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Intravenous stem cell dose and changes in quantitative lung fibrosis and DLCO in the AETHER trial: a pilot study

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Abstract. – OBJECTIVE: Our purpose was to compare quantitative CT-derived changes in lung fibrosis with pulmonary function, including DLCO, in human subjects with idiopathic pulmonary fibrosis who received an injection of one of two different intravenous doses of human bone-marrow-derived mesenchymal stem cells.

PATIENTS AND METHODS: Two three-subject cohorts from the AETHER trial (Allogeneic Human Cells in subjects with Idiopathic Pulmonary Fibrosis via Intravenous Delivery) underwent high-resolution CT and clinical testing at baseline, 24 weeks, and 48 weeks after injection. Cohort 1 received $2x10^7$ stem cells, and cohort 2 received $1x10^8$ stem cells. CT scans were quantitatively analyzed for lung fibrosis using 510K cleared validated software. The percent predicted DLCO and other pulmonary function studies were obtained.

RESULTS: The cohorts were well matched in lung fibrosis at baseline as assessed by CT scan and lung function. The mean QLF in cohort 1 increased from 13.1% at baseline to 17.1% at 48 weeks, while mean QLF in cohort 2 increased from 15.4% at baseline to 16.5% at 48 weeks. The subjects in cohort 2 progressed more slowly in whole lung fibrosis by a mean of 2.87% compared with cohort 1 (p=0.001 with adjustment of baseline covariates) during the baseline to the 48-week interval. The baseline DLCO was lower in cohort 2 than in cohort 1 (p<0.0001). Over 48 weeks of the study, cohort 2 subjects demonstrated a mean DLCO decline of only 2% compared with a decline of 17% in cohort 1 subjects (p=0.02).

CONCLUSIONS: In this pilot study, the subjects receiving $1x10^8$ stem cells demonstrated slower progression in quantitative lung fibrosis and a smaller decrease in DLCO than subjects receiving $2x10^7$ stem cells.

Key Words
Idiopathic pulmonary fibrosis, Pulmonary fibrosis, Mesenchymal stem cells, Tomography, X-ray computed, Pulmonary function tests.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease characterized by interstitial fibrosis with decreasing lung volumes and declining pulmonary function. Although two drugs are approved by the Food and Drug Administration (FDA) for patients with IPF, neither is curative[1,2]. In preclinical studies, mesenchymal stem cells (MSCs) have shown promise as a potential novel treatment for lung disease[3-5]. We recently reported on the safety of a single infusion of bone marrow-derived MSCs in nine subjects with IPF (The Allogeneic Human Cells hMSC) and in patients with Idiopathic Pulmonary Fibrosis via Intravenous Delivery (AETHER) trial[6]. Quantitative high-resolution chest CT (HRCT) analysis is of increasingly important use in IPF; subtle changes in image-derived biomarkers of IPF disease status may be unapparent by qualitative visual analysis[7,8]. In this report, we provide preliminary data suggesting that MSC dose may be associated with quantitative HRCT and DLCO changes in subgroups of the AETHER cohort.

Patients and Methods

AETHER was a single-center, non-randomized, non-placebo-controlled phase I study of subjects with mild to moderate IPF conducted at the University of Miami Miller School of Medicine Interstitial Lung Disease Program/Pulmonary Division of the Interdisciplinary Stem Cell Institute (ClinicalTrials.gov Identifier: NCT02013700, IRB protocol approval #20120924). The study design and safety results have been previously described[6]. Imaging analysis was conducted on three subjects in cohort 1 (single infusion of $2x10^7$ hMSCs) and three in cohort 2 (single in-

