

# Relationship Between Pulmonary Gas Transfer and Biomarker Levels in Patients with Autoimmune Pulmonary Alveolar Proteinosis (aPAP)

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## OBJECTIVE

To report serum biomarker levels in patients with aPAP from the IMPALA-2 Phase 3 clinical trial

## CONCLUSIONS

Levels of biomarkers associated with aPAP disease severity decreased in patients treated with molgramostim

Decreased levels of biomarkers were associated with improvements in pulmonary gas transfer

## Background

- Autoimmune pulmonary alveolar proteinosis (aPAP) is a rare lung disease caused by autoantibodies to granulocyte-macrophage colony-stimulating factor (GM-CSF)<sup>1</sup>
- aPAP is characterized by the accumulation of surfactant in the alveoli, leading to respiratory distress, hypoxemia, and increased infection risk<sup>2,3</sup>
- Biomarkers for the assessment of aPAP disease severity and therapeutic response are well-reported, and include serum cytokeratin 19 fragments (CYFRA21-1), carcinoembryonic antigen (CEA), Krebs von den Lungen-6 (KL-6), and lactate dehydrogenase (LDH)<sup>4-10</sup>
- Molgramostim inhalation solution (molgramostim), an investigational recombinant human GM-CSF, is being evaluated for the treatment of aPAP in a Phase 3 clinical trial (IMPALA-2)
- IMPALA-2 achieved statistical significance on its primary endpoint, change from baseline in hemoglobin-adjusted percent predicted diffusing capacity of the lungs for carbon monoxide (DLco%) at Week 24, and multiple secondary endpoints
- Here we report biomarker results from the IMPALA-2 clinical trial double-blind period

## Methods

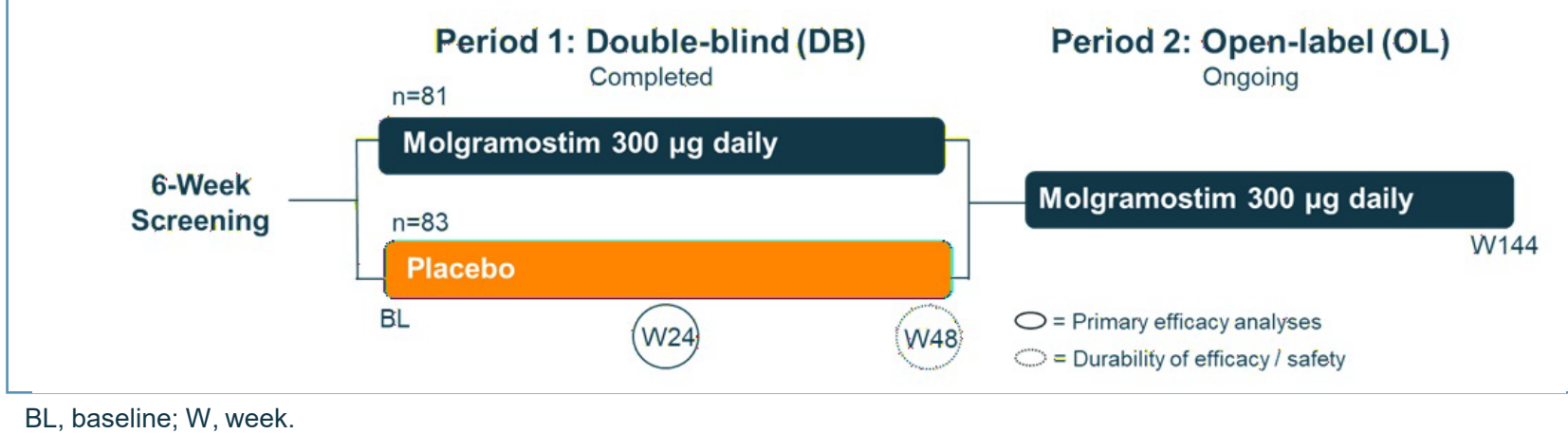
### Patients

- Patients were required to have:
  - A positive (abnormal) anti-GM-CSF autoantibody test result
  - DLco% ≤70% at the first screening and baseline visits
  - Change in DLco% of <15 percentage points during the screening period to ensure stability of impaired patients

### Study Design

- IMPALA-2 is a randomized, double-blind, placebo-controlled Phase 3 clinical trial being conducted at 43 clinical sites across 16 countries
- The trial consists of a 48-week DB period followed by a 96-week OL period (Figure 1)

