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Preclinical Evaluation of new Antifibrotics in IPF Rodent Models

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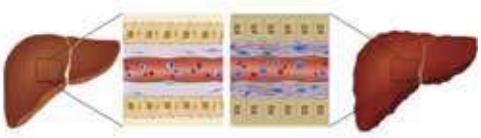
(Sources: Public information and Case studies from Aragen)

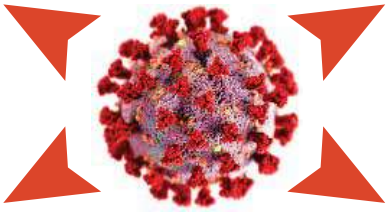
Normal **Fibrosis**

LUNG
BLM-Induced: Young
BLM-Induced: Aged Mice
Interstitial Lung Disease
Silica-Induced

Normal **Fibrosis**

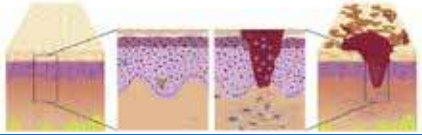


LIVER
CCl4, TAA
NASH fibrosis: CDAHFD,
WD+CCl4, AMLN Diet
DDC induced biliary fibrosis



Mouse Corona Virus Model

Normal **Fibrosis**



SKIN
Bleo-Induced
Scleroderma

Normal **Fibrosis**

KIDNEY
Sodium Oxalate induced
Adenine Induced
UUO

Executive Summary

According to the Pulmonary Fibrosis Foundation (PFF), there are about 200 types of interstitial lung diseases (ILD), which are characterized by inflammation, scarring, or both, lung damage and decrease in its ability to absorb oxygen from the air. Majority of ILD manifest as Idiopathic Pulmonary fibrosis (IPF) due to scarring of the lung tissues. Nearly, 250,000 Americans are living with IPF and ILD with more than 50,000 new cases diagnosed annually. Main factors that cause IPF, include airborne contaminants, radiation treatments, some medications, genetics, autoimmune diseases. With no known cure, IPF initiates mortality within 3-5 years from diagnosis. Presently, only two approved antifibrotic drugs pirfenidone and nintedanib are present in the market but both fail to stop disease progression. Therefore, urgent attempt is required to develop new therapies aided by effective and relevant IND-enabling preclinical models for clinical evaluation of new drugs. Recent reports implicate SARS-CoV 2 infection in causing lung fibrosis through multiple signaling pathways and TGF-β activation. Aragen scientists have developed a mouse corona virus model to study lung fibrosis along with other 17 fibrosis models, including lung, liver, kidney, scleroderma, NASH-Fibrosis and Biliary Fibrosis. Our scientists have extensive experience and expertise with preclinical bleomycin induced- IPF rodent models for testing new antifibrotic drugs. For more information, contact us by visiting: Fibrosis - Aragen Life Sciences

Key words: Interstitial lung diseases (ILD), Idiopathic Pulmonary fibrosis (IPF), bleomycin-induced IPF, antifibrotics, Preclinical and clinical development, Inflammatory response, pharmacology, toxicology

Introduction

A recent study found that among seventeen methodologically heterogenous studies that examined the incidence, prevalence, and relative frequencies of ILDs, the incidence of ILD ranged from 1 to 31.5 per 100,000 person-years and prevalence ranged from 6.3 to 71 per 100,000 people (1). Nearly, 250,000 Americans are living with interstitial lung diseases (ILD) and idiopathic pulmonary fibrosis (IPF). Main factors causing IPF, include airborne contaminants, radiation treatments, some medications, genetics, and autoimmune diseases. Presently, there are only two approved antifibrotic drugs pirfenidone and nintedanib. However, both drugs partially slow down the rate in lung function decline but do not stop disease progression (2). New antifibrotics need to be tested through IND-enabling preclinical models for eventual clinical evaluation in humans. Recent clinical reports implicate SARS-CoV infection in causing lung fibrosis (3) and kidney fibrosis (4) through multiple signaling pathways. Aragen has developed a mouse corona virus model to study lung fibrosis for BSLII studies, along with other 17 fibrosis models, including lung, liver, kidney, scleroderma, NASH-Fibrosis and Biliary Fibrosis. Our scientists have extensive experience and expertise with preclinical IPF rodent models for testing new antifibrotic drugs. In summary, this article includes in-house as well as client-sponsored study data developed over several years using rodent models. These studies generated clinically pertinent information for proper testing of new vaccines and antivirals, including few that are already in various phase of clinical trials. Aragen will continue to support your Antifibrotics development projects by providing reliable and effective preclinical services in this important disease area.

