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Effects of inhaled human insulin on airway lining fluid composition in adults with diabetes

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ABSTRACT:

Inhaled human insulin (Exubera® (human insulin [rDNA origin]) Inhalation Powder) causes small, early and reversible changes in pulmonary function in subjects with diabetes mellitus. These studies assessed whether changes occur in cellular and soluble constituents in airway lining fluid consistent with inflammation as a possible cause for Exubera-associated lung function alterations.

Two 31-week, open-label, sequential-design phase 2 studies were conducted, one with 20 subjects with type 1 and one with 24 subjects with type 2 diabetes. After run-in, all subjects received subcutaneous insulin for 12 weeks, followed after 1 week by 12 weeks of Exubera. Bronchoalveolar lavage fluid cell counts and protein constituents were determined at baseline, after 12 weeks of subcutaneous insulin and after 12 weeks of Exubera.

Baseline cellular and soluble constituents of lavage fluid were similar to those reported for non-diabetic adults. Exubera produced no consistent clinically or statistically significant changes in total or differential lavage fluid cell counts or protein concentrations, even though Exubera-associated changes in pulmonary function are known to be fully manifest within 12 weeks.

Thus, 12 weeks of Exubera are not associated with evidence of pulmonary inflammation. The treatment effects on lung function observed in Exubera trials are not caused by lung inflammation.

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KEYWORDS: Bronchoalveolar lavage, bronchoscopy, Exubera, glycemic control, pulmonary function tests

INTRODUCTION

Inhaled human insulin (EXU; Exubera® human insulin [rDNA origin]) Inhalation Powder) was approved for use in adult patients with type 1 or 2 diabetes mellitus in the U.S. and European Union in January 2006.* Clinical trials with EXU have shown similar efficacy and tolerability to subcutaneous (SC) insulin in subjects with diabetes [1-4]. EXU is a premeal insulin with a time-action profile closer to meals than subcutaneous (SC) regular insulin [5].

Clinical trials have also revealed that subjects treated with EXU have a small decline in some pulmonary function measurements relative to those treated with SC insulin [1-4, 6, 7]. These treatment group differences in forced expiratory volume in one second (FEV_1) and carbon monoxide diffusing capacity (DL_{CO}) were observed within 1 to 3 weeks of initiation of therapy, were fully manifest within 12 weeks of therapy, were non-progressive, and reversed on discontinuation of EXU [3, 4, 6, 7]. A similar magnitude of change in FEV_1 and DL_{CO} has been reported for a different inhaled insulin product [8].

The etiology of this decline is presently unclear, although indirect evidence suggests that it is unlikely to be secondary to lung inflammation. Extensive non-invasive evaluation, including high-resolution computed tomography, has failed to demonstrate any associated radiographic abnormalities of the lung parenchyma. Furthermore, the time course, pattern, and prompt reversibility of the Exubera-associated changes in pulmonary function are distinct from the known time course and pattern of FEV_1 changes produced from stimuli associated with chronic

* On October 18, 2007, Pfizer Inc announced that it was returning the worldwide rights for Exubera to Nektar, the company from which it licensed the inhaled insulin technology.

inflammation; however, no studies have directly examined whether the EXU-associated changes in lung function are caused by lung inflammation.

Bronchoalveolar lavage (BAL) is a well-tolerated, minimally invasive technique that samples cells and solutes from the lower respiratory tract and is used as a standard diagnostic procedure in pulmonary medicine [9, 10]. BAL fluid assessments provide a direct indication of the cellular response to inflammatory stimuli within the lung. Changes in absolute cell counts within BAL fluid, shifts in leukocyte differential (percentage of lymphocytes, neutrophils, eosinophils, or macrophages) in BAL fluid, or changes in BAL protein composition can reveal subtle inflammatory reactions in the lungs.

We conducted two studies (one in subjects with type 1 and one in subjects with type 2 diabetes) with the primary objective to assess the effects of EXU on airway lining fluid composition in comparison with SC insulin, and to secondarily assess routine efficacy and safety parameters during 12 weeks of short-acting SC insulin, followed by 12 weeks of EXU treatment. BAL was performed by a standardised protocol in all patients. Since the onset of the lung function alterations associated with EXU therapy occurs within 1 to 3 weeks of initiation of therapy and the changes are fully evident within 12 weeks of therapy [6], the 12-week treatment duration in these studies is an optimal time interval to assess whether or not inflammation plays a role in mediating these changes.

METHODS

Patients

For inclusion in the study, subjects aged 18 to 55 years with type 1 diabetes or aged 30 to 55 years with type 2 diabetes had to have normal lung function defined as DL_{CO} <120% and >80% of predicted; total lung capacity <120% and >80% of predicted; forced vital capacity (FVC) and FEV_1 >80% of predicted; FEV_1/FVC >70%. They also had to have glycosylated hemoglobin (HbA_{1c}) between 5.5 and 11.0%, fasting insulin C-peptide <0.2 pmol/ml with type 1 diabetes and >0.2 pmol/ml with type 2 diabetes, and body mass index ≤30 kg/m^2 with type 1 diabetes and ≤35 kg/m^2 with type 2 diabetes. Subjects needed to have been on a stable insulin regimen for 2 months prior to screening. Those with a history of smoking (>5 pack-yrs), atopy or pulmonary disease, brittle diabetes, or a predisposition to severe hypoglycaemia were excluded. All subjects with type 2 diabetes were SC insulin-using at study entry.

Study design

Two 31-week, non-randomised, comparator-controlled, open-label, sequential-design, multicentre phase 2 studies were conducted, one in subjects with type 1 and one in subjects with type 2 diabetes (fig. 1). Following screening, subjects began a 4-week run-in period during which diabetes control was optimised by SC insulin therapy consisting of two to three pre-meal daily doses of short-acting insulin plus administration of intermediate/long-acting insulin [6]. During the study, subjects received the SC insulin regimen for 13 weeks, followed by pre-meal EXU plus intermediate/long-acting SC insulin for 12 weeks, followed by a 2-week wash-out period. Patient consent and site information are provided in the online data supplement.

