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Inhaled interferon and diffusion capacity in idiopathic pulmonary fibrosis (IPF)

S.D. Skaria[1], Jie Yang[2], Rany Condos[3], G.C Smaldone[1]

[1]Stony Brook University Medical Center, Pulmonary, Critical Care & Sleep Medicine; [2]Stony Brook University Medical Center, Department of Preventive Medicine; [3]New York University Medical Center, NY, Pulmonary, Critical Care & Sleep Medicine; [4]Stony Brook University Medical Center, Pulmonary, Critical Care & Sleep Medicine

Abstract. *Background:* Using data from a previously reported phase 2 safety trial, testing inhaled interferon gamma (IFN-γ) for IPF, we analyzed effects on full pulmonary function tests (PFTs) for efficacy before and after therapy and designed a randomized controlled trial of inhaled IFN-γ to treat IPF. *Methods:* Ten patients with IPF had received inhaled IFN-γ (Actimmune, InterMune) for 80 weeks. Full PFTs were available 20-50 weeks before Rx and monthly during Rx. Eighty-nine observations were used in the analysis. Linear mixed models for modeling longitudinal data were used to test if the PFT change over time was significantly different before and after IFN-γ. Autoregressive dependence structure with order one was consistently selected as the best one to model the intra-patient correlation over time. Normality assumption was confirmed. Significance level was set at 0.05. Using published literature and our data we performed a sample size calculation based on simulated data. *Results:* The change over time in DLCO was significantly different before and after IFN-γ treatment. DLCO decreased over time before treatment but increased after treatment (p-value=0.03). Changes in TLC, FRC, RV and FVC were not statistically significant. With a sample size of 60, a placebo controlled, randomized trial has about 90% power to detect a significant difference in the change rate of DLCO in the groups of patients treated with IFN-γ vs placebo. *Conclusions:* DLCO was significantly improved following inhaled (IFN-γ) as treatment for IPF. Our data suggest that previous studies utilizing parenteral IFN-γ may have failed because of the mode of delivery. Future randomized, controlled, phase 3 trials, comparing the difference in PFT behavior (specifically DLCO) longitudinally may be more sensitive to drug effect and serve as a valuable clinical endpoint. *(Sarcoidosis Vasc Diffuse Lung Dis 2015; 32: 37-42)*

Key words: Aerosol, Clinical Endpoint, Pulmonary Function Testing

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive form of restrictive lung disease that involves fibrosis and distortion of lung architecture. Most patients die from progressive respiratory failure (1). Lack of clinical response to immunosuppressive therapies has led to the concept that repetitive epithelial injury, fibroblast activation, microvascular injury, and dysregulation of normal wound repair

Received: 26 March 2014
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Correspondence: G.C. Smaldone MD, PhD
Stony Brook University Medical Center, Pulmonary,
Critical Care & Sleep Medicine
100 Nicolls Rd
HSC T-17-040
Stony Brook, NY 11794-8172
Phone: 631-444-3869 - Fax: 631-444-7501
Email: gerald.smaldone@stonybook.edu

lead to fibrosis (2-6). Primary Endpoints for treatment protocols have included mortality as well as pathophysiologic surrogates e.g. the 6-minute walk and Forced Vital Capacity (FVC), as a substitute for mortality but many patients exhibit relatively small changes in all of these parameters over time compared to placebo (7-9).

Identification of short-term predictors of disease progression and mortality i.e. 'disease surrogates' would be very valuable in assessing potential drug therapies and prognosis. For example in this Journal, Luppi and colleagues measured endogenous interferon-gamma (IFN-γ) production in seven patients before and after treatment with subcutaneous IFN-γ.

They found that 3 of the 7 with the lowest baseline levels of endogenous IFN-γ had worsening pulmonary function over time, the other patients remained stable or improved (10).

In general, most investigators follow serial pulmonary function testing to monitor the clinical course of the disease with a combination of small changes in multiple physiological endpoints used to predict prognosis (7). Currently, antifibrotic agents for IPF in phase 3 trials are focusing on the surrogate endpoint, FVC (6,9,11).