Understanding mechanism of Fibrosis disease progression for developing new Antifibrotics:

Pathogenesis of fibrosis is complex. The repetitive and constant injury “scarring” leads to a sustained and self-perpetuating activation of fibroblasts, leading to their trans differentiation into synthetic and highly contractile α–smooth muscle actin (αSMA). This leads to expression of myofibroblasts that deposit in extracellular matrix (ECM) causing stiffening and altering the normal lung architecture leading to decreased lung function (5). The matrisome of fibrotic ECM is a major process towards chronic disease progression. Growth factor–β1 (GFβ1) binding to the TGFβ1 receptor triggering downstream signaling by posttranslational modifications of cytoplasmic members of the SMAD family, which act as transcription factors in the cell nucleus, regulating the expression of common profibrotic genes, including ECM proteins (5). Plasminogen activator inhibitor-1 (PAI-1) is considered as a therapeutic target option for fibrosis because it is a key signaling molecule in the TGFβ1 pathway. Also, in IPF, profibrotic interleukin-8 (IL-8) was found to be secreted by a special fibrogenic mesenchymal progenitor cell population with autocrine effects on proliferation and motility and paracrine effects on macrophage recruitment. Target-based antifibrotic drug discovery failed to go to clinics because many molecules are required to block multiple signaling pathways involved in fibrosis disease pathogenesis. On the contrary, pathway-unbiased phenotypic drug screening succeeded in discovery of first-in-class drugs (5). This success is attributed to the application of physiological relevant stimuli, and a readout close to the clinical end- point observed in patient-derived primary cells (5). Multifunctional transforming TGFβ1 is shown to be a key factor in various fibrotic diseases capable of triggering trans differentiation of fibroblasts into myofibroblasts (6). These developments resulted in rigorous effort for developing new and effective treatment strategies.

How Aragen is different from other CROs?

Aragen is working on the current and clinically relevant models including, rodent models, *ex vivo* models, and *in vitro* models of pulmonary fibrosis for drug discovery efforts. In consultation with clients and partners, our scientists use the appropriate preclinical model system that is required to improve your drug development pipeline for pulmonary fibrosis. Aragen has over 75 combined years of experience in fibrosis preclinical service with more than 600 successful fibrosis studies for 50+ customers. Out of which >10 programs are in advanced in Phase II clinical studies.

A very small number of efficacious compounds against IPF move to clinical trials, although several compounds show efficacy against pulmonary fibrosis in animal models. Hence relevant preclinical animal models are key to increase in success of preclinical IND-enabling process. We need to better identify, characterize, and select clinically useful targets in the animal models that CROs offer. Aragen scientists understand this dilemma and consequently offer most appropriate animal models. Our preclinical fibrosis services help in improving the identification and characterization of clinically relevant molecules or pathways responsible for progressive fibrotic diseases. We combine appropriate preclinical models, including 17 models- Fibrosis of Lungs, Liver, Kidney, Scleroderma, NASH-Fibrosis, biliary fibrosis, bleomycin induced IPF, and *ex vivo* (precision-cut lung slices) or *in vitro* models to assist your high-throughput drug discovery or validation of drug effects. For more information and to contact us, please visit: Fibrosis - Aragen Life Sciences

Case study 1: The bleomycin mouse model for testing antifibrotics against IPF

Over the years, numerous agents have been shown to inhibit fibrosis in this model (7,8). It is critical to distinguish between drugs interfering with the inflammatory and early fibrogenic response from those preventing progression of fibrosis, the latter likely much more meaningful for clinical application. All potential antifibrotic compounds should be evaluated in the phase of established fibrosis rather than in the early period of bleomycin-induced inflammation for assessment of its antifibrotic properties. Further care should be taken in extrapolation of drugs successfully tested in the bleomycin model due to partial reversibility of bleomycin-induced fibrosis, which is slightly different over time in young and aged animals. The use of alternative and more robust animal models, which better reflect human IPF, is warranted.