Measurements

Fibreoptic bronchoscopy was performed at baseline (Week 0), after 12 weeks of SC therapy (Week 12), and following 12 weeks of EXU (Week 25) using a standardised protocol. BAL was performed on subjects who had fasted overnight and prior to the first daily dose of short-acting insulin using standard bronchoscopic techniques via a transoral approach. Visual inspection of the trachea and bronchi bilaterally was performed prior to lavage, and the mucosal surface was graded using the Bronchitis Index scale [11]. BAL was performed in one non-dependent segment (anterior segment of right upper lobe, right middle lobe, left lingula) by instillation of five lavages of 20 ml normal saline. Lavages were collected with low suction and pooled. If the total lavage fluid collected after all five aliquots was <50 mL, a second site in the same lung was lavaged, and the fluid combined. Bronchoscopists were instructed to use the same segment for each subsequent BAL procedure. BAL was postponed if the subject had an intercurrent respiratory tract illness at that time.

Initial processing of the BAL fluid was conducted at the local sites. Total cell counts were performed using cell preparations of BAL fluid diluted 1:1 with trypan blue on a hemacytometer. Cytoprep slides were stained with the leukostat stain kit (Fisher Scientific, Pittsburgh, PA, USA). Differential cell counts were performed at a central laboratory (Mark Liu, MD, Baltimore, MD, USA) as previously described [12]. BAL fluid albumin, fibrinogen, and total protein assays were conducted at IBT Reference Laboratories (Lenexa, KS, USA).

Pulmonary function tests (PFTs; spirometry, lung volumes by helium dilution, and DL_{CO}) were conducted at screening (Week -4) and at Weeks -2, -1, 0, 11, 24, and 27 using standardised methodology [6,7,13]. HbA_{1c} and fasting plasma glucose (FPG) levels were collected at screening, baseline (Week 0), and at Weeks 11 and 24. General safety monitoring, including adverse events (AEs) and hypoglycemic events, was conducted throughout the trial.

Statistical methods

The primary endpoints in these studies were lung lining fluid cell count and differentials within subjects after 12 weeks of EXU therapy compared with 12 weeks of SC ((EXU-SC)-(SC-Baseline)). For the BAL fluid parameters of total cell count and differential cell counts, a linear statistical model that included centre, gender, atopic disease status, and smoking history (never versus ex-smoker) was used to calculate adjusted least squares means for the change from baseline (Week 0) to Week 12 (SC), the change from Week 12 to Week 25 (EXU), and the difference between the changes for EXU and SC, along with its 95% confidence interval.

The primary analysis set consists of all patients who had cell differentials from all three BAL fluid samples. The full analysis set consists of all patients who received any study treatment and had at least one BAL cell differential. Although the sample size of 20 patients was determined from practical considerations, this sample size was estimated to provide a 95% confidence interval for the estimate of the mean within subject difference between treatment arms that is ±3.9% for lymphocytes and ±1.0% for neutrophils. This estimate was based on data derived from normal (non-diabetic), non-smoking population [1]. For additional statistical methods, see online data supplement.

RESULTS

Twenty-four patients with type 1 diabetes and 26 patients with type 2 diabetes were treated during the SC insulin phase. Twenty-one patients with type 1 and 24 patients with type 2 diabetes were subsequently treated during the EXU phase (table 1).

Twenty patients with type 1 diabetes and 24 patients with type 2 diabetes completed all three bronchoscopy procedures and are included in the primary analysis set. The patient demographics at screening are shown in table 2. Discontinuations are described in the *Adverse events* section in the online supplement.

BAL total cell counts, leukocyte differential, and protein concentrations

Changes in total leukocyte count and BAL fluid leukocyte differential were small after 12 weeks of SC insulin or 12 weeks of EXU and showed no apparent trends favouring either treatment group among subjects with type 1 (fig. 2A) or type 2 (fig. 2B) diabetes. In patients with type 1 diabetes, analysis of the changes in total cell count and leukocyte differential during the SC insulin and EXU treatment periods showed no statistically significant or clinically meaningful differences between the SC insulin and EXU treatment periods (table 3). In patients with type 2 diabetes, analysis of the changes in total cell count, lymphocytes, or eosinophils during treatment periods showed no statistically significant or clinically meaningful differences between the SC insulin and EXU treatment periods (table 3); however, for macrophages and neutrophils, there was a small, but statistically significant, treatment group difference with an increase in percentage of neutrophils associated with SC insulin administration and an increase in percentage of macrophages associated with EXU therapy (table 3). These differences appear to be due to changes from Week 0 to Week 12 (SC insulin treatment period) and not from changes between Weeks 12 and 25 (EXU), and result from a small increase in neutrophils (percentage of total cells) that occurred between Weeks 0 and 12. The mean observed values for both parameters returned to near baseline mean values by Week 25. Among patients with type 1 or type 2 diabetes, there was no change in BAL fluid albumin, fibrinogen, or

total protein concentrations associated with either SC insulin or EXU treatment (figs. 3A and 3B).

Pulmonary function

Small decreases in FEV_1 and DL_{CO} were observed in patients with either type 1 or type 2 diabetes during the EXU treatment period (Table 4). Patients with type 1 diabetes experienced a mean decline in FEV_1 from Week 11 to Week 24 (EXU treatment period) of 0.067 L and a mean decline in DL_{CO} from Week 11 to Week 24 of 0.699 ml/min/mmHg. Patients with type 2 diabetes exhibited a decline in FEV_1 from Week 11 to Week 24 0.027 L, and a decline in DL_{CO} of 0.902 ml/min/mmHg during the same EXU treatment period. These changes are entirely consistent with previous, larger EXU studies, which more fully characterized Exubera-associated alterations in lung function (1-4, 6, 7). In the larger pulmonary safety studies the mean Exubera-associated declines in FEV_1 were approximately 30-40 mL and in DL_{CO} were approximately 0.4-0.6 mL/min/mmHg. The less consistent changes observed in FEV_1 and DL_{CO} during the SC treatment and 2-week follow-up phases are likely secondary to the expected variability associated with these measurements in a relatively small group of patients.