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fusion of $1x10^8$ hMSCs). The subjects underwent HRCT at baseline (n=6), 24 weeks (n=5), and 48 weeks (n=6) post-infusion using a protocol enabling quantitative fibrosis evaluation. HRCT was performed on a Siemens Definition 64 slice CT scanner (Siemens Healthineers, Malvern, PA). Continuous changes in the HRCT parameters of quantitative lung fibrosis (QLF) and quantitative ground glass (QGG) from baseline to 24 weeks, 24 to 48 weeks, and baseline to 48 weeks were evaluated[7]. Continuous changes are expressed as the percentage of lung involvement. Each cohort's mean percentage of QLF and QGG in the entirety of both lungs was measured. QLF changes in different lung regions were calculated as each cohort's mean of summed percentage involvement of the bilateral upper lobes plus right middle lobe (upper lung zone, ULZ) and the mean of summed percentage involvement of right and left lower lobes (lower lung zone, LLZ). The changes in the total lung capacity (TLC), forced vital capacity (FVC), 6-minute walk test (6MWT), and the percent-predicted diffusing capacity of the lungs for carbon monoxide (DLCO) were also measured at baseline, 24, and 48 weeks post-infusion.

Statistical Analysis

The baseline and follow-up characteristics were compared for cohort 1 and 2 using two-group *t*-tests or Wilcoxon rank-sum tests. The subtraction of QLF scores at two-time points was used for reporting the mean differences. Subject 2 of cohort 1 did not undergo HRCT at 24 weeks.

Results

Quantitative Lung Fibrosis

The total amount of QLF at baseline and follow-up in all subjects is shown in Table I and Figure 1. Baseline QLF was well matched between the cohorts. Cohort 1 *vs.* cohort 2 had mean fibrosis scores in 13.1% *vs.* 15.4% of total lungs, 7.8% *vs.* 9.8% of combined upper and mid lung zones, and 33.4% *vs.* 34.6% of lower lung zones. The mean QLF in cohort 1 changed from 13.1% at baseline to 15.1% at 24 weeks, and 17.1% at 48 weeks (absolute increase over baseline of 4.0%, relative increase over baseline of 30.5%). The mean QLF in cohort 2 changed from 15.4% at baseline to 16.7% at 24 weeks and 16.5% at 48 weeks (absolute increase of 1.1%, relative increase of 7.1%). The subjects in cohort 2 progressed more slowly by a mean of 2.87% at week 48 in whole lung QLF scores compared with cohort 1 (overall changes p=0.001 with adjustment of baseline covariates; p=0.021 without adjustment) during the baseline to the 48-week interval (Figure 2). During the 24 to 48-week interval, in particular, all three cohorts 2 subjects demonstrated nearly unchanged total lung fibrosis. The segmented CT images of a mid-thoracic slice showing portions of both ULZ and LLZ in a cohort 1 and a cohort 2 subject at baseline and 48 weeks are shown in Figure 3. For cohort 1, the ULZ involvement changed from 21.2% at baseline to 29.5% at 48 weeks (relative increase of 39.2%), while for cohort 2 ULZ involvement changed from 31.7% at baseline to 34.1% at 48 weeks (relative increase

Table I. Summary statistics of quantitative HRCT and pulmonary function tests at baseline and follow-up.

	Cohort	Baseline	Baseline to 24 weeks	Baseline to 48 weeks
Quantitative Lung Fibrosis (QLF)	1	13.10% ± 4.69	3.75 ± 2.19	4.00 ± 2.19
	2	15.37% ± 11.31	1.33 ±1.80	1.13 ± 2.32
Quantitative Ground Glass (QGG)	1	14.17% ± 5.96	2.35 ±1.63	1.00 ± 1.35
	2	15.77% ± 7.33	2.40 ± 1.47	0.60 ± 1.31
Forced Vital Capacity (FVC)	1	2.92 L ± 0.45	-2.75% ± 7.99	-5.85% ± 4.11
	2	2.84 L ± 0.82	-3.28% ±3.35	-3.84% ± 3.14
Total Lung Capacity (TLC)	1	4.15 L ± 0.59	-2.50% ±11.67	-1.24% ± 15.64
	2	4.39 L ± 1.22	-9.66% ± 4.01	9.95% ± 10.52
Diffusing Capacity of the Lung for Carbon Monoxide (DLCO), % predicted	1	69.67% ± 21.78	-10.33 ± 2.52	-17.00 ± 5.00
	2	44.33% ± 4.62	0.00 ± 5.29	-2.00 ± 5.29
6 Minute Walk Test (6MWT)	1	415 m ± 58.66	-10.92% ± 25.06	1.63% ± 14.93
	2	493 m ± 48.77	-2.81% ± 7.54	-3.73% ± 10.58