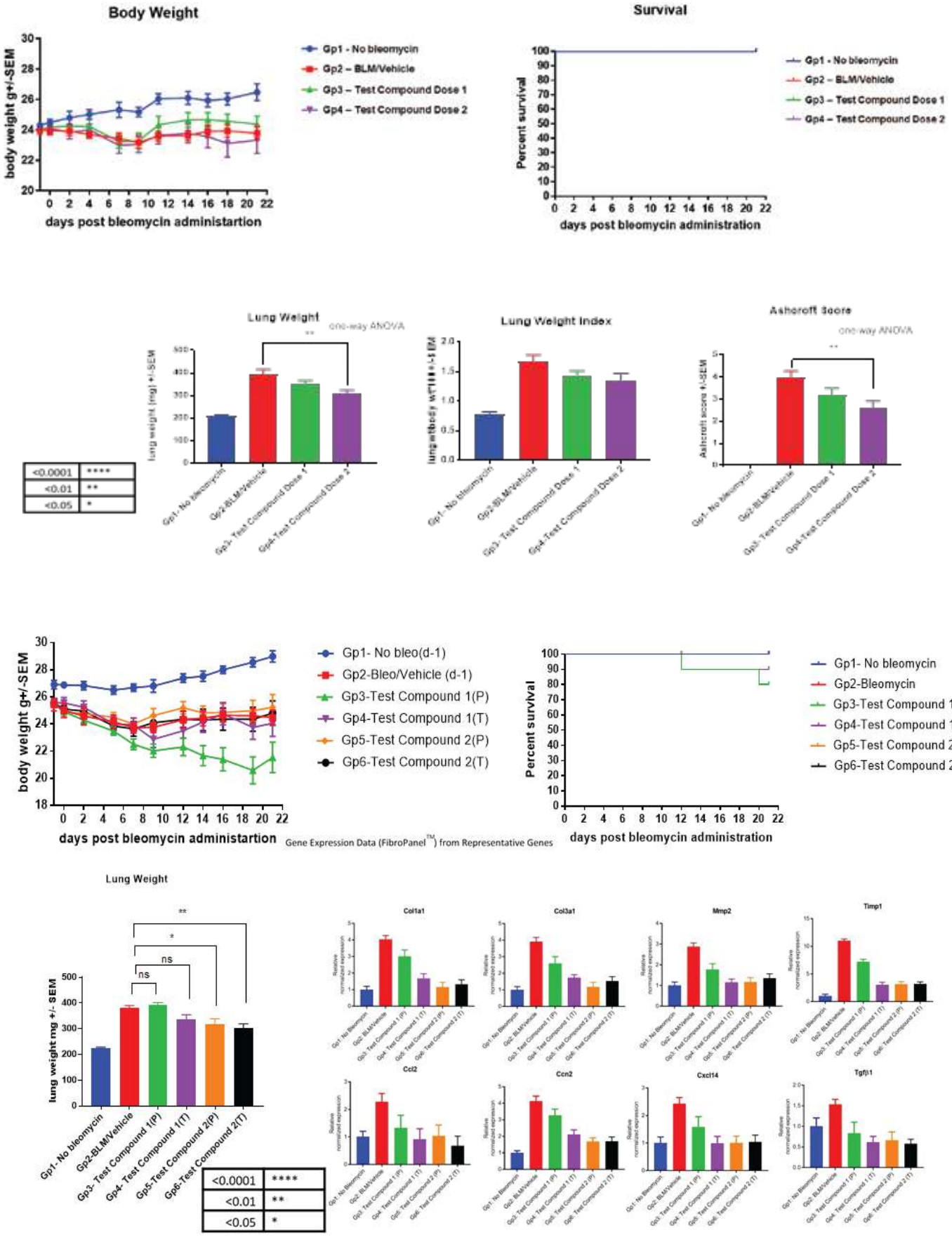
Standard methodology

Fibrosis induced in C57BL/6 mice with infusion of clinical grade Bleomycin via oropharyngeal route or through an osmotic pump. Various routes of administration of test articles (PO, IP, IV, IM, SC, nebulization, and osmotic pumps) and option to treat animals therapeutically or prophylactically are available.

Standard readouts include body weight, survival, lung weight, leukocyte count in bronchoalveolar lavage (BAL) and lung fixation for histology (H&E and trichrome staining).

Fibrotic readouts include lung hydroxyproline, serum/BAL soluble mediators, lung FibroPanelTM Gene expression, lung fixation for histology (H&E and trichrome staining at third party CRO) and differentials from BAL cells. Lung measurements include flexiVentTM, hypoxia related parameters and whole-body plethysmography.

