Ob(nox)ious fibrosis

By Benjamin Boettner, Associate Editor

Although Esbriet pirfenidone, the only marketed drug for idiopathic pulmonary fibrosis, has shown a mortality benefit, the effects are modest and there remains room for improvement. New preclinical findings suggest that inhibiting NADPH oxidase 4 could both resolve and reverse fibrosis.¹

Based on the results, **Genkyotex S.A.** plans to advance its GKT137831 NADPH oxidase 4 (NOX4) inhibitor to treat IPF.

IPF, like other forms of fibrosis, is the consequence of a failed epithelial repair process triggered by inflammation or injury. The process begins when transforming growth factor β 1 (TGFB1) activates myofibroblasts.

Normally, this allows for the production of extracellular matrix components like collagen that serve as a substrate for epithelial repair. But in patients with IPF, activated myofibroblasts overproliferate and produce excessive amounts of extracellular matrix components.

The result is fibrotic scars that prevent re-epithelialization and ultimately render organ systems dysfunctional.²

InterMune Inc.'s Esbriet pirfenidone is approved in multiple countries. The drug is a small molecule inhibitor of proinflammatory cytokines and profibrotic cytokines that targets p38 mitogen-activated protein kinase (p38 MAPK; MAPK14).

The next drug likely to be marketed for IPF is Vargatef nintedanib from **Boehringer Ingelheim GmbH**. Vargatef is a small molecule inhibitor of multiple proangiogenic kinases that is in Phase III testing for IPF. The compound significantly slowed declines in forced vital capacity; however, pooled Phase III data show that it produced only nonsignificant improvements in all-cause mortality.

IPF's incidence and prevalence increase with age—most patients are more than 65 years old at the time of diagnosis.

Now, a team led by **The University of Alabama at Birmingham** researchers Louise Hecker and Victor Thannickal thinks NOX4 may be at the root of IPF's age association.

Thannickal is a professor of medicine and pathology and director of the Division of Pulmonary, Allergy and Critical Care at The University of Alabama at Birmingham. Hecker recently moved to **The University of Arizona**, where she is an assistant professor of medicine. The study was coauthored by former Genkyotex CSO Eric Meldrum.

The group already had clues that the target was involved in IPF. In

2009, they showed that NOX4 expression was stimulated in fibroblastic foci by TGFB1 in a mouse model of IPF and in samples from patients with IPF. siRNAs targeting *Nox4* were able to prevent fibrogenesis.³

In the new study, the group compared chemically induced lung fibrosis in young and aged mice. Although both sets of animals developed fibrosis, only the young animals were able to resolve the fibrotic lesions.

Additional studies pointed to Nox4-generated oxidative stress as the key difference between the young and old animals. Fibrotic areas in old but not young mice were characterized by an imbalance between two redox systems: profibrotic superoxide-producing Nox4 and anti-fibrotic nuclear factor (erythroid-derived 2)-like 2 (Nfe2l2; Nrf2).

NRF2 is a transcriptional regulator that activates a battery of genes that cooperate to reduce reactive oxygen species.

Intranasal delivery of a *Nox4*-specific siRNA reduced senescence markers and eliminated apoptosis resistance—both of which are associated with fibrosis in aged animals—and restored the ability to resolve fibrosis. The researchers saw similar results when they used GKT137831.

The University of Alabama team found the NRF2 response to elevated superoxide levels to be impeded specifically in fibrotic cells of aged mice.

Accordingly, sulforaphane, an activator of Nrf2, was able to resensitize lung myofibroblasts isolated from aged, injured mice to apoptosis. This could potentially reduce collagen-producing cell numbers in fibrotic foci.

Data were published in *Science Translational Medicine*.

"The data provide clear evidence of the fundamental role of NOX4 in lung fibrosis in a model which best reflects the disease in patients. It points the way to the use of a NOX4 inhibitor for the treatment of IPF," said Genkyotex CEO Ursula Ney.

GKT137831 is a dual inhibitor of NOX4 and NOX1. The molecule is in Phase II testing to treat diabetic nephropathy.

Both Ney and Yves Gorin think that inhibiting NOX4 will be a better option than stimulating NRF2 in fibrotic disease. "The targeted inhibition of NOX4 is likely to be safer than the chronic activation of hundreds of NRF2-responsive genes," said Ney.

Gorin is an associate professor of medicine at **The University of Texas Health Science Center at San Antonio** whose work is focused on redox systems including NOX4 in different diseases.

Resolving the mechanisms

Scott Turner, EVP of R&D at **KineMed Inc.**, wanted to see more evidence that Nox4 inhibition helps to actively resolve fibrosis in aged mice.

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—Scott Turner, KineMed Inc.

of the inhibitor, as would be a demonstration of an influx of resolving cell types and increased matrix metalloproteinase activity,” he said.

KineMed is developing biomarkers and assays, including dynamic measurements of collagen in fibrotic contexts.

Gorin said that it would be useful to identify the molecular mechanisms in young mice that keep a healthy balance of Nox4- and Nrf2-dependent biological responses.

Gorin and Bruno Crestani think that NOX4 inhibitors could have utility beyond IPF because myofibroblasts are activated in virtually every fibrotic organ.

Crestani is a professor at **Paris Diderot University**, at **Institut National de la Santé et de la Recherche Médicale** (INSERM) unit 1152 and in the pneumology department and Centre for Rare Lung Diseases at the **Hospital Bichat**.

“It would be interesting to also study the effect of NOX4 inhibition and the modulation of NOX4/NRF2 coupling on fibrosis-associated complications in the kidney or the heart in elderly diabetes patients,” said Gorin.

Paul Higgins, a professor and director at **Albany Medical College**, agreed. “Other models of experimental fibrosis particularly in the heart and renal systems should be evaluated with respect to age,” he said. Higgins did caution that the precise NOX proteins involved in each tissue, however, might differ.

Thannickal told *SciBX* that he has applied for funding from the **NIH**’s Centers for Advanced Diagnostics and Experimental Therapeutics program to develop additional NOX4 inhibitors. He said that he is looking for industry partners.

The University of Alabama at Birmingham has a patent covering NOX4-specific siRNAs that block fibrotic processes.

Genkyotex owns IP covering GKT137831’s composition of matter.

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