To assess whether the changes in lung function were associated with changes in total cell counts and leukocyte counts, scatter plots were created correlating the change in FEV_1 with the changes in total cell counts and leukocyte subset counts during the EXU treatment phase. There were no consistent correlations observed between the change in FEV_1 and changes in total cell counts and leukocyte subset counts in the BAL fluid (fig. 4) in patients with either type 1 or 2 diabetes mellitus,

These data provide further evidence that inflammation is not mediating the changes in lung function observed during EXU therapy.

Airway evaluations

Bronchoscopy airway evaluation total scores revealed near normal values in subjects with type 1 or type 2 diabetes (see table 1 in the online supplement). The mean total score at baseline was 1.30 in patients with type 1 diabetes and 2.04 in patients with type 2 diabetes. No significant changes were observed at Week 12 or Week 25 in either patient population, indicating there was no change following SC insulin or EXU therapy. The lack of changes seen in the airway evaluation is consistent with the lack of changes observed in BAL total cell counts, leukocytes differentials, and protein composition.

Efficacy

HbA_{1c} and FPG measurements taken at the end of each treatment period showed ongoing maintenance of glycaemic control (see table 2 in the online supplement).

DISCUSSION

These are the first studies that directly assessed whether a potential inflammatory response could play a role in mediating the small, early, non-progressive, and reversible changes lung function observed during EXU therapy [1-4, 6, 13]. Since the onset of these EXU-associated changes in lung function occurs within one to three weeks of initiation of therapy and the changes are fully manifest within 12 weeks of therapy, the 12-week EXU treatment duration in these studies is the optimal time

period to assess the potential cause of these effects. The data presented clearly show no measurable inflammatory response within the lung during EXU therapy, indicating that inflammation is not playing a role in the EXU-associated changes in lung function.

The BAL fluid total cell count and leukocyte differentials from the diabetic populations in these studies fell within the range found in normal, non-smoking individuals [9, 14-18] for all treatment periods. This indicates the presence of diabetes itself does not alter the cellular composition of the lung lining fluid in non-smoking patients without lung disease.

There was no consistent change in BAL fluid total cell counts, leukocyte differentials, or protein composition following 12 weeks of EXU therapy in patients with type 1 or type 2 diabetes. In contrast, active cigarette smokers exhibit a 2.5- to 7-fold increase in BAL fluid total cell counts that is secondary to a marked influx of macrophages [9, 14, 19]. Subjects with asthma display an increase in eosinophils or neutrophils [20, 21]. In allergic subjects with asthma, segmental allergen challenge causes an 8.6-fold increase in BAL fluid total counts, an 8.4-fold increase in fibrinogen concentration, and eosinophils increase from approximately 0.0×10^6 cells/ml to 0.7×10^6 cells/ml [22]. This increase in eosinophils is markedly greater than the number of eosinophils observed at any time point during the current study.

Significant alterations in BAL fluid are also observed in patients with interstitial lung processes and following inhalation of ozone and diesel exhaust. Cell counts rose 52% in subjects with idiopathic pulmonary fibrosis and 91% in subjects with interstitial lung disease secondary to connective tissue disorders [14]. Subjects with acute lung

injury and acute respiratory distress syndrome exhibited an 8-fold increase in BAL fluid total cell counts, indicative of a brisk neutrophil influx within the lung, and a 35-fold increase in BAL fluid total protein concentration, indicating a marked increase in alveolar permeability [23]. Smaller, but significant, increases in BAL fluid neutrophils, total protein, and fibrinogen occur 1 to 24 hours following inhalation of ozone [24-27]. Inhalation of diesel exhaust results in significant increases in bronchial wash neutrophils and BAL fluid lymphocytes [28]. Importantly, the current studies show no neutrophil infiltration as a result of EXU therapy, indicating the absence of an acute inflammatory response.

Semi-quantitative grading of the airway mucosal surface using the Bronchitis Index Scale [13] failed to reveal any clinically significant changes during EXU therapy. These findings are consistent with the lack of alterations in BAL fluid total cell counts, leukocyte differentials, and protein composition. They are also in contrast to the airway evaluation scores of 8.5 in asymptomatic smokers and 13.2 in patients with chronic bronchitis compared with 2.3 in normal subjects [11].

The exact mechanism mediating the EXU-associated changes in FEV_1 and DL_{CO} is currently unknown; however, the lack of acute changes in FEV_1 10 and 60 mins after EXU dosing in patients with type 1 diabetes suggests that acute smooth muscle contraction with resulting bronchoconstriction is not the mechanism mediating these changes [6]. Furthermore, data reported here provide direct evidence that inflammation also is not the causative mechanism. One hypothesis is that the repeated osmotic load of the pre-meal dry powder EXU formulation could result in physiologic adaptation and in subtle physiologic fluid shifts within the airways and

alveoli, giving rise to the observed small, non-progressive, and reversible changes in lung function.

Since EXU therapy is associated with small changes in lung function, EXU should not be used in patients with lung disease such as asthma or chronic obstructive pulmonary disease (COPD), and EXU is contraindicated in patients with severe (GOLD stage III or IV) COPD or poorly controlled, unstable or severe asthma [29]. Ongoing studies will assess the safety and efficacy of EXU therapy in patients with mild-to-moderate asthma or COPD.

It is important to note that the findings and interpretation of these studies are constrained to the actual formulation of the EXU powder. The EXU formulation consists of human insulin in a homogeneous powder formulation containing sodium citrate (dihydrate), mannitol, glycine, and sodium hydroxide. Other inhaled insulin formulations containing alternative excipients may engender a different response within the lung.