Figure 1. Study Design



### Measurement of Serum Biomarkers

- Blood samples for assessment of the following aPAP-related biomarkers were taken at baseline and Weeks 4, 12, 24, and 48: KL-6, CYFRA21-1, CEA, LDH, hemoglobin (Hb), and hematocrit (Hct)
- Analysis of KL-6, CYFRA21-1, and CEA was performed by a central laboratory using validated methods
- LDH was analyzed from the standard biochemistry safety laboratory sample, and Hb and Hct from the standard hematology safety laboratory sample

### Post-Hoc Statistical Analyses

- Changes from baseline in biomarker levels over the double-blind period were analyzed using a general linear mixed model for repeated measures adjusted for baseline biomarker as a continuous covariate and region, DLco% severity at randomization (≤50%, >50%), treatment, visit, and treatment by visit interaction as factors
- Spearman's rank correlation coefficients (r) were calculated to determine the relationship between change from baseline in DLco% and each biomarker

## Results

### Patients

- A total of 164 patients with autoimmune PAP underwent randomization; 81 patients were assigned to receive molgramostim and 83 to receive placebo
- At baseline, biomarker levels were similar between treatment groups (Table 1)

Table 1. Baseline biomarker levels

Biomarker	Molgramostim n=81	Placebo n=83	Total N=164
<b>LDH (U/L)</b>			
n	81	83	164
Mean (SD)	281.9 (79.7)	289.4 (103.0)	285.7 (92.0)
Median (min, max)	270.0 (137, 646)	267.0 (167, 893)	267.5 (137, 893)
<b>CYFRA21-1 (ng/mL)</b>			
n	79	76	155
Mean (SD)	10.2 (6.8)	9.5 (7.3)	9.8 (7.0)
Median (min, max)	8.7 (2.1, 36.2)	8.0 (0.8, 47.7)	8.5 (0.8, 47.7)
<b>KL-6 (U/mL)</b>			
n	79	80	159
Mean (SD)	4946.5 (3105.4)	5129.3 (2859.9)	5038.5 (2976.4)
Median (min, max)	4334.0 (143, 10,000)	5270.5 (575, 10,000)	4569.0 (143, 10,000)
<b>CEA (ng/mL)</b>			
n	78	79	157
Mean (SD)	7.6 (5.6)	8.8 (7.1)	8.2 (6.4)
Median (min, max)	6.3 (1.8, 28.3)	7.1 (1.8, 37.2)	6.4 (1.8, 37.2)
<b>Hb (g/L)</b>			
n	81	83	164
Mean (SD)	148.2 (21.9)	152.5 (20.7)	150.4 (21.4)
Median (min, max)	149.0 (88, 197)	155.0 (83, 207)	152.5 (83, 207)
<b>Hct (%)</b>			
n	81	83	164
Mean (SD)	46.6 (5.7)	47.5 (5.4)	47.0 (5.6)
Median (min, max)	46.0 (31, 59)	47.0 (33, 60)	47.0 (31, 60)

CEA = carcinoembryonic antigen; CYFRA21-1 = cytokeratin 19 fragments; Hb, hemoglobin; Hct, hematocrit; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; Max, maximum; Min, minimum; n, number of patients; SD, standard deviation.

### Serum Biomarker Levels During the Double-Blind Treatment Period

- Changes from baseline in levels of CYFRA21-1, KL-6, CEA, and LDH in the molgramostim and the placebo groups over the 48-week DB treatment period are shown in Figure 2
- Least-squares mean (LSM) CYFRA21-1, KL-6, and LDH levels decreased significantly more in the molgramostim group than in the placebo group at Weeks 24 and 48; there were no significant differences in levels of CEA (Figure 2)
- LSM changes from baseline in Hb and Hct were similar between the treatment groups (data not shown)

### Relationship Between Serum Biomarkers and Pulmonary Gas Transfer

- Figure 3 shows the relationship between change from baseline in DLco% and changes from baseline in CYFRA21-1, KL-6, CEA, and LDH biomarker levels at Week 24 and at Week 48; correlation coefficients are for all patients (pooled data from molgramostim and placebo groups)
  - Interpretation of strength of correlations: <0.3 is weak, 0.3-0.5 is moderate, and ≥0.5 is strong<sup>11</sup>
- Strong negative correlations (increase in DLco% was associated with decreases in biomarker levels) were observed between DLco% and KL-6, DLco% and CYFRA21-1, DLco% and CEA, and DLco% and LDH (Figure 3)
- Moderate negative correlations were observed between DLco% and Hct (Table 2)
- Weak negative correlations were observed between DLco% and Hb (Table 2)