Data are presented as mean ± standard deviation. Changes in QLF, QGG, and DLCO % predicted are the differences from the baseline where the baseline units are percentages. Changes in FVC, TLC, and 6MWT are percent changes from the baseline in the specified units.

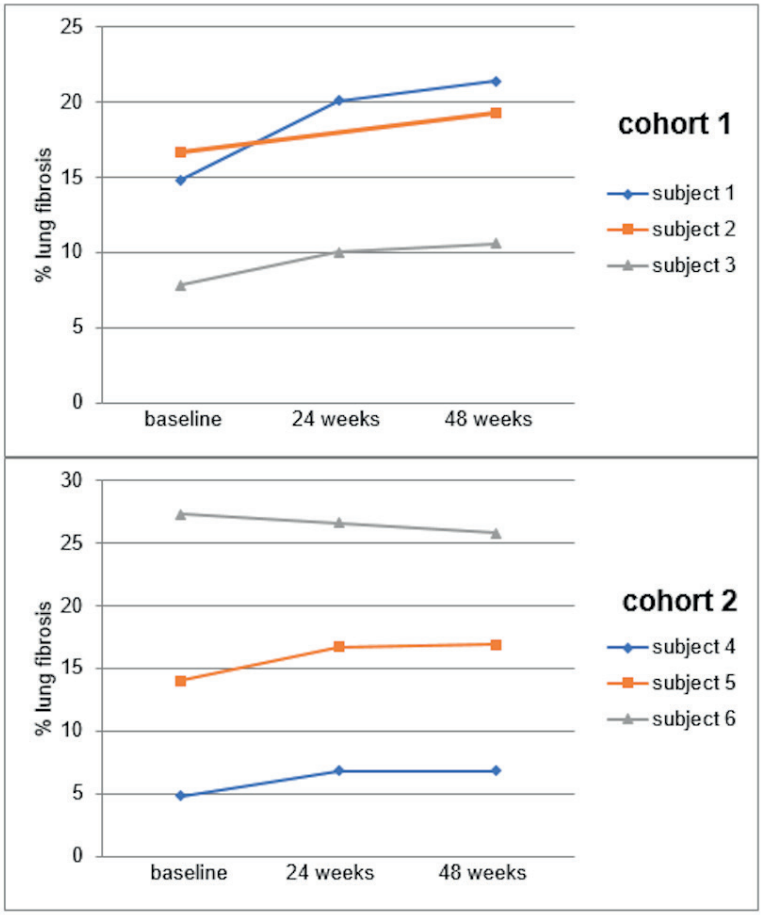

Figure 1. Total quantitative lung fibrosis in cohorts 1 and 2 at baseline, 24 weeks, and 48 weeks after injection. Subject 2 of cohort 1 did not undergo HRCT at 24 weeks.

of 7.6%). For cohort 1, LLZ involvement changed from 48.6% at baseline to 64.3% at 48 weeks (relative increase of 32.3%), while for cohort 2 LLZ involvement changed from 50.0% at baseline to 56.9% at 48 weeks (relative increase of 13.8%).

Quantitative Ground Glass

The baseline QGG was well matched between the cohorts. The changes in mean QGG scores were not different between the two cohorts over time (p=0.935).