One potential limitation to the interpretation of these studies is the open-label design. All phase 2/3 studies in the EXU development program were open-label design. Subjects with diabetes mellitus are required to carefully titrate their premeal insulin doses (both SC and EXU) based on premeal blood glucose levels, meal size and expected activity levels to adequately control blood glucose while avoiding hypoglycemia. Based on these considerations, an open-label design was deemed necessary to assure patient safety. This may result in bias to some study endpoints especially concerning patient and physician-reported adverse events, although bias in BAL fluid total cell count and differentials are less likely to occur.

A second potential limitation to these studies is the duration of the treatments, particularly in regards to longer-term EXU effects. Prior studies show that the EXU-associated changes in pulmonary function occur as early as 1-2 weeks following initiation of therapy and is fully manifest by the 3-month time point (3, 4, 6). The primary goal of these studies was to evaluate whether changes in BAL fluid cell count and differentials consistent with inflammation occur within this same time period as the changes in PFTs. Therefore, 12 weeks was chosen as the optimal treatment duration to detect these changes. This duration of treatment may not be sufficient to detect potential longer-term EXU effects. One theoretical effect of inhaled insulin would be its mitogenic potential via interaction with the insulin-like growth factor-1 (IGF-1) receptor, although native insulin is a relatively weak ligand for this receptor, requiring 50 to 100 times the concentration of IGF-1 to have the same effect (30). Longer-term studies are needed to address these concerns.

In summary, treatment of type 1 or type 2 diabetes with EXU does not appear to have any effect on lung lining composition as assessed by visual inspection, BAL fluid total cell counts, and leukocyte cell differentials (lymphocytes, neutrophils, eosinophils, or macrophages). Similarly, no effect on protein composition of lung lining fluid was apparent as assessed by BAL fluid total protein, albumin, and fibrinogen concentrations. Based on these studies, there is no evidence that 12 weeks of treatment with EXU causes clinically meaningful cellular changes within the lung that would indicate inflammation or other clinically important lung processes. These results indicate that pulmonary inflammation is not driving the small, non-progressive, reversible treatment effect of EXU on pulmonary function that has been consistently observed in randomised controlled studies.

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Figure 1. Study Design. SC = subcutaneous insulin; BAL: bronchoalveolar lavage; PFTs: pulmonary function tests; LSV: last subject visit.

Figure 2. Box plot showing BAL fluid cell counts and leukocyte cell differentials at baseline (BSL) and following 12 weeks of treatment with either SC insulin or EXU in subjects with type 1 *(A)* or type 2 *(B)* diabetes. The box plot represents the median (centre horizontal line), mean (+), and the 25th and 75th percentiles (bottom and top edges of the box). Whiskers extend from the box to the farthest point within 1.5 times the interquartile range (75th percentile − 25th percentile); values beyond those points are represented by an X. *Statistically significant treatment group difference with an increase in percentage of neutrophils associated with SC administration and an increase in percentage of macrophages associated with EXU therapy (see table 3B).

Figure 3. Box plot showing the concentration of BAL fluid proteins at baseline (BSL) and following 12 weeks of treatment with either SC insulin or EXU in subjects with type 1 (*A*) or type 2 diabetes (*B*). The box plot represents the median (centre horizontal line), mean (+), and the 25th and 75th percentiles (bottom and top edges of the box). Whiskers extend from the box to the farthest point within 1.5 times the interquartile range (75th percentile−25th percentile); values beyond those points are represented by an X. The units for albumin and total protein are μg/ml (left y-axis) and the units for fibrinogen are ng/ml (right y-axis).

Figure 4. Scatter plots correlating the change in FEV_1 with changes in total cell counts (A, F) and leukocyte subset counts (B-E, G-J) during the EXU treatment phase in patients with either type 1 (A-E) or type 2 (F-J) diabetes mellitus.

TABLE 1. Disposition of patients with type 1 or type 2 diabetes

	Type 1 Diabetes		Type 2 Diabetes	
	SC Insulin	**EXU**	**SC Insulin**	**EXU**
Number screened	42		48	
Assigned to treatment				
Treated	24	21	26	24
Completed	21	20	24	24
Discontinued	3	1*	2	0
Analysed for BAL (Primary analysis set)	20	20	24	24
Analysed for safety (Adverse events)	24	21	26	24
Discontinuations				
Not related to study drug	3	1	2	0
Other	2	0	1	0
Subject defaulted	1	1	1	0
Total	3	1	2	0
Study visit completion				
Baseline (Week 0)	23		25	
SC insulin treatment (Week 12)	22		24	
EXU insulin treatment (Week 25)	21		24	

SC: subcutaneous insulin; EXU: Exubera.

*Patient completed the Week 25 visit and was lost to follow-up in the 2-week run-out phase.

TABLE 2. Patient demographics at screening: full analysis set

	Type 1 Diabetes N = 24	**Type 2 Diabetes N = 26**
Male n, %	17 (71)	21 (81)
Female n, %	7 (29)	5 (19)
Age, y, mean (range)	37.9 (18–58)	45.1 (21–59)
Body mass index, kg/m^2, mean (range)	26.4 (22.3–32.3)	30.2 (24.3–37.0)
Diagnosis, y, mean (range)	18.2 (2.9–46.2)	9.7 (1.4–35.1)
HbA_{1c},%, mean (± SD)	7.8 (±1.2)	8.2 (±1.2)
C-peptide, pmol/ml, mean (± SD)	0.166 (±0.001)	0.528 (±0.334)

TABLE 3. Statistical analysis for primary endpoints*: type 1 and 2 diabetes, primary analysis set

	Type 1 Diabetes Mellitus			Type 2 Diabetes Mellitus		
		95% CI on Within-Subject Difference			95% CI on Within-Subject Difference	
	Within-Subject Difference[#]	Lower	Upper	Within-Subject Difference[#]	Lower	Upper
Total cell count, 10^6cells per ml	0.041 (0.029)	–0.022	0.104	0.039 (0.033)	–0.030	0.108
Lymphocytes, % of total cells	0.150 (2.167)	–4.498	4.798	–2.475 (1.935)	–6.510	1.560
Neutrophils, % of total cells	–2.070 (1.449)	–5.178	1.038	–1.788 (0.834)	–3.527	–0.048[§]
Macrophages, % of total cells	1.840 (3.095)	–4.798	8.478	5.125 (2.113)	0.718	9.532[§]
Eosinophils, % of total cells	–[+]	–[+]	–[+]	–0.846 (0.606)	–2.109	0.417

SC: subcutaneous insulin; EXU: Exubera; CI: confidence interval.