Although subcutaneous IFN-γ failed to modify the course of IPF in large clinical trials, IFN-γ is the signature Th1 cytokine endogenously produced by T cells and natural killer cells which, exhibits antifibrotic, antiproliferative, and immunomodulatory properties (5,12-14). Our group feels that the failure of subcutaneous therapy may be due to the difficulty in getting much of the agent into the lung.

We have hypothesized that targeting the lung directly with inhaled IFN-γ may be more successful. In a recently published Phase I/II safety study, Diaz et al. (12), successfully administered aerosolized IFN-γ to 10 patients with IPF for over 80 weeks.

They demonstrated that it was safely and effectively delivered to the lung parenchyma with no systemic side effects. Similar to the study of Luppi et al, we are looking for a treatment signal by analyzing the serial full pulmonary function data (PFTs) measured by Diaz et al, before and after inhaled IFN-γ therapy.

The paper by Diaz et al was not an efficacy study and it was not intended to look for changes in PFTS. Diaz et al. measured serial PFTs, 6 min walk, plasma levels, bronchoalveolar drug and cytokine levels for safety.

Those authors reported PFT data for 80 weeks of therapy. Besides the fact that inhaled IFN-γ was found to be safe, the PFT data suggested a possible drug effect.

The present paper presents the results of a formal analysis of that data.

We found evidence for a drug effect following inhaled IFN-γ.

We also performed a power analysis of that data to help design future placebo controlled clinical trials.

Methods

We performed a statistical assessment of the results reported in Diaz et al and developed a model to design phase 3 treatment trials. Full description of materials and methods of the Diaz study are described in detail in Diaz, et al (12).

Ten patients with IPF received inhaled IFN-γ (Actimmune, InterMune) 100μg 3x/week for 80-130 weeks delivered with I-neb [Philips Respironics], using a monitored slow and deep breathing pattern. Deposition of radiolabeled IFN-γ in the lungs was determined using a gamma camera. Full PFTs were measured 20-50 weeks before treatment and monthly during treatment (average 13 tests/patient).

Statistical analysis

Effects of inhaled IFN-γ on PFTs

Our approach was to compare trends of the available PFT data before and after inhaled therapy. While there was no placebo group the patient's own data acted as a control. Linear mixed models for modeling longitudinal data were used to test if the PFT change over time was significantly different before and after inhaled IFN-γ.

Autoregressive dependence structure with order one was consistently selected as the best one to model the intra-patient correlation over time based on Akaike Information Criteria (AIC). Normality assumption was confirmed. Eighty-nine observations were used to build models. Significance level was set at 0.05 and all analysis was done in SAS 9.3 (SAS Institute Inc., Cary, NC).

Statistical consideration for randomized controlled trial design

We designed randomized control trials comparing interferon vs placebo and comparing interferon vs pirfenidone, a therapy approved in Europe and under evaluation in the US based on longitudinal PFT data.

These calculations provide an estimate of the power of the effects of inhaled IFN-γ compared to placebo or, if needed, for a comparative trial with pirfenidone.

Statistical Study design

Patients will be randomized to two treatment arms on a 1:1 ratio; both treatments last for 48 weeks. Patients' PFT measurements will be recorded at baseline and every 4 weeks till week 48. The primary endpoint is the longitudinal DLCO measurement (% predicted).

Historical data

In both PIPF-004 and PIPF-006 studies (Noble *et al.* (2011) (11) the change in FVC over time can be well approximated by a linear function (Figure 2 in ref 11). We assume that the change pattern in DLCO is similar to that in FVC, that is, it also has a linear changing pattern over time. Table 2 in Noble *et al.* (2011) suggested that the change rates in DLCO over 72 weeks were -8.8/72=-0.122%/week and -9.6/72= -0.133%/week in patients who were treated with pirfenidone 2403 mg/day and placebo, respectively.

Average baseline DLCO in both studies were about 47% predicted.

Our in-house data suggested that the change rate in DLCO was estimated about -0.2579 %/week before interferon treatment and +0.06%/week after interferon treatment started. Average baseline DLCO were estimated at 49% predicted.

