Fighting fibrosis

Improving target selectivity and defining cell phenotypes may improve the odds of finding therapies for fibrosis.

Melanie Senior

Two recent sets of positive clinical data have bucked the trend in the failure-strewn idiopathic pulmonary fibrosis (IPF) field: Boehringer Ingelheim’s phase 2 data for phosphodiesterase 4B (PDE4B) inhibitor BI 1015550 in May 2022 and and Pliant Therapeutics’ phase 2a data for dual-selective integrin inhibitor PLN-74809 in July. The good news reflects renewed optimism in a space with huge unmet need. IPF is the deadliest and fastest-progressing form of fibrosis, wreaking havoc with the alveolar space in the lungs. Median survival, at just three to five years, is worse than for many cancers. The only treatment options are Roche’s transforming growth factor-β (TGFβ) inhibitor Esbriet (pirfenidone) and Boehringer’s repurposed cancer drug Ofev (nintedanib), a multi-kinase inhibitor approved in the United States nearly a decade ago (2014). Neither drug stops disease progression, a third of patients can’t tolerate them and it isn’t even clear how Esbriet works.

Despite setbacks, developers haven’t given up. R&D efforts are becoming more targeted, as single-cell genomics and other ‘-omics’ tools help unravel the molecular and cellular mechanisms underpinning fibrosis. Today’s IPF pipeline includes more selective, potent iterations of drug classes that have previously stumbled. Newer approaches are emerging too, including targeting the collagen-rich extracellular matrix (ECM), whose disorderly deposition is the hallmark of fibrosis, or the RNA that regulates fibroblast differentiation.

“We’re now chasing drugs that target plausible, known biological mechanisms rather than just seeing what sticks,” says Dean Sheppard, director of the Lung Biology Center at the University of California, San Francisco.

It’s welcome news for patients with IPF. Though classified as a rare disease, IPF is becoming more widespread, as it’s associated with aging, smoking and pollution. Any drug that works in IPF could have wider potential: fibrosis can affect almost any tissue or organ and is central to a string of disorders, from kidney disease to
nonalcoholic steatohepatitis, heart failure, rheumatoid arthritis and even some cancers.

**Healing gone wrong**

Put simply, fibrosis is wound repair gone awry, leading to tissue scarring, stiffness and malfunction. Yet there is nothing simple about this disorder. Fibrosis is a complex, dynamic process, involving multiple cell types and differentiation pathways, genes and signaling mechanisms, feedback and feedforward loops.

Several cellular and molecular protagonists are well known. Fibroblasts and their α-smooth muscle actin–expressing derivatives, myofibroblasts, are responsible for the excessive ECM deposition characteristic of many fibrotic conditions. Injured epithelial cells—including, in lung fibroses, alveolar type II cells responsible for secreting lung surfactant—are believed to trigger or exacerbate fibroblast activity, and endothelial cells, too, are implicated in this and other pro-fibrotic processes. Immune cells including macrophages, inflammatory monocytes, T cells and neutrophils also play parts in fibrosis's intricate cellular dance, though the precise relationship between immune-mediated inflammation and fibrosis remains inadequately understood.

On the signaling side, TGFβ is considered a ‘master regulator’ in IPF, triggering the differentiation of fibroblasts into myofibroblasts. Stored in an inactive form in the ECM, TGFβ is switched on by cell surface receptors called integrins. The enzyme autotaxin activates myofibroblasts further upstream via the lysophosphatidic acid (LPA) signaling pathway, which is implicated in a wide range of cellular processes. Galectin-3, a carbohydrate-binding lectin, is another promiscuous signaling molecule shown to influence integrin and TGFβ activity and to impact all the key IPF cell types, including pro-inflammatory macrophages, pro-fibrotic fibroblasts and epithelial cells (Fig. 1).

More recently, single-cell genomic, proteomic and transcriptomic techniques have uncovered further cellular subtypes and interactions involved in fibrosis pathobiology, including functionally distinct subpopulations of mesenchymal, fibroblast and macrophage cells. Computational techniques and diffusion maps are also allowing scientists to capture these cells’ evolution and differentiation during the fibrotic process.