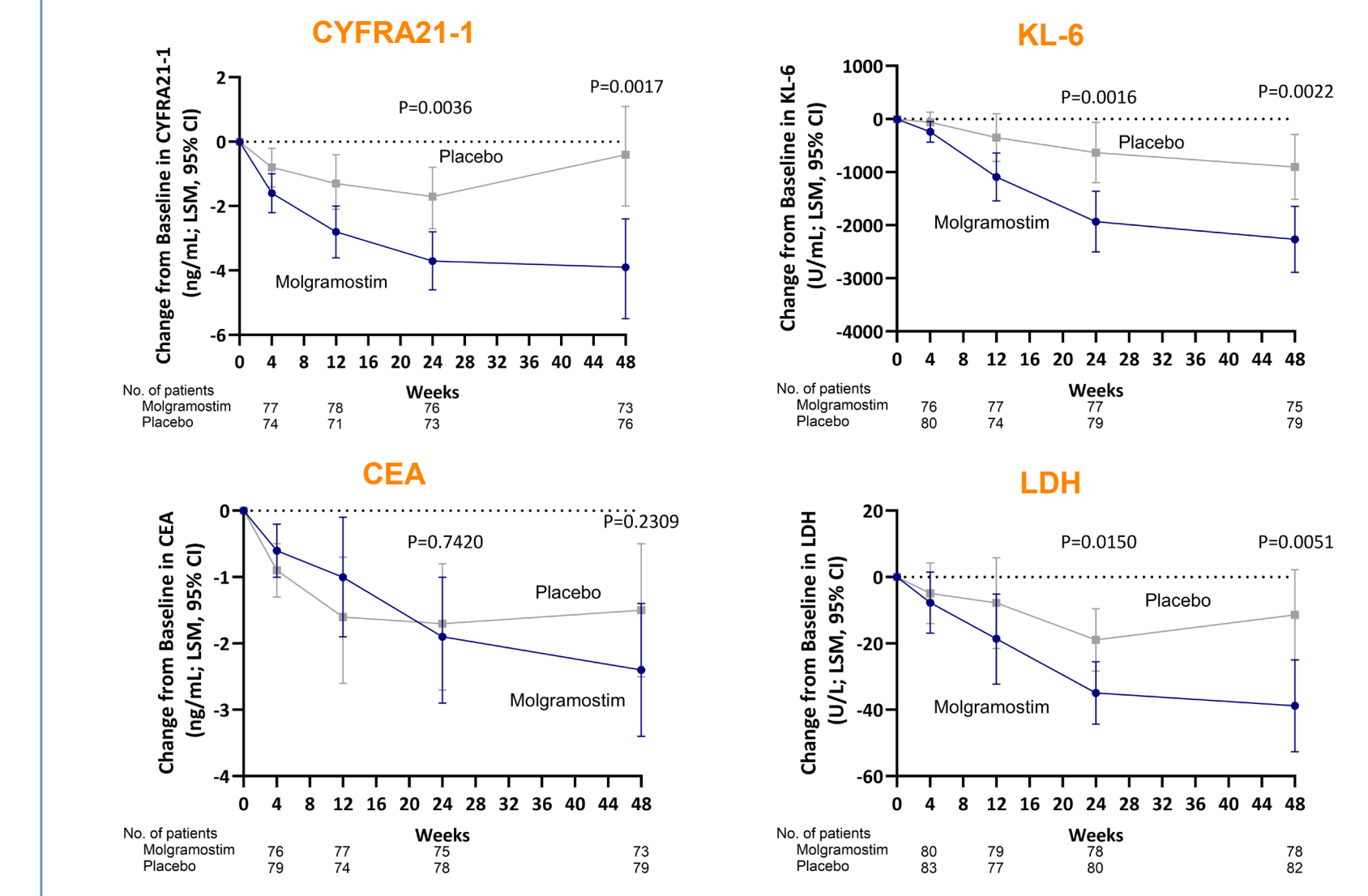
Table 2. Correlations\* between change in DLco% and biomarkers

Time point	Hct r (P-value) n	Hb r (P-value) n
<b>Week 24</b>	-0.32 (P<0.0001); 152	-0.27 (P=0.0009); 153
<b>Week 48</b>	-0.32 (P<0.0001); 148	-0.25 (P=0.0019); 150

\*Correlation coefficients are for all patients (pooled data from molgramostim and placebo groups) Hct, hematocrit; Hb, hemoglobin; n, number of patients; r, Spearman's Rank correlation coefficient.