DLCO, TLC, FVC, and 6MWT

At baseline, the subjects were reasonably well matched except for baseline percent predicted DLCO, which was significantly better in cohort 1 than cohort 2: 69.7 ± 21.8% *vs.* 44.3 ± 4.6% (p<0.0001) (Table I). Over 48 weeks of cohort 1 subjects demonstrated a 17% relative decrease in DLCO *vs.* a 2% decrease in cohort 2 subjects (p=0.02). Over 48 weeks, the mean TLC decreased by 1.2% in cohort 1 and improved by 10.0% in cohort 2 (p=0.37). The differences between the cohorts in other PFT values were of smaller magnitude.

Discussion

The AETHER study was the first reported trial of allogeneic mesenchymal stem cells administered to subjects with IPF. In this sub-study, we have used the methods not reported in our original study. We incorporated quantitative HRCT analysis of total, upper lung zone, and lower lung zone fibrosis at baseline and follow-up scans up to 48 weeks post-infusion. The quantitative analysis has higher reproducibility and better correlation with clinical measures than qualitative or semi-quantitative evaluation[7,8]. We also examined

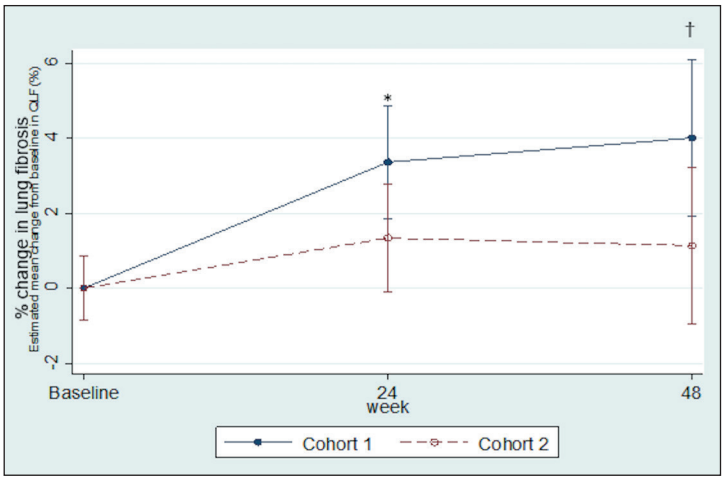

Figure 2. Mean percentage whole lung QLF changes from baseline at 24 and 48 weeks after adjusting the baseline characteristics, by cohort. *Mean (±SE) differences and [95% CI] in cohort 2 minus cohort 1 from baseline to 24 weeks are -1.89% (±0.90), [-3.66%, -0.13%]. †Mean (±SE) differences and [95% CI] in cohort 2 minus cohort 1 from baseline to 48 weeks are -2.87% (±1.17), [-5.17%, -0.57%].

the response during the first 24 weeks, and the subsequent 24 to 48-week interval, after infusion.

As is expected in the course of IPF, all cohort 1 subjects (dose $2x10^7$ hMSCs) showed worsening QLF from both baselines to 24 weeks, and 24 to 48 weeks. On the other hand, one cohort 2 subject (dose $1x10^8$ hMSCs) showed a decrease in QLF both in the baseline to 24-week interval and the 24-48 week interval. We recognized that with six subjects, the relative effect of a possible outlier is magnified in the pooled data. However, a decrease in pulmonary fibrosis with any type of intervention is unusual. The other two cohorts 2 subjects had nearly unchanged total QLF between 24-48 weeks. Image analysis also permits comparison between QLF changes in the upper lung zones (bilateral upper lobes plus right middle lobe) and lower lung zones (bilateral lower lobes). Over the course of 48 weeks, the difference in average percentage increase in QLF between the cohorts was greater in upper lung zone fibrosis than in lower lung zone or whole lung fibrosis.