*Analysis of variance with week and subject as fixed effects and an unstructured within-subject covariance matrix and the following covariates: centre, gender, atopic disease status, and smoking history (never versus ever smoked).

[#]Adjusted mean difference (SE) between successive differences: (EXU – SC), (SC – Baseline). These differences are obtained through contrasts on the weekly values.

[+]Statistical analysis of eosinophils (percentage of total cells) was not possible because the data consisted mainly of values of 0.0.

[§]Statistically significant treatment group difference with an increase in percentage of neutrophils associated with SC administration and an increase in percentage of macrophages associated with EXU therapy

Table 4. FEV_1 and DLco Measurements

	Type 1 Diabetes		Type 2 Diabetes	
	N	Mean (SD)	N	Mean (SD)
FEV_1 (L)				
Baseline	20	3.65 (0.79)	24	3.49 (0.59)
SC	20	3.66 (0.78)	23	3.63 (0.52)
Δ from Baseline	20	0.011 (0.16)	23	-0.061 (0.16)
EXU	20	3.60 (0.77)	21	3.39 (0.60)
Δ from SC	20	-0.067 (0.11)	20	-0.027 (0.14)
2-Week follow-up	19	3.58 (0.82)	24	3.45 (0.61)
DLco (mL/min/mmHg)				
Baseline	20	28.32 (6.33)	24	28.91 (5.43)
SC	20	28.02 (6.36)	23	27.76 (4.55)
Δ from Baseline	20	-0.303 (1.86)	23	-0.676 (1.41)
EXU	20	27.32 (6.16)	21	26.95 (4.74)
Δ from SC	20	-0.699 (2.11)	20	-0.902 (1.21)
2-Week follow-up	19	27.86 (6.12)	24	27.96 (4.53)

FIGURE 1.

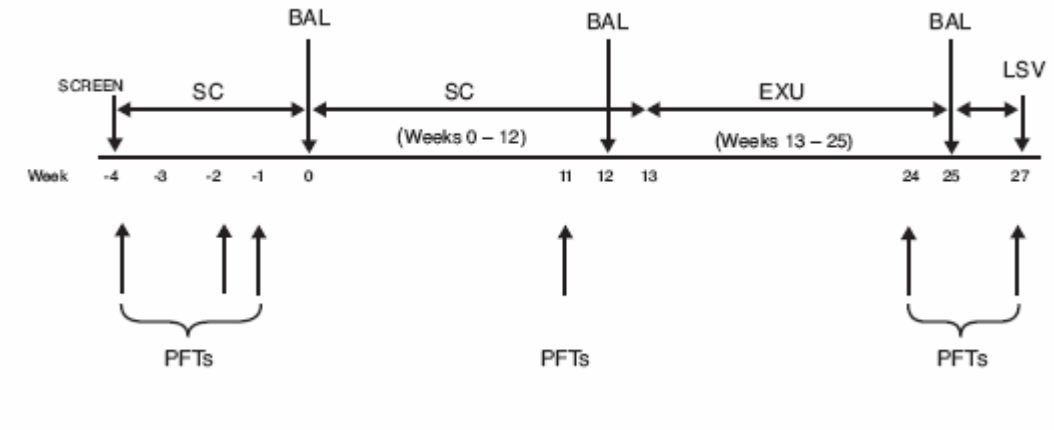

BAL
BAL
BAL
LSV
SCREEN
SC
SC
EXU
(Weeks 0 – 12)
(Weeks 13 – 25)
Week
-4
-3
-2
-1
0
11
12
13
24
25
27
PFTs
PFTs
PFTs

FIGURE 2.