Therefore, in the sample size calculation based on simulation, it is assumed that the change rates in DLCO in interferon, pirfenidone, and placebo groups are +0.06%/week, -0.125%/week, and-0.25%/week, respectively and Baseline DLCO was assumed at 50% predicted.

Variance components values used in simulation were all based on our in-house data.

Results

Detailed PFT data are listed in the table. Patients were treated for a maximum of 132 weeks. On average there was an obvious decline in TLC and

Table 1. Patient demographics and pulmonary function tests before and after treatment with inhaled IFN-γ. Inhaled therapy started at baseline.

Demographics			FVC as % predicted				TLC as % predicted				DLCO as % predicted			
Pt	Age	Sex	-20 weeks	Baseline	Final % FVC	Final Week	-20 weeks	Baseline	Final % TLC	Final Week	-20 weeks	Baseline	Final % DLCO	Final Week
1	58	M	76.5	82	82	132	76.0	70	74	132	66.6	58	82	132
2	75	M	86.7	85	78	109	78.0	78	65	109	35.2	33	20	109
3	67	M	64.0	58	55	118	64.5	59	83	118	56.0	48	57	118
4	71	M	84.2	75	96	103	99.5	79	89	103	77.7	62	71	103
5	67	F	67.5	68	43	109	56.7	60	46	109	51.0	47	40	109
6	75	M	63.3	69	83	107	78.6	72	79	107		44	52	107
7	63	F	70.0	72	46	87	61.0	61	50	87	36.1	38	37	87
8	64	M	85.5	93	90	88	75.9	74	78	88	67.8	63	65	88
9	75	M	103.5	104	107	97	92.0	92	85	97	47.1	43	43	97
10	69	M	84.2	86	69	81	78.8	75	60	81	69.1	52	53	81
MEAN	**68.4**		**78.5**	**79.2**	**74.9**		**76.1**	**72.0**	**70.9**		**56.3**	**48.8**	**52.0**	
SEM	**1.8**		**4.0**	**4.3**	**6.8**		**4.2**	**3.2**	**4.7**		**5.0**	**3.2**	**5.7**	

DLCO during the period of observation before start of inhaled therapy with IFN-γ (inhaled therapy initiated at baseline).

At the end of treatment TLC was unchanged from baseline, and DLCO increased. Figure 1 is a spaghetti plot of individual PFT data for all 10 patients.

The figure illustrates an inflection point at baseline for TLC and DLCO with an increase in DLCO soon after initiation of inhaled IFN-γ.

The rate of change in DLCO was about -0.2579 %/week before interferon treatment and +0.06%/week after interferon treatment started. The average baseline DLCO was estimated at 49% predicted.

The change over time in DLCO was significantly different before and after interferon treatment. More specifically, DLCO decreased over time before treatment but increased after treatment (p-value=0.03). Changes in TLC, FRC, RV and FVC were not statistically significant in this small pilot study.

Figures 2 and 3 have the estimated powers for different sample sizes based on 1000 simulation runs at each sample size. Solid lines result from assuming all patients are fully evaluable while dashed lines assume 10% patients have partial PFT measurements because of early withdrawal or death. For example, with a sample size of 60, a placebo controlled, randomized trial has about 90% power to detect significant difference in the change rate of DLCO in the groups of patients treated with Interferon and those treated with placebo. Figure 3 represents the power curves for a trial comparing interferon vs pirfenidone.

Discussion

Our analysis of the data reported by Diaz et al demonstrates that inhaled IFN-γ significantly improved DLCO, an effect that may be important in the long-term treatment of the disease.

This effect has not been seen in trials using subcutaneous IFN-γ and our results support the idea of targeted therapy direct to the lungs via aerosol (15-17). Further, because the effect was significant in such a small group the changes in DLCO have a strong influence in the design of a randomized placebo controlled study (e.g. 90% power in 60 patients).

