CE (ES-SCLC, $n = 100$). Bev, bevacizumab; CE, carboplatin + etoposide; CnP, carboplatin + nab-paclitaxel; CP, carboplatin + paclitaxel; ES-SCLC, extensive-stage small cell lung cancer; NSCLC, non-small cell lung cancer; OS, overall survival; PP, carboplatin or cisplatin + pemetrexed. **Figure S4:** Protein expression analysis of CR/PR vs PD before the second dose of atezolizumab. (A) Atezolizumab + CnP (NSCLC, $n = 42$), (B) atezolizumab + PP (NSCLC, $n = 72$), (C) atezolizumab + bev + CP (NSCLC, $n = 135$), (D) atezolizumab + CE (ES-SCLC, $n = 100$). Bev, bevacizumab; CE, carboplatin + etoposide; CnP, carboplatin + nab-paclitaxel; CP, carboplatin + paclitaxel; CR, complete response; ES-SCLC, extensive-stage small cell lung cancer; NSCLC, non-small cell lung cancer; PD, progressive disease; PP, carboplatin or cisplatin + pemetrexed; PR, partial response. **Figure S5:** Protein expression analysis of OS at baseline in older patients who received (A) atezolizumab + bev + CP (NSCLC, $n = 135$) and (B) atezolizumab + CE (ES-SCLC, $n = 100$). Bev, bevacizumab; CP, carboplatin + paclitaxel; ES-SCLC, extensive-stage small cell lung cancer; NSCLC, non-small cell lung cancer; OS, overall survival. **Figure S6:** Protein expression analysis of protein changes for patients with *EGFR*mt vs non-mutant who received atezolizumab + bev + CP (NSCLC, $n = 135$) between baseline and before the second dose of atezolizumab. Bev, bevacizumab; CP, carboplatin + paclitaxel; mt, mutant; NSCLC, non-small cell lung cancer. **Figure. S7.** Protein expression analysis of OS for patients with *EGFR*mt who received atezolizumab + bev + CP (NSCLC, $n = 135$) of protein changes between baseline and before the second dose of atezolizumab. Bev, bevacizumab; CP, carboplatin + paclitaxel; mt, mutant; NSCLC, non-small cell lung cancer; OS, overall survival.