Correspondence

Is Warfarin the Right Anticoagulant in Idiopathic Pulmonary Fibrosis?

To the Editor:

We have with great interest read the article by Noth and colleagues from The Idiopathic Pulmonary Fibrosis Clinical Research Network (IPFnet) in a recent issue of the *Journal*. They report on the results from the Placebo-Controlled Randomized Trial of Warfarin in IPF (ACE-IPF) (1). The ACE-IPF study was aborted before study endpoint due to a low probability of benefit and an increase in mortality in the warfarin-treated subjects (14 warfarin vs. 3 placebo deaths; $P = 0.005$).

We compliment the researchers for a well-conducted study, but at the same time we caution the idiopathic pulmonary fibrosis (IPF) community not to abandon the principle of anticoagulation in IPF solely due to the results from the present study. Besides coumarins, there are other anticoagulant agents, and among these, unfractionated and low-molecular-weight heparins are the most commonly used anticoagulant and antithrombotic agents. In the only other clinical trial with anticoagulation, a combination of warfarin, prednisolone, and heparin showed that systemic anticoagulant therapy may have a beneficial effect on survival in patients with IPF (2).

Many of the pathways that are involved in fibrogenesis in IPF are unknown, but the initial event involves alveolar epithelial injury; the mechanisms of lung injury and fibrogenesis have been explored by the use of animal models, and increased procoagulant activity (tissue factor/VIIa) as well as suppression of the normal fibrinolytic activity through up-regulation of several protease inhibitors in the alveoli seems of great importance (3). The same mechanisms have been observed in patients with IPF, acute lung injury, and sarcoidosis, and thus anticoagulation might be beneficial in IPF as summarized in References 1 and 3.

However, in addition to their well-known anticoagulant effects, heparins have documented antiinflammatory and antifibrotic properties, as shown in multiple laboratory, animal, and human studies (4).

Normally, heparin and low-molecular-weight heparin are administered either intravenously or subcutaneously, which very likely will represent a barrier for self-administration for many patients. However, several studies have investigated the intrapulmonary route, and studies have also proven antiinflammatory effects after inhalation of heparin (5). Inhaled heparin has been shown to be safe in both healthy volunteers and patients with IPF with respect to pulmonary function, gas exchange, exercise capacity, and local and systemic anticoagulant effects (3, 6).

Therefore, based on the multiple antiinflammatory and antifibrotic effects of heparin, the encouraging results from Kuno and colleagues, and the disappointing results from Noth and coworkers, we question the choice of anticoagulant drug (warfarin) for treatment of IPF and suggest that studies with inhaled heparin should be prosecuted in the future.

Author disclosures are available with the text of this letter at www.atsjournals.org.

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References

1. Noth I, Anstrom K, Calvert SB, de Andrade J, Flaherty KR, Glazer C, Kaner RJ, Olman MA. A placebo-controlled randomized trial of

warfarin in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2012;186:88–95.

2. Kubo H, Nakayama K, Yanai M, Suzuki T, Yamaya M, Watanabe M, Sasaki H. Anticoagulant therapy for idiopathic pulmonary fibrosis. *Chest* 2005;128:1475–1482.
3. Markart P, Nass R, Ruppert C, Hundack L, Wygrecka M, Korfei M, Boedeker RH, Staehler G, Kroll H, Scheuch G, et al. Safety and tolerability of inhaled heparin in idiopathic pulmonary fibrosis. *J Aerosol Med Pulm Drug Delivery* 2010;23:161–172.
4. Lever R, Page C. Non-anticoagulant effects of heparin: an overview. *Handb Exp Pharmacol* 2012;207:281–305.
5. Bendstrup KE, Jensen JI. Inhaled heparin is effective in exacerbations of asthma. *Respir Med* 2000;94:174–175.
6. Bendstrup KE, Gram J, Jensen JI. Effect of inhaled heparin on lung function and coagulation in healthy volunteers. *Eur Respir J* 2002;19:606–610.

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Reply

From the Authors:

We thank Drs. Bendstrup and Hilberg for their comments regarding the IPFnet ACE trial examining the use of warfarin in idiopathic pulmonary fibrosis (IPF), and agree with their premise for the need for further investigation of anticoagulant therapy in IPF (1).