While preliminary, comparison with the reported pirfenidone data reported by Noble et al indicate that inhaled IFN-γ may be a more potent drug be-

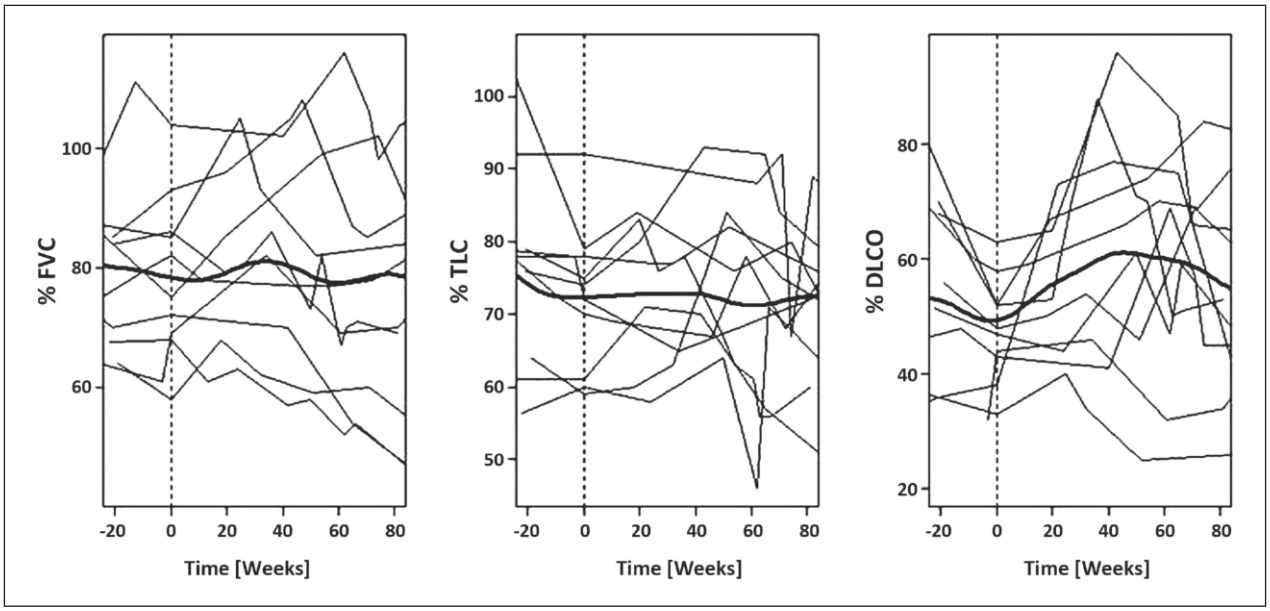

Fig. 1. Spaghetti plot of pulmonary functions before and after initiation of inhaled interferon therapy (inhaled therapy started at "baseline". O weeks). Data are plotted as percent change from baseline. Mean data shown as bold line. There is an inflection point after therapy for TLC and DVCO. The change in slope of the DLCO was significant.

cause the proposed pirfenidone vs inhaled IFN-γ RCT requires only 180 patients to demonstrate a significant superior effect.

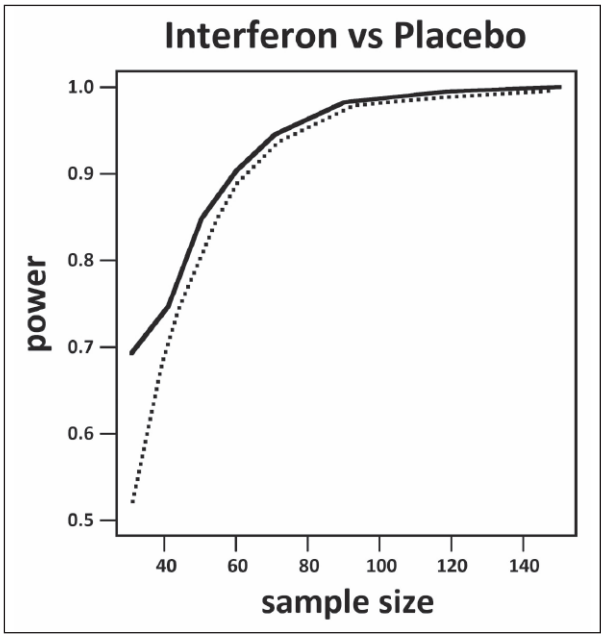

Fig. 2. Power curve to detect a significant different change rate in DLCO using a placebo controlled, randomized trial. Dashed line assumes 10% dropouts.