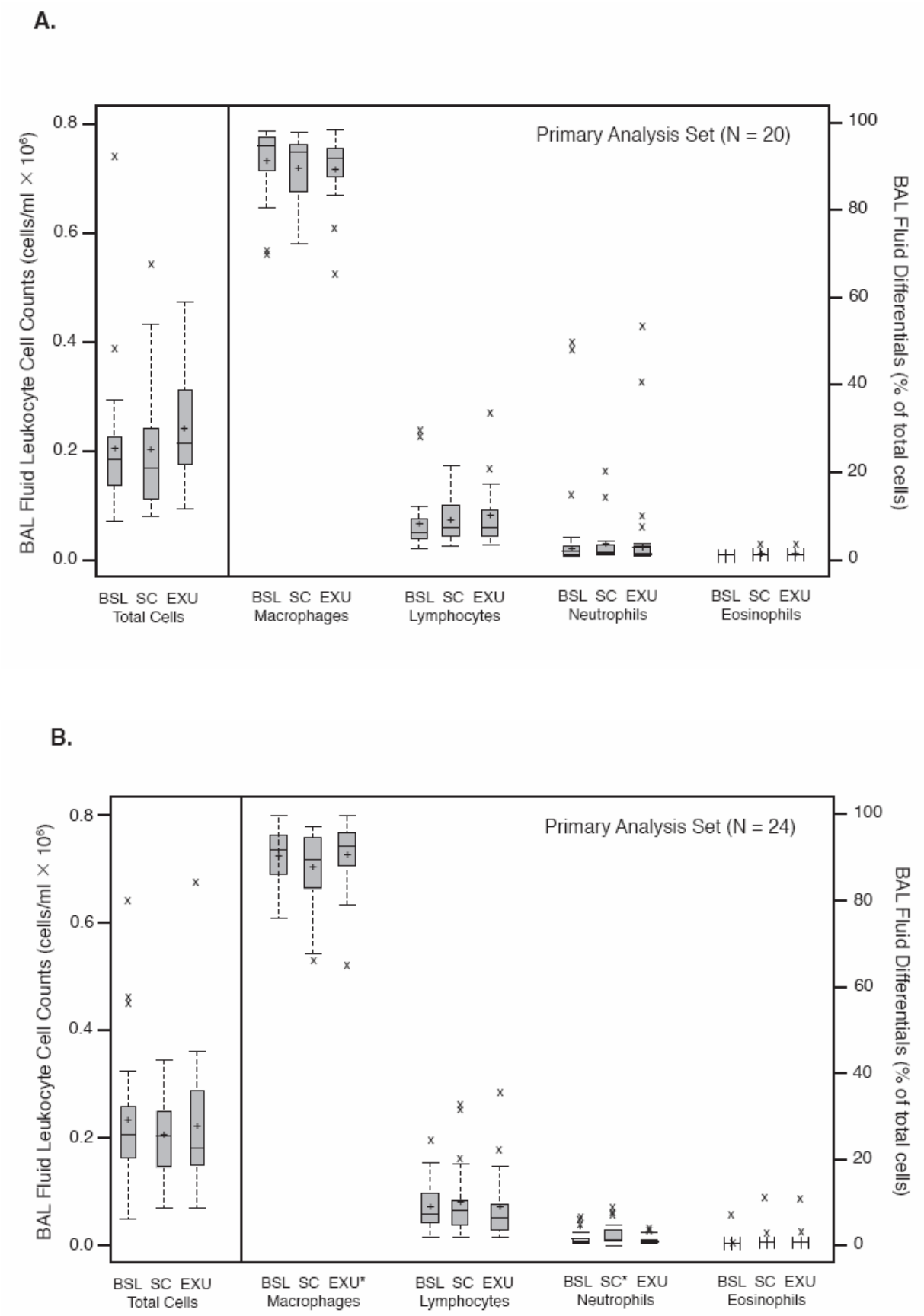

A.
Primary Analysis Set (N = 20)
BAL Fluid Leukocyte Cell Counts (cells/ml × 10⁶)
BAL Fluid Differentials (% of total cells)
0.0
0.2
0.4
0.6
0.8
0
20
40
60
80
100
BSL SC EXU
Total Cells
BSL SC EXU
Macrophages
BSL SC EXU
Lymphocytes
BSL SC EXU
Neutrophils
BSL SC EXU
Eosinophils
B.
Primary Analysis Set (N = 24)
BAL Fluid Leukocyte Cell Counts (cells/ml × 10⁶)
BAL Fluid Differentials (% of total cells)
0.0
0.2
0.4
0.6
0.8
0
20
40
60
80
100
BSL SC EXU
Total Cells
BSL SC EXU*
Macrophages
BSL SC EXU
Lymphocytes
BSL SC* EXU
Neutrophils
BSL SC EXU
Eosinophils

FIGURE 3.