We agree that the ACE study addresses the use of only warfarin in IPF. Importantly, there are several differences between ACE and the previous study examining the utility of anticoagulation in IPF by Kubo and coworkers (2). In the prior study, much less physiologically impaired patients with IPF were enrolled at hospitalization and placed on warfarin and prednisolone in combination, as opposed to warfarin alone. In fact, the number of subjects on both agents in ACE was very small. Furthermore, dalteparin, a low-molecular-weight heparin, was added during subsequent hospitalizations for acute exacerbation events. By allowing sicker patients in ACE, we both enhanced event rates, which was the objective, but may not have allowed sufficient time for a treatment effect to occur in altering the course of fibrosis.

There is a plethora of evidence demonstrating antifibrotic effects of anticoagulants, both dependent and independent of their anticoagulant actions (3). Factor VII activity has been demonstrated to be elevated in patients with interstitial lung disease, and specifically in IPF (3). Also recently demonstrated, factor VIIa stimulates proliferation of human lung fibroblasts and extracellular matrix in a PAR-2–dependent manner. This effect is factor Xa and thrombin independent (4). Additionally shown after ACE's inception is that increased local expression of coagulation factor X contributes to the fibrotic response in human and murine lung injury (5). Furthermore, evidence supports a protective role for protein C, a vitamin K–dependent anticoagulant protein, on the endothelial cell barrier function in acute lung injury (6). This growing body of evidence supports the concept that anticoagulants targeting specific coagulation factors or their “fibrosis-signaling” receptors may exert antifibrotic effects independent of their anticoagulant activities.

Heparin shares anticoagulant action with warfarin, but its coagulation factor targets only partially overlap with that of warfarin. Furthermore, different heparin size fractions, and biochemical modifications, differ in their potencies, anticoagulant targets (such as thrombin or Factor X), and in their antiinflammatory and antiproliferative capacities (7–9). There is much evidence *in vitro* and *in vivo* to suggest that all anticoagulants are not equal with respect to their antifibrotic therapeutic potential. Thus, we agree that the

compelling basic science and animal model observations would provide a solid foundation upon which to implement additional trials of “anti-coagulants” in IPF. In conclusion, we have answered the question of the utility of warfarin in progressive IPF, but many questions remain.

Author disclosures are available with the text of this letter at www.atsjournals.org.

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References

1. Noth I, Anstrom K, Calvert SB, de Andrade J, Flaherty KR, Glazer C, Kaner RJ, Olan MA. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2012;186:88–95.
2. Kubo H, Nakayama K, Yanai M, Suzuki T, Yamaya M, Watanabe M, Sasaki H. Anticoagulant therapy for idiopathic pulmonary fibrosis. *Chest* 2005;128:1475–1482.
3. Gunther A, Mosavi P, Ruppert C, Heinemann S, Temmesfeld B, Velcovsky HG, Morr H, Grimminger F, Walmrath D, Seeger W. Enhanced tissue factor pathway activity and fibrin turnover in the alveolar compartment of patients with interstitial lung disease. *Thromb Haemost* 2000;83:853–886.
4. Wygrecka M, Kwapiszewska G, Jablonska E, von Gerlach S, Henneke I, Zakrzewicz D, Guenther A, Preissner KT, Markart P. Role of protease-activated receptor-2 in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011;183:1703–1714.
5. Scotton CJ, Krupiczkoj MA, Königshoff M, Mercer PF, Lee YC, Kaminski N, Morser J, Post JM, Maher TM, Nicholson AG, et al. Increased local expression of coagulation factor X contributes to the fibrotic response in human and murine lung injury. *J Clin Invest* 2009;119:2550–2563.
6. Finigan JH, Dudek SM, Singleton PA, Chiang ET, Jacobson JR, Camp SM, Ye SQ, Garcia JG. Activated protein C mediates novel lung endothelial barrier enhancement: role of sphingosine 1-phosphate receptor transactivation. *J Biol Chem* 2005;280:17286–17293.
7. Fryer A, Huang YC, Rao G, Jacoby D, Mancilla E, Whorton R, Piantadosi CA, Kennedy T, Hoidal J. Selective O-desulfation produces nonanticoagulant heparin that retains pharmacological activity in the lung 1,2. *J Pharmacol Exp Ther* 1997;282:208–219.
8. Koenig A, Norgard-Sumnicht K, Linhardt R, Varki A. Differential interactions of heparin and heparan sulfate glycosaminoglycans with the selectins: implications for the use of unfractionated and low molecular weight heparins as therapeutic agents. *J Clin Invest* 1998;101:877–889.
9. Garg HG, Thompson BT, Hales CA. Structural determinants of antiproliferative activity of heparin on pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L779–L789.