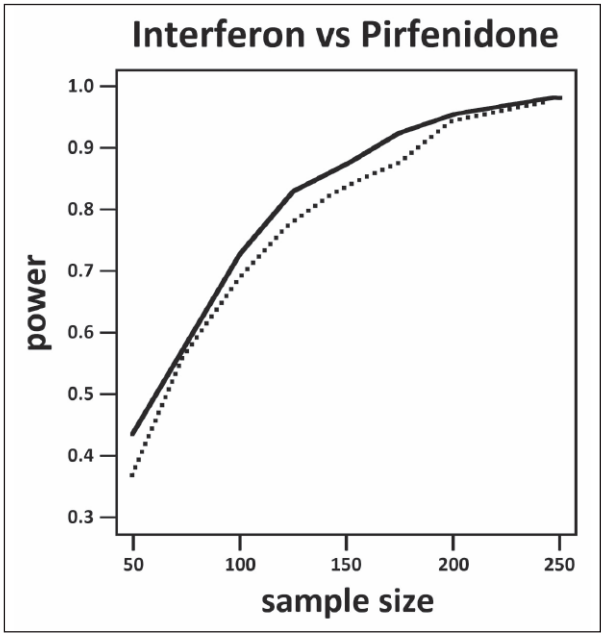

Fig. 3. Power curve to detect a significant different change rate in DLCO using a randomized trial with pirfenidone as active control. Dashed line assumes 10% dropouts.

It is difficult to conclude with certainty that a change over time in FVC or DLCO is a valid surrogate for survival (18). However, IPF is a rare disease and using survival as a primary endpoint raises practical and ethical questions (19,20).

Although FVC is used as a surrogate marker for treatment effect, baseline FVC is of unclear predictive value (9). Baseline and serial decline in PFT values have shown mixed associations with survival and therapeutic effect in IPF. Our analysis differs from those reported because we looked at serial tests before and after an intervention.

Abnormal DLCO levels are important in patients with restrictive lung disease. The DLCO declines with restrictive lung disease because of the reduced area for gas exchange (2).

Therefore, DLCO has been shown as a reliable predictor of survival at baseline, and a threshold of approximately 40 percent predicted has been associated with an increased risk of mortality (1,2). Hanson et al was able to demonstrate that patients with an improved or unchanged DLCO over 1 year had a enhanced survival as compared to those with a greater than or equal to 20% decrease in DLCO (21) DLCO has been shown as a critical factor for evaluating disease status and prognosis in IPF patients (21,22).

Why did IFN-γ affect the DLCO in our patients? Inhibition of TGF-β is desirable on a cellular level but connecting cellular responses to a measured clinical parameter is difficult. However, there are other possible effects of IFN-γ that may be important. Folicik et al have found evidence for repeated viral infection in patients with IPF (23). In 21 patients with IPF lung biopsies were examined for a series of γ-herpesviruses' DNA/RNA and related proteins using in situ hybridization and reverse transcriptase-polymerase chain reaction (RT-PCR)-based methods. They detected four proteins known to be in the genome of several γ-herpesviruses (cyclin D, thymidylate synthase, dihydrofolate reductase, and interleukin-17). IFN-γ has antiviral effects and this may be an additional potential mechanism for therapy in this disease.

Our results are preliminary and limited by the small number of patients. In addition, the DLCO, as a test, is more difficult to perform than simple spirometry. However, if there was random variation in the DLCO test for technical reasons then we

should not have been able to detect a significant therapeutic effect in only 10 patients.

Our results suggest a rationale for using serial changes in measured PFT before and after treatment as criteria for comparing therapeutic regimens (12). A decreasing DLCO before treatment may be a useful parameter for selecting the cohort of individuals declining more rapidly over time.

These patients may have a more readily identifiable disease pattern before and after treatment with inhaled IFN-γ.

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