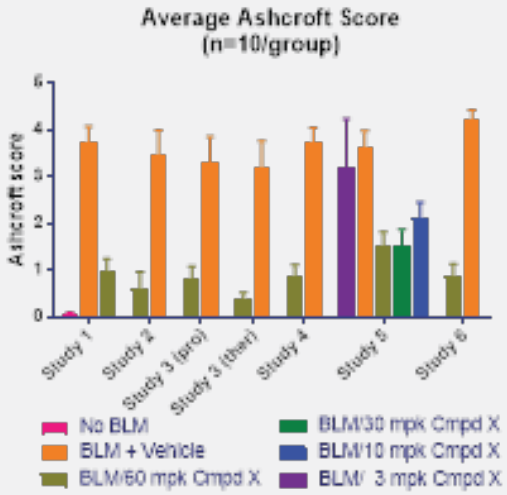
Case study 1 showing the readouts using the above-mentioned protocol

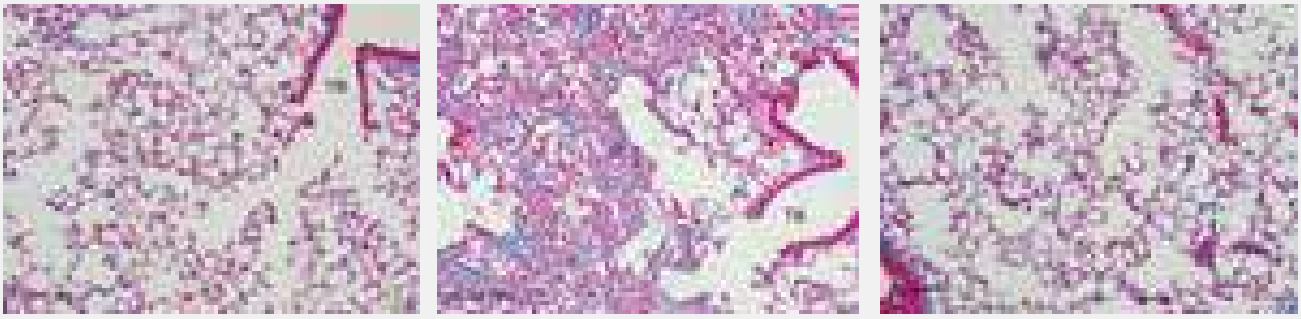


The above graphs show results from flexiVentTM analysis. It was observed that bleomycin instillation increases resistance and elastance while decreasing compliance and enhanced Respiratory pause (Penh) increased by Bleomycin. Pirfenidone treatment improved lung function measurements in mice.

Case Study 2: Consistency of fibrotic induction in lungs from multiple studies

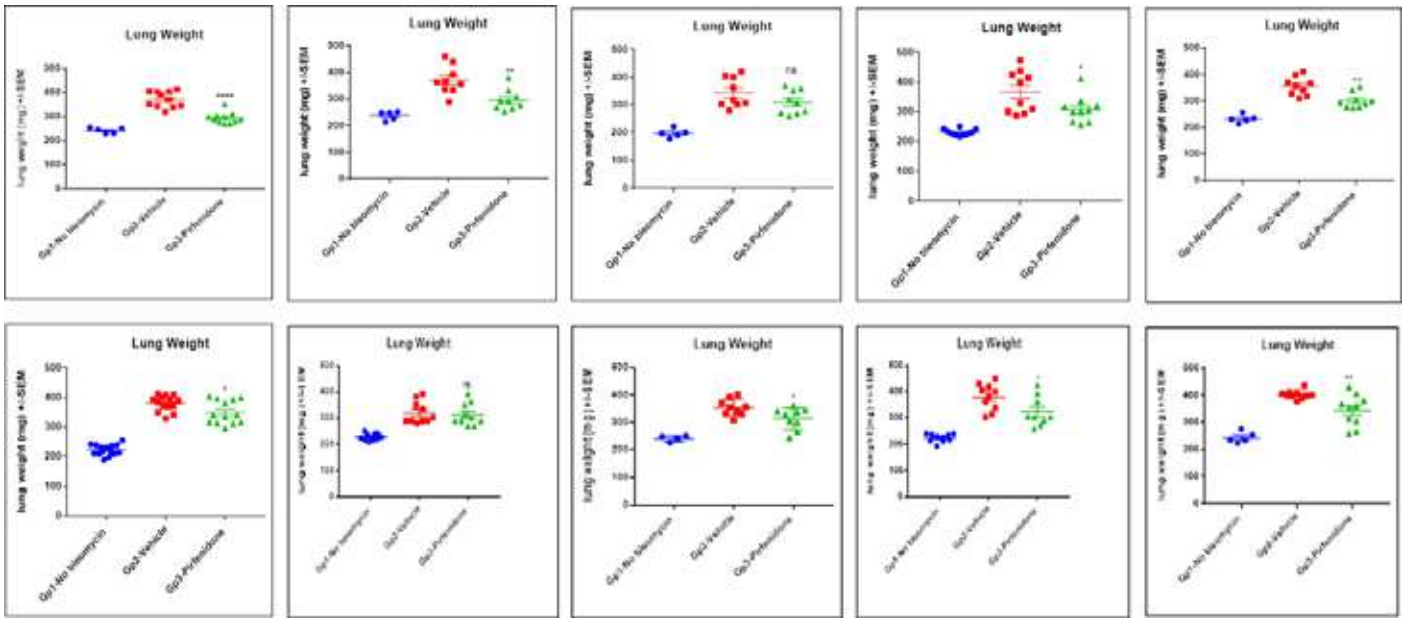
The following pictures shows that we achieved highly reproducible and consistent fibrotic induction (Ashcroft score) in lungs and pathology of tissues from six studies. Hence our established models allow for robust evaluation of candidate drugs.