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A Young Hispanic with c.1646G>A Mutation Exhibits Severe Cystic Fibrosis Lung Disease: Is Ivacaftor an Option for Therapy?

To the Editor:

Cystic fibrosis (CF) is a lethal autosomal recessive inherited disease caused by the loss or dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride (Cl[−]) channel activity resulting from mutations (1, 2). More than 1,600 mutations, which can be broadly grouped into six classes, have been identified in the CFTR gene (3). The incidence of CF and the frequency of specific mutations have been found to vary among ethnic populations (4, 5).

We presented a clinical case of a young Hispanic patient with CF heterozygous for c.1521_1523delCTT (p.Phe508del or F508del)/c.1646G>A (p.Ser549Asn or S549N) mutations (please note that the legacy names for the mutations will be used in this letter). This individual was diagnosed with CF at 5 months of age after multiple hospitalizations due to dehydration, failure to thrive, and emesis. She also had a prolonged and persistent cough thought secondary to a diagnosis of pertussis at 4 months of age. At diagnosis, she was also noted to have a hypochloremic, hypokalemic metabolic alkalosis. She was below the fifth percentile on all aspects of her growth curve. Sweat chloride levels were abnormal with values of 87.1 mmol/L (right arm) and 78.2 mmol/L (left arm). She was empirically given pancreatic replacement enzymes and fat soluble vitamin replacement. By the age of 3 years, she was found to be colonized by two strains of nonmucoid *Pseudomonas aeruginosa* as well as a mucoid *Pseudomonas* that was her predominant isolate by 4 years of age. Over the past 2 years, she has had three additional hospitalizations for pulmonary exacerbations (April 2010, April 2011, and October 2011). Pulmonary function tests (PFTs) have continued to worsen with FEV₁ values of 50 to 60% predicted (the most recent FEV₁ prior to hospitalization in October 2011 was 51% predicted) and FEF_{25–75} values in the low end of 20 to 40% predicted (the most recent value was 20% predicted). She has had increasing pulmonary symptoms and has developed worsening sinusitis. Chest computed tomography scan completed in October 2011 showed areas of extensive bronchiectasis and fibrotic change, predominantly affecting the upper lobes. Areas of disease were observed in the lower lobes as well (Figure 1A and Figure E1 in the online supplement). There are numerous areas of “tree-in-bud” opacities present essentially involving all lobes of both lungs (Figure 1A).

F508del is the most common CFTR mutation, with more than 90% of patients with CF carrying it on at least one allele. F508del-CFTR protein is insufficiently folded and is trapped in the endoplasmic reticulum and targeted for degradation (1). Like c.1652G>A (p.Gly551Asp or G551D), S549N mutation occurs in the signature sequence (LSGGQ) of the first nucleotide-binding domain on the CFTR protein (Figure E2A). S549N mutation was first reported by Curtis and colleagues in a Pakistani individual who was homozygous for S549N and demonstrated severe malnutrition, growth retardation, and advanced pulmonary disease (6). S549N mutation was found to be prevalent among Hispanic, African American, and Asian populations (4).

We characterized S549N-CFTR at the protein level to understand its molecular characteristics and the associated severe disease phenotype and, more importantly, to explore using mutation-specific therapy for medical interventions. S549N-CFTR was expressed as a mature form of CFTR (band C) with levels comparable to wild-type CFTR (Figure 1B). Surface labeling assay and fluorescence microscopy data showed that S549N-CFTR was expressed at the plasma membrane of cells (Figures E3A and E3B). However, S549N-CFTR lacks Cl[−] channel function (Figures E2B and E2C). These molecular characteristics correlate well with the severe CF phenotype we observed and suggest that, like G551D, S549N-CFTR is probably a regulation mutant.

Author Contributions: The manuscript was written by S.Y. and W.Z. supervised by A.P.N. and D.C.S. The project was designed and supervised by A.P.N. S.Y. conducted site-directed mutagenesis and surface labeling, F.A.I.K. performed iodide efflux assays, and H.P. performed immunoprecipitation and Western blotting experiments. A.R. conducted *I_{sc}* measurements. K.A. performed immunofluorescence microscopy studies. C.A.D., S.S., and D.C.S. did the clinical studies. J.C.K. assisted in site-directed mutagenesis. All authors discussed the results and commented on the manuscript.

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