**The selectivity challenge**

Because many of the players implicated in fibrosis are also central to other processes, targeting them with selectivity and sensitivity is key. TGFβ, for example, also controls inflammation and cell growth, differentiation and apoptosis. A systemic shutoff is nastily toxic, as seen in several cancer trials targeting the molecule. Biogen tried hitting an important ‘on’ switch for TGFβ, αvβ6 integrin, with an antibody from its 2012 acquisition of Stromedix. That didn’t work either: the antibody was axed in 2019 due to safety signals, and so was an inhaled integrin candidate from GlaxoSmithKline. In early 2022, AbbVie axed two αvβ6 integrin inhibitors licensed two years earlier from Morphic Therapeutic because of on-target safety signals found in preclinical testing.

The autotaxin system also has broad-ranging effects. Galapagos’s phase 3 autotaxin inhibitor ziritaxestat was shut down in early 2021 by an independent data-monitoring committee in part because of toxic interactions with Ofev, now part of the standard of care (SOC). This had a knock-on effect on Galecto, which in the same year to exclude patients with IPF also of toxic interactions with Ofev also on SOC from its phase 2b trial of GB0139. That compound inhibits galectin-3, a multifunctional protein that is widespread inside and outside many cell types.

“We’re good at identifying pathways [involved in fibrosis] but not their hierarchy of importance—which are critical or redundant downstream,” says Toby Maher, professor of medicine at the University of Southern California’s Hastings Center for Pulmonary Research, who has been involved in several past and current IPF trials.
Another challenge for IPF drug developers is the lack of a clear, noninvasive biomarker for the disease, which is diagnosed by lung function tests, chest X-rays and high-resolution CT scans.

**Fibrosis and inflammation**

Two members of the thinning mid- to late-stage IPF pipeline are best known for their anti-inflammatory properties: Boehringer Ingelheim's PDE4B inhibitor BI 1015500 and Roche's phase 3 hopeful zinpentraxin alfa (Table 1).

Until about 15 years ago, IPF was considered primarily an inflammatory disorder. Corticosteroids were (and still are) used off-label in some interstitial lung conditions. But when steroids were found to have little or no impact on IPF, scientists inferred that inflammation wasn't the culprit. Focus shifted to abnormal tissue remodeling—the fibrotic aspect of the disease. It is believed to stop macrophages from differentiating into a pro-fibrotic variety and to inhibit neutrophil adhesion to ECM proteins, which contributes to the faulty ECM structure found in fibrosis. (Pentraxin may also dampen the activity of collagen-producing cells called fibrocytes, though their role in fibrosis is disputed, according to Sheppard.) A phase 3 trial of zinpentraxin alfa, including patients on existing SOC (Ofev or Esbriet), is due to complete by the end of 2023.

PDE4 inhibitors such as Daxas (roflumilast) or Ortega (apremilast) are approved for inflammatory airway diseases and psoriatic arthritis, respectively. But animal and in vitro experiments over the last few years suggest that the class may also have a direct antifibrotic effect. In rodent lung extracts, roflumilast reduced expression of TGFβ, connective tissue growth factor (CTGF) and various extracellular matrix proteins, such as collagen α1 and pro-fibrotic endothelin-1.

Boehringer's BI 1015500, by virtue of preferentially inhibiting the PDE4B subtype, may avoid emesis, a well-known class side-effect associated with the D subtype.

Table 1 | Selected clinical IPF candidates

| Compound/company | Stage of development | Mechanism of action |
|------------------|----------------------|---------------------|
| Zinpentraxin alfa/Roche (previously Promedior) | Phase 3 | Recombinant human pentraxin-2; regulates immune cell-ECM interaction |
| Pamrevlumab/Fibrogen | Phase 3 | Targets CTGF, involved in tissue remodeling and ECM synthesis |
| BI 1015550/Boehringer Ingelheim | Phase 3 (not yet recruiting) | PDE4B inhibitor with anti-inflammatory effect; may have direct antifibrotic effect through TGFβ and CTGF |
| Inhaled treprostinil (sold already as Tyvaso for PAH)/United Therapeutics | Phase 3 | Analog of prostacyclin, which has anti-inflammatory properties in the lung but can be pro-inflammatory elsewhere. |
| GB0139/Galecto | Phase 2b | Inhibits galectin-3, expressed on inflammatory and fibrotic cell types and acts as a multifunctional signaling molecule |
| PLN-74809/Pliant | Phase 2a | Inhibits integrins αvβ3 and αvβ6, found in fibrosis-linked fibroblasts and epithelial cells. |
| BMS986278/Bristol Myers Squibb | Phase 2 | Inhibits the LPA1 receptor found on multiple fibrosis-implicated cell types |
| HZN-825/Horizon Therapeutics | Phase 2b | Inhibits LPA receptor 1 |
| Taladegib/Endeavor Biomedicines | Phase 2 | Small-molecule hedgehog signaling inhibitor |
| Cudetaxestat/Blade Therapeutics | Phase 1b/2 | Allosteric inhibitor of autotaxin |