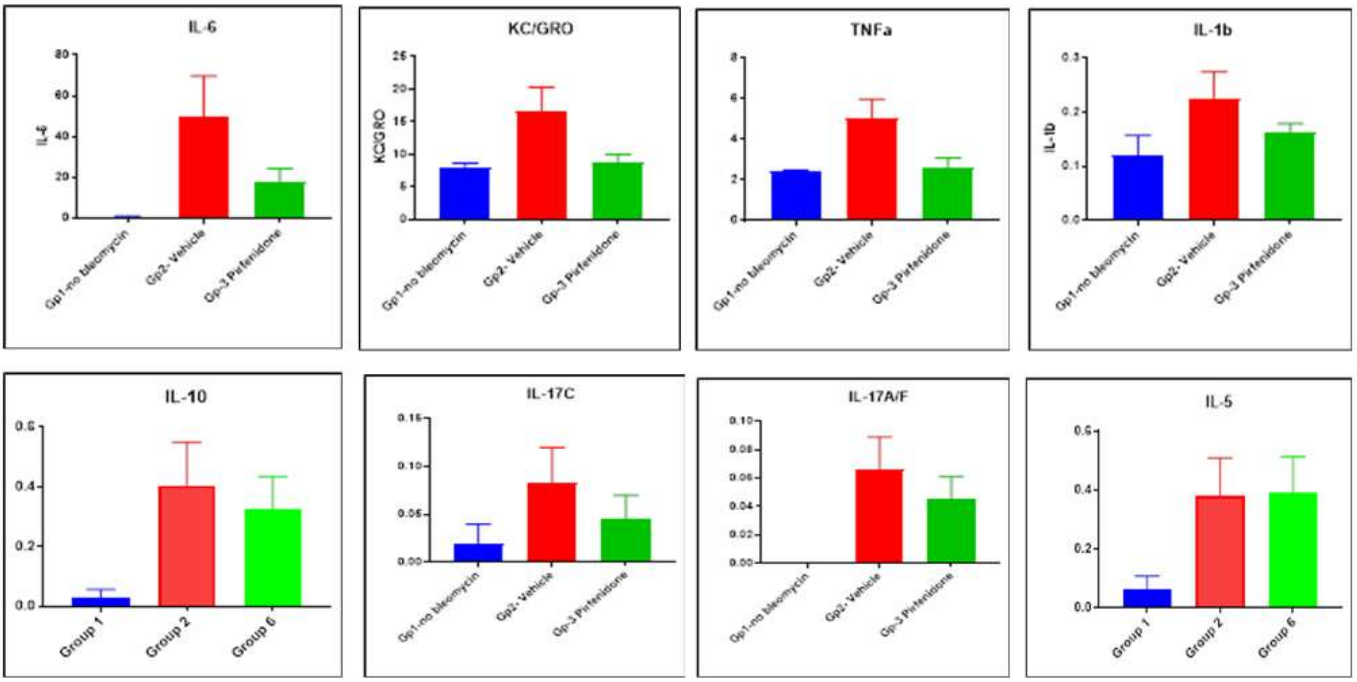
Case Study 3: Consistency of Bleomycin-induced pulmonary fibrosis

Multiple studies were performed to achieve consistency in our methodology and the lung weight measurements as shown in the graphs below.