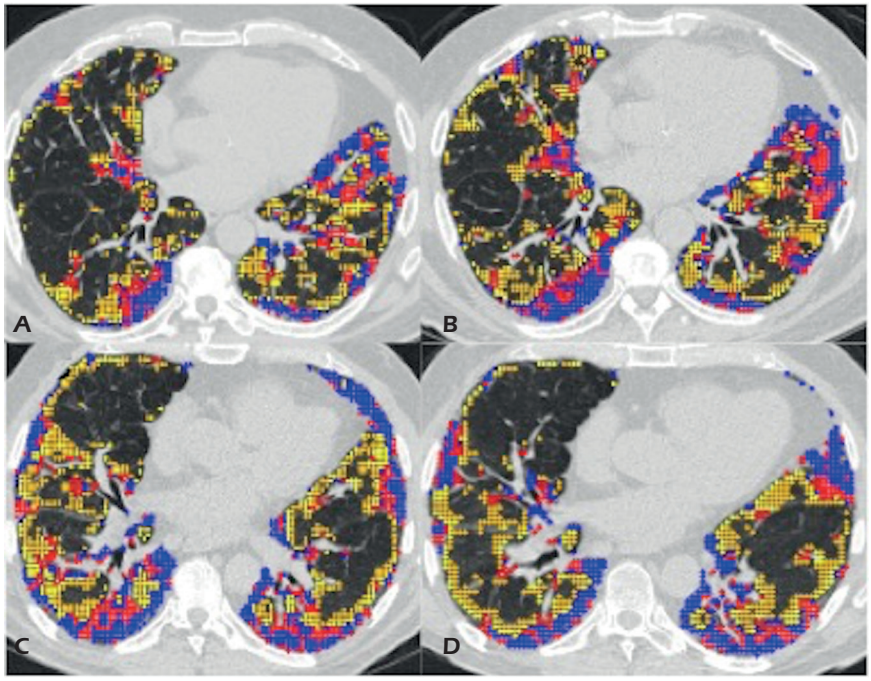

Figure 3. HRCT Images Segmented for QLF and QGG. ***A-B,*** Subject 1 from Cohort 1: (***A***) QLF (blue + red dots) 14.8% and QGG (yellow dots) 13.8% at baseline; (***B***) QLF 21.4% and QGG 15.2% at Week 48. ***C-D,*** Subject 6 from Cohort 2: (***C***) QLF 27.3% and QGG 20.0% at baseline; (***D***) QLF 25.8% and QGG 19.4% at Week 48.

Similar to QLF, all cohort 1 subjects demonstrated worsening DLCO in each measured interval. Baseline measurement of DLCO was significantly worse in cohort 2 than in cohort 1, but subsequent DLCO changes favored cohort 2. While all three cohort 1 subjects met the criteria for clinical worsening in % predicted DLCO (≥15% decline) over the course of 48 weeks, cohort 2 did not. We also noted a difference in the direction of change in TLC, which declined in cohort 1 and improved in cohort 2. The improvement occurring in the 24-48 week interval, although statistical significance was not reached.

Conclusions

We have applied a quantitative image analysis to whole lung, upper lung zone, and lower lung zone pulmonary fibrosis in subjects with IPF receiving one of two doses of hMSC. Our preliminary data suggests the possibility of an hMSC cell dose influence on changes in QLF and DLCO, and the possibility of different magnitude changes depending on time post-injection. However, we recognize that this is a small and non-randomized sub-study in a small number of subjects; a larger study is planned. An optimal injected cell dose, and an optimized timing regimen if multiple doses are given, are key questions for research. The limitations of the current study include its small sample size, lack of randomization, and lack of a placebo arm. Carefully conducted phase II/III clinical trials of hMSCs for the treatment of IPF will be required to more optimally evaluate their efficacy as a potential therapeutic modality for this fatal disease.

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ClinicalTrials.gov Identifier

NCT02013700.

Declaration of funding interests

This study was funded by the Lester and Sue Smith Foundation.

Conflict of Interests

Grace-Hyun J. Kim holds patent UC-2015-0324982-A1 for CT analysis software.
All other authors declare no relevant personal interests.

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