CTGF, connective tissue growth factor; ECM, extracellular matrix; LPA, lysophosphatidic acid; PDE4B, phosphodiesterase 4B; TGFβ, transforming growth factor β.

Phase 2 data (147 patients, 12 weeks) published in the *New England Journal of Medicine* in May 2022 showed a promising reduction in the rate of lung function decline (measured as forced vital capacity, FVC), both in patients on SOC and in those not, with no severe side effects. Phase 3 is expected to start later this year.

Fibrogen's pamrevlumab is another member of the dwindling phase 3 class of IPF candidates. It targets CTGF, which acts downstream of TGFβ in tissue remodeling and ECM synthesis and is also pro-inflammatory. A cross-trial comparison published in 2020 suggested the antibody may beat both Ofev and Esbriet in reducing lung function decline, though neither of Fibrogen's ongoing phase 3 trials include patients on either SOC treatment. The company recently completed enrollment in a 356-patient phase 3 trial in those ineligible for or intolerant of current IPF therapies; a second trial is underway in previously treated individuals.

**An inhaled first-line therapy?**

Copenhagen-based Galecto had hoped its inhaled once-daily galectin-3 inhibitor GB0139 would become an easy-to-use add-on to SOC. The company's phase 2b program began with patients also taking either of the existing IPF drugs. But in March 2021, an independent data-monitoring committee found an "imbalance" of serious adverse events in trial patients taking both SOC and GB0139 and in those taking only the higher (10-mg) dose of the Galecto drug (which was well tolerated in a small phase 1/2a study).

Coming just a few weeks after a similar committee axed Galapagos's phase 3 autotaxin inhibitor due to drug interactions with Ofev, the restrictions on Galecto's trial point to regulators on high alert. Galecto's phase 2b study resumed in July 2021, but only in patients taking a lower-dose monotherapy. The trial, already delayed by Covid-19 and the war in Ukraine (where one study site is located), is expected to report in mid-2023.

GB0139 acts on both fibroblasts and macrophages, hitting both the fibrotic and inflammatory aspects of the disease. It also appears to significantly depress a range of plasma biomarkers associated with fibrosis, including PDGF, PAI-1, YKL40 and MCP1. No single biomarker has yet been validated as sufficiently predictive of disease to be used as a surrogate endpoint, though companies such as Copenhagen-based Nordic Bioscience have identified a range of biomarkers that help quantify pathological changes in fibrotic tissue. "Hitting a range of [fibrosis-associated] biomarkers is a stronger
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signal than just one biomarker standing out," claims Galacto CEO Hans Schambye, adding that both existing IPF drugs, and many of those in development, have little or no impact on these markers. Nonetheless, galectin-3’s pleiotropic action increases the risk of unwanted effects.

If it succeeds, GB0139 could reach a sizeable share of patients with IPF, even if restricted to those not on SOC. up to 40% discontinue treatment with Ofev or Esbriet. Schambye also believes Galacto's candidate could offer a first-line alternative for the estimated 20–30% of patients newly diagnosed with IPF each year, though designing an appropriate phase 3 trial for first-line use could be challenging.

Selective integrin inhibition

For Pliant CEO Bernard Coulie, any new IPF drug “has to be safe on top of SOC”— even though there may be little or no biological rationale for the combination. Pliant has doubled down on integrins, hoping that its α,β, and α,β, dual-integrin inhibitor PLN-74809, currently in phase 2 for IPF and primary sclerosing cholangitis (fibrosis of the bile ducts), can escape the fate of its predecessors thanks to its selectivity and improved pharmacokinetics.