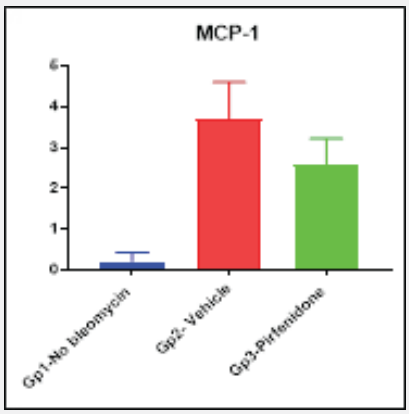
Case study 4: Pro-inflammatory cytokines measured in bronchoalveolar lavage fluid (BALF) of mice

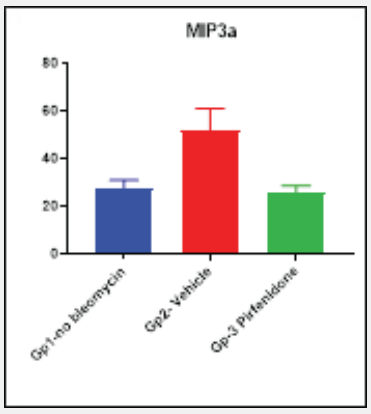
Bleomycin administration increased pro-inflammatory cytokines in group 2 (red) compared to no-bleomycin (control-blue) group. Pirfenidone treatment attenuated the cytokine response in group 3 (green) compared to bleomycin-administered group 2 (blue).

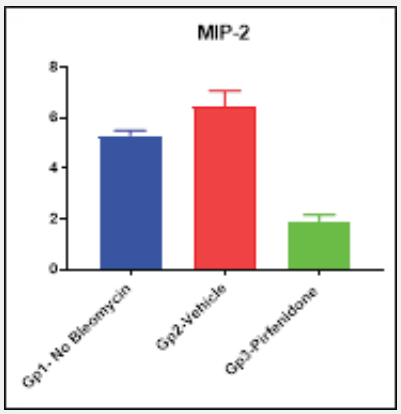


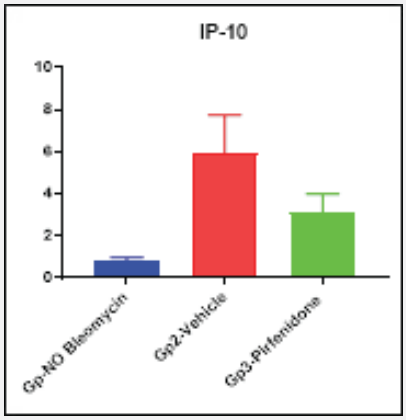
Case study 5: Pro-inflammatory chemokines measured in bronchoalveolar lavage fluid (BALF) of mice

Bleomycin administration increased pro-inflammatory chemokines (MCP-1, MIP3a, MIP-2, IP-10) in group 2 (red) compared to no-bleomycin (control-blue) group. Pirfenidone treatment affected the chemokine response in group 3 (green) compared to groups 2 and 3.









Summary

Recent published work helped us understand pathways responsible for progressive fibrotic diseases. The identification and characterization of clinically relevant molecules or pathways and targeting them with effective anti-fibrotics is essential to improve the drug development pipeline for fibrosis, including bleomycin induced- IPF. Selection of appropriate preclinical models supported by specific ex vivo (precision-cut lung slices) and in vitro assays should trigger high-throughput drug discovery or validation of drug efficacy and safety.

For more information and to contact us, please visit: Fibrosis - Aragen Life Sciences

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About the Author

Dr. Nagendra has held progressive leadership roles in pre-clinical and clinical oncology research and development. He worked at academic centers like-University of Kansas, Cedars-Sinai Medical Center, Mercer University Medical Center, Vanderbilt University Medical center, Anderson Cancer Institute. He led research teams on brain tumor and breast cancer biology. He had extensively published in peer-reviewed journals and secured US, EU and Japan patents. He directed the Human Tissue banking and biorepository and New Animal Facility. Later he worked in pharmaceutical companies such as Dr. Reddy's Labs, Scintilla BioMarc, PPD/ Thermo Fisher Scientific with focus on in vitro diagnostics, clinical pharmacology and toxicology, medicinal chemistry aspects of clinical drug development. He is presently a Global Senior Director of Scientific Affairs at Aragen Life sciences, USA.