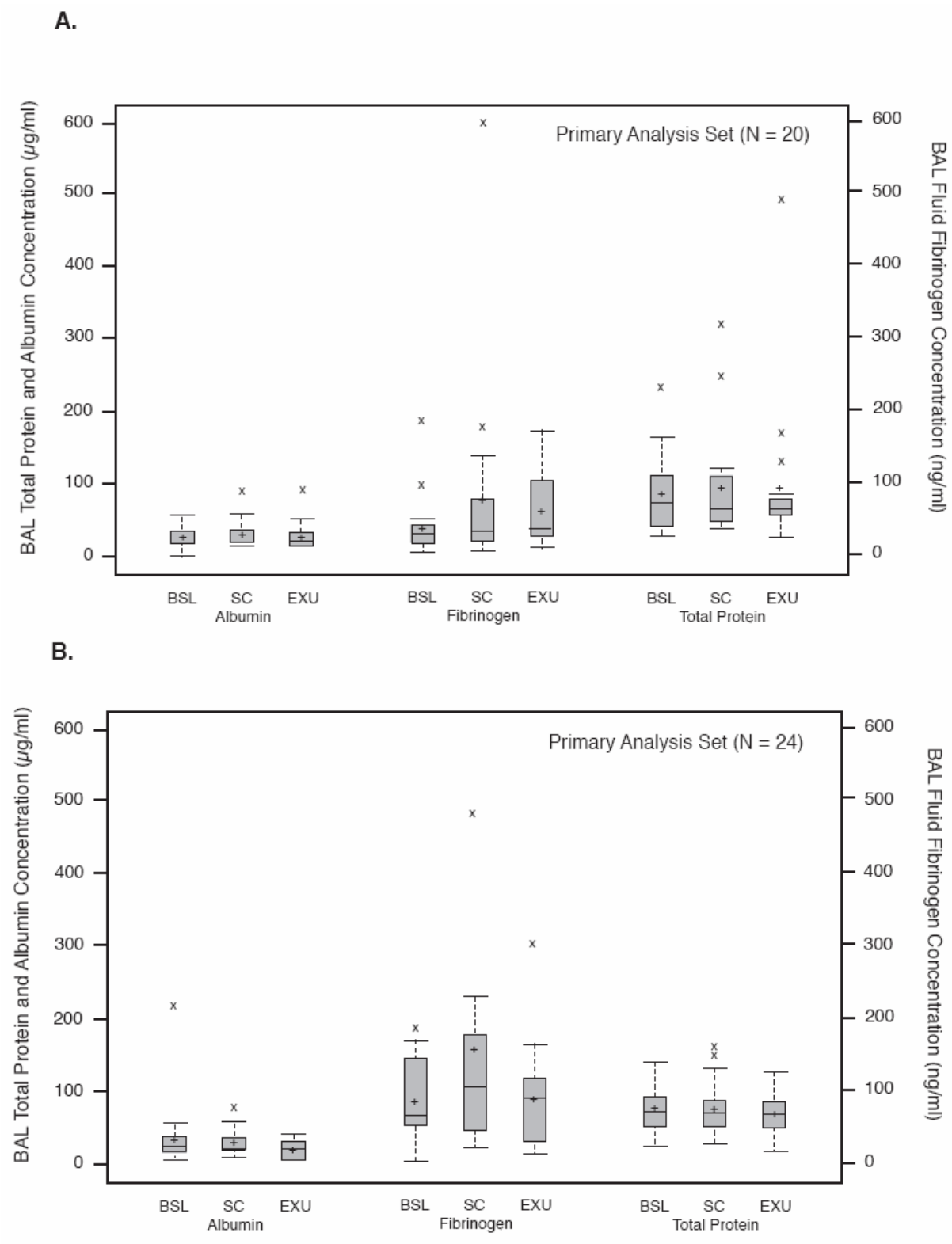
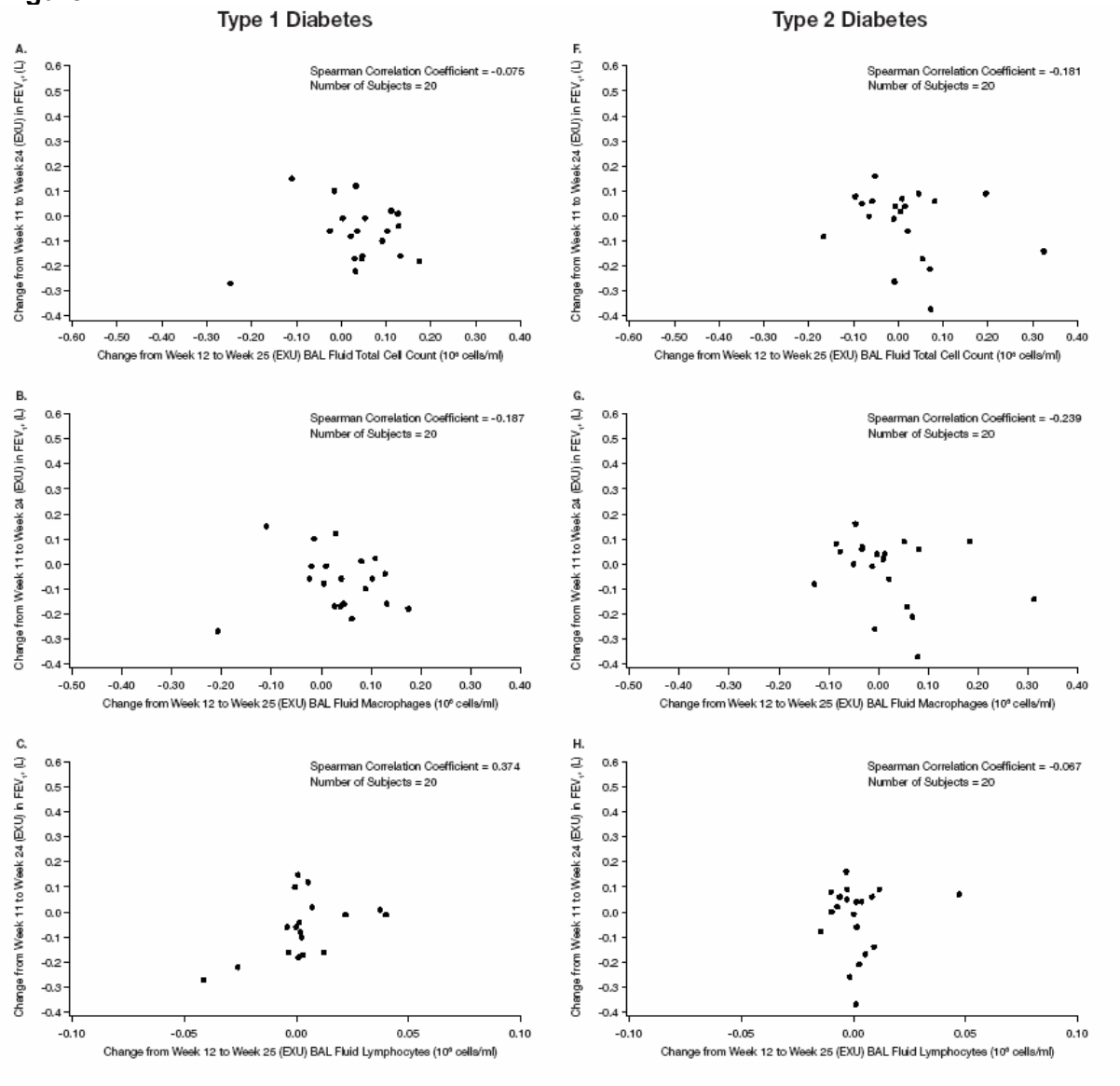
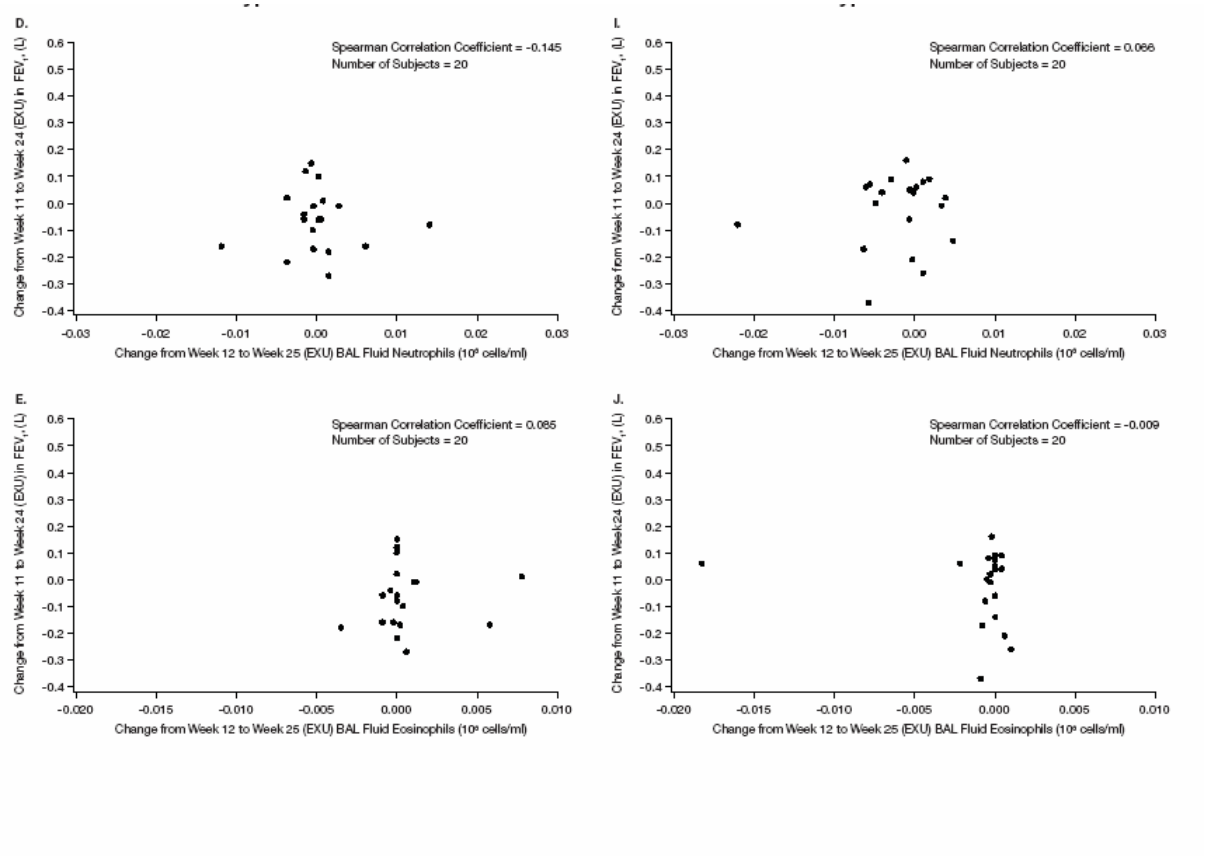