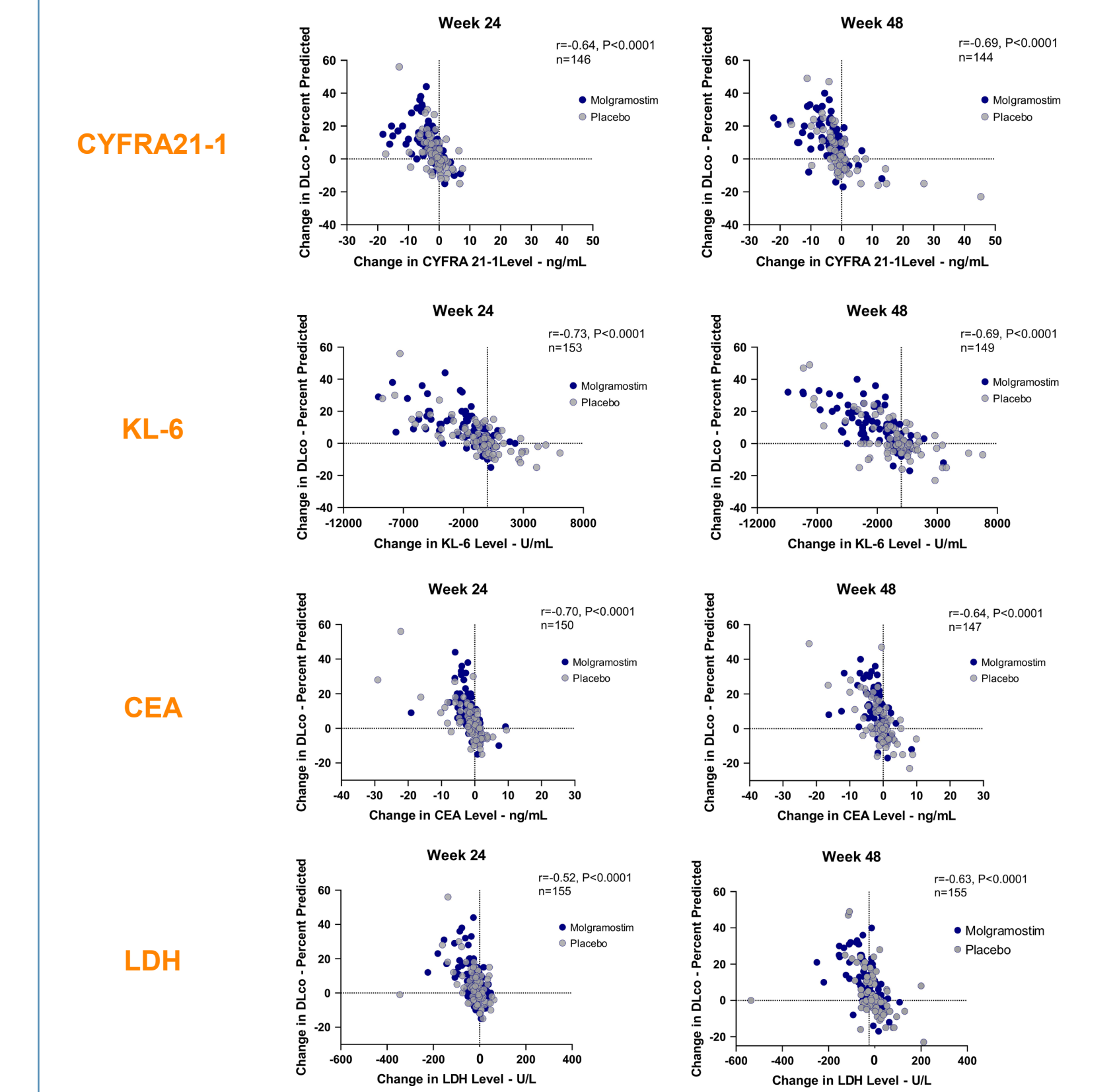
## Results

Figure 2. LSM Change From Baseline in Biomarker Levels Over Time



CEA, carcinoembryonic antigen; CI, confidence interval; CYFRA21-1, cytokeratin 19 fragments; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; LSM, least-squares mean; No., number.

Figure 3. Correlations Between Change from Baseline in DLco% and Biomarker Levels



CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin 19 fragments; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; r, Spearman's Rank correlation coefficient.

### DISCLOSURES

- IMPALA-2 is sponsored by Savara Inc.
- YI has received financial compensation from Savara Inc. for consultant services.

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