Pliant cofounder Dean Sheppard singled out the α,β, and α,β, integrin subtypes as most relevant, as they activate TGFβ only in fibrotic tissue, rather than systemically. α,β, is solely expressed in connective tissue fibroblasts, and α,β, only on epithelial cells. Both are scarce in healthy individuals. Plant synthesized a small molecule, PLN-74809, that blocks these two subtypes by occupying the integrin RGD (arginine-glycine-aspartic acid)-binding pockets normally used to activate TGFβ. The result, according to Coulie, is antifibrotic activity “approaching complete TGFβ blocking but limited to fibrotic tissue.”

The effect is more powerful because it cuts the feedforward loop that occurs when activated TGFβ, sensing a stiffening ECM, in turn ramps up integrin expression and recruits more fibroblasts, leading to worsening fibrosis and mortality. “It’s the first time a drug [for IPF] addresses the full complexity of the disease and not just one element,” says Coulie. A 50-hour half-life and high bioavailability should also help the compound stand out from other integrin blockers, the company hopes (Box 1).

A phase 1b proof-of-mechanism trial in February 2022 reported strong evidence of TGFβ inhibition, as measured by downstream Smad protein signaling and phosphorylation, with only mild adverse events. The more recent phase 2a data backed that up and went further. Besides confirming safety and tolerability (the primary endpoints), over 12 weeks of treatment, the trial showed dose-dependent effects on FVC and on qualitative lung fibrosis (as measured by high-resolution CT imaging). FVC, used as a registration endpoint in IPF, was only an exploratory endpoint in the 90-patient trial, which was underpowered to show a significant effect. Still, the 80% reduction in FVC decline (across all doses) and 26.5-ml increase in FVC in patients receiving the middle dose of 80 mg “is quite spectacular,” said Coulie, on a July 10 call announcing the results. About 80% of patients were also on SOC. (Pliant went on to raise $230 million in an upsized public offering on July 15.)

Unlike Galacto’s GB0139, Pliant’s PLN-74809 didn’t raise any safety concerns when the data-safety monitoring board looked at the ongoing placebo-controlled phase 2 trial in February 2022. Instead, the board “allowed us a higher dose level, and longer treatment” duration, says Coulie. (The highest daily dose in the recently reported trial was 160 mg; data from patients taking 320 mg will be reported in early 2023.)

Pliant’s compound already appears more tolerable than either Ofev or Esbriet and has proven safe in many more patients than Biogen’s α,β, integrin antibody, which threw up negative signals in far smaller trials, according to Maher, enlisted as a key opinion leader on the Pliant call and not involved in the trial. For him, the data offer “reassurance that this [integrin] pathway can be blocked safely.”

But even with these promising phase 2a results, the US Food and Drug Administration (FDA) is unlikely to allow Pliant to jump directly to phase 3 trials, given the safety issues hampering IPF candidates. “The problems with integrin blockers tend to show up in larger trials,” says Galecto’s Schambye. “They all end up being toxic.”

Threading the needle with autotaxin inhibition

Prior class failures can also provide valuable lessons. San Francisco, CA-based Blade Therapeutics believes its autotaxin inhibitor cudetaxestat can avoid the toxicity issues that befell Galapagos’s ziritaxestat in 2021.

Autotaxin catalyzes the conversion of lysophosphatidylcholine (LPC) to LPA, a signaling lipid that triggers myofibroblast activation and differentiation. LPA is present on both epithelial cells and fibroblasts, working upstream of TGFβ to activate it via integrins, but also activating other pro-fibrotic genes and mediators (Fig. 1).

After testing of ziritaxestat was halted due to suspected interactions with Ofev, the FDA advised Blade to assess the likelihood of similar issues with cudetaxestat. Blade found in pre-clinical tests that ziritaxestat strongly inhibits p-glycoprotein, a transmembrane pump for which Ofev is a substrate. That activity ramps up Ofev plasma concentrations, exposure and hence toxicity. Cudetaxestat, in contrast, does not inhibit or bind p-glycoprotein at physiological concentrations, potentially making it safer to coadminister with currently approved therapies.