A.
Primary Analysis Set (N = 20)
BAL Total Protein and Albumin Concentration (µg/ml)
BAL Fluid Fibrinogen Concentration (ng/ml)
0
100
200
300
400
500
600
BSL
SC
EXU
Albumin
Fibrinogen
Total Protein
B.
Primary Analysis Set (N = 24)
BAL Total Protein and Albumin Concentration (µg/ml)
BAL Fluid Fibrinogen Concentration (ng/ml)
Albumin
Fibrinogen
Total Protein

Figure 4.


Type 1 Diabetes
Type 2 Diabetes
A.
Spearman Correlation Coefficient = -0.075
Number of Subjects = 20
Change from Week 11 to Week 24 (EXU) in FEV1 (L)
Change from Week 12 to Week 25 (EXU) BAL Fluid Total Cell Count (10⁶ cells/ml)
F.
Spearman Correlation Coefficient = -0.181
Number of Subjects = 20
Change from Week 11 to Week 24 (EXU) in FEV1 (L)
Change from Week 12 to Week 25 (EXU) BAL Fluid Total Cell Count (10⁶ cells/ml)
B.
Spearman Correlation Coefficient = -0.187
Number of Subjects = 20
Change from Week 11 to Week 24 (EXU) in FEV1 (L)
Change from Week 12 to Week 25 (EXU) BAL Fluid Macrophages (10⁶ cells/ml)
G.
Spearman Correlation Coefficient = -0.239
Number of Subjects = 20
Change from Week 11 to Week 24 (EXU) in FEV1 (L)
Change from Week 12 to Week 25 (EXU) BAL Fluid Macrophages (10⁶ cells/ml)
C.
Spearman Correlation Coefficient = 0.374
Number of Subjects = 20
Change from Week 11 to Week 24 (EXU) in FEV1 (L)
Change from Week 12 to Week 25 (EXU) BAL Fluid Lymphocytes (10⁶ cells/ml)
H.
Spearman Correlation Coefficient = -0.067
Number of Subjects = 20
Change from Week 11 to Week 24 (EXU) in FEV1 (L)
Change from Week 12 to Week 25 (EXU) BAL Fluid Lymphocytes (10⁶ cells/ml)


D.
Spearman Correlation Coefficient = -0.145
Number of Subjects = 20
Change from Week 11 to Week 24 (EXU) in FEV1 (L)
Change from Week 12 to Week 25 (EXU) BAL Fluid Neutrophils (10⁶ cells/ml)
I.
Spearman Correlation Coefficient = 0.066
Number of Subjects = 20
Change from Week 11 to Week 24 (EXU) in FEV1 (L)
Change from Week 12 to Week 25 (EXU) BAL Fluid Neutrophils (10⁶ cells/ml)
E.
Spearman Correlation Coefficient = 0.085
Number of Subjects = 20
Change from Week 11 to Week 24 (EXU) in FEV1 (L)
Change from Week 12 to Week 25 (EXU) BAL Fluid Eosinophils (10⁶ cells/ml)
J.
Spearman Correlation Coefficient = -0.009
Number of Subjects = 20
Change from Week 11 to Week 24 (EXU) in FEV1 (L)
Change from Week 12 to Week 25 (EXU) BAL Fluid Eosinophils (10⁶ cells/ml)