Blade believes cudetaxestat may also be more potent than previous autotaxin inhibitors because of where it binds autotaxin. Unlike ziritaxestat, it does not compete with LPC for the active site, but instead binds autotaxin allosterically, leading to a conformation change that reduces the enzyme’s ability to bind LPC. In fibrotic tissue, high LPC concentrations may overwhelm competitive-binding autotaxin inhibitors, limiting their potency. Cudetaxestat escapes this issue. “Our molecule stays on target a long time,” says Robbins, citing a 10-hour half-life and good tissue penetration. “We are threading the needle, with [a compound that’s] allosteric, very potent and clean.”

Yet autotaxin, like TGFβ, is involved in many other systems that have nothing to do with fibrosis. “There’s no fibrosis-specific

Box 1 | Pan-integrin inhibition

The jury’s still out on whether singling out specific integrins is advisable. Scientists at Merck are investigating a pan-α, inhibiting antibody, Ab-31, found to have potent antifibrotic activity in lung fibroblast cells from patients with IPF (more so than normal lung fibroblasts) and, possibly, to dampen TGFβ not only by blocking its activation but also through other downstream mechanisms. Given “the complexity of integrin biology, and the overlapping roles of multiple integrins in fibrosis progression,” a broader inhibition approach may work best, the researchers write in a 2021 paper in Scientific Reports. Yet they acknowledge the safety concerns associated with integrin-blocking. For UCSF’s Sheppard, pan-integrin inhibition is a bad idea. "Why target them all, if only two are important? Each of the others brings additional toxicity."
The ECM is made up of collagens and elastic fibers in a gel of proteoglycans and glycoproteins. Far from being a passive framework filling up the space between tissue cells, it is a dynamic, bioactive physical scaffold that receives incoming signals and modulates behavior for the worse, says Mazza. An unhealthy ECM has also been shown to encourage cancer cell growth.

Because most TGFβ is stored in an inactive form in the ECM, any changes to ECM structure influence TGFβ activation, plus “a lot more besides,” including TGFβ-independent pathways, says Mazza. “We’ve spent ten years trying to deconvolute these mechanisms.” Nordic Bioscience is also focused on the ECM in its quest for fibrosis biomarkers: its “protein fingerprint” markers pick up fragments of collagen released into the bloodstream as ECM proteins are cleaved or degraded.

Engitix uses its ECM-based discovery platform—which includes a human tissue biobank, in vitro, and in vivo disease models and AI-powered structure-based design and screening—to find targets and biomarkers across fibrosis and solid tumors. The company can probe what a disease-specific ECM looks like, and how it influences the cells around it, by removing cells from a diseased organ or tissue sample and performing proteomic analysis of the decellularized tissue scaffold. Re-seeding cells types into the ECM can help identify ECM-driven targets using comparative RNA sequencing.

The company, spun out of University College London in 2016, is also working with Takeda to find drug targets in liver fibrosis and fibrostenotic inflammatory bowel disease. In January 2022, Engitix raised $54 million and announced a partnership with Dompe Farmaceutici SPA, which also invested in the round.

Early activity continues, but licensors await data

Single-cell RNA sequencing and other tools have reinvigorated IPF R&D and startup activity. Sheppard’s team at UCSF have uncovered new subsets of collagen-producing cells involved in fibrosis and mapped out fibroblasts’ stepwise path from normal to pro-inflammatory and pro-fibrotic, throwing open more potential drug targets and intervention sites. Lausanne, Switzerland-based Haya Therapeutics, seeded in 2021 and a resident of JLABS San Diego, is hitting long noncoding RNAs to regulate the conversion of fibroblasts to myofibroblasts. Anteros was spun up in 2020 by Bristol Myers Squibb and accelerator BioMotiv with IP from Yale University, though it’s not revealing the mechanism behind its small-molecule approach to IPF and liver fibrosis.

As for when the next IPF therapy may emerge, “I feel hopeful,” says Sheppard, given the rapid progress in our understanding of the condition. Galecto’s Schambye is equally optimistic. But, he adds, “IPF is IPF.”