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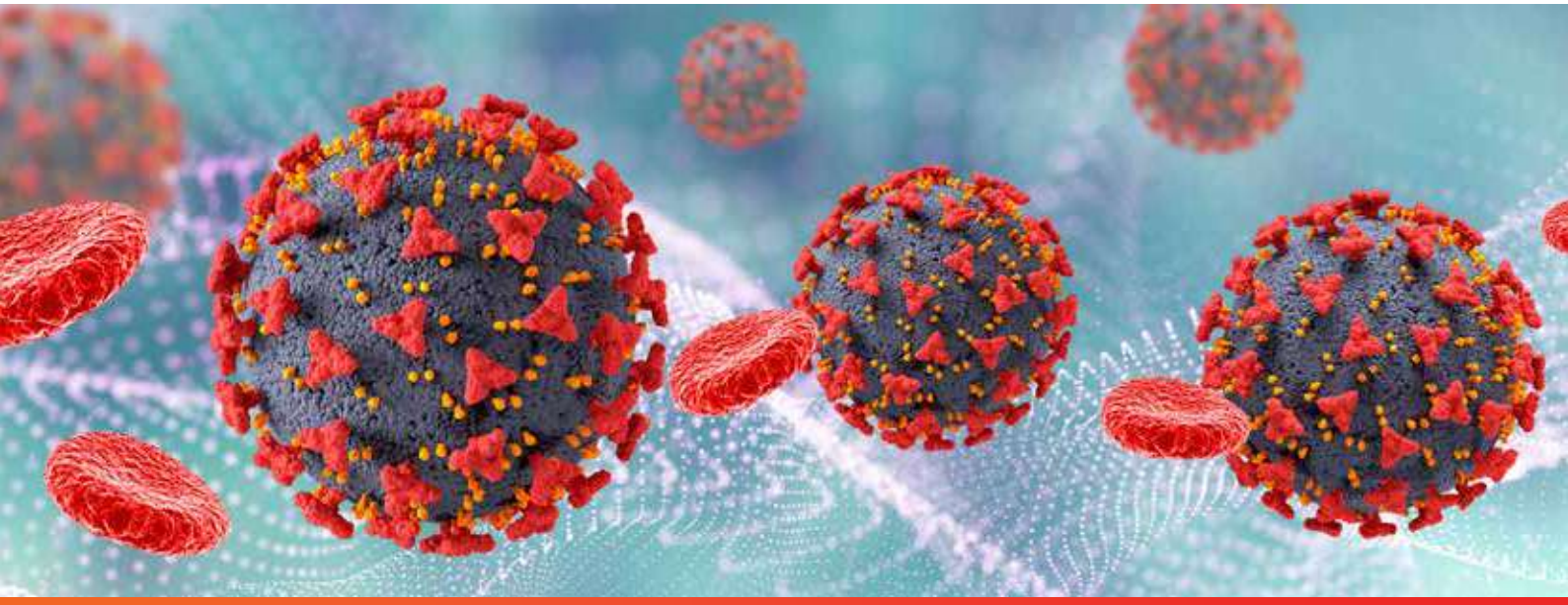

# Preclinical Evaluation of new Antifibrotics in Corona mouse Virus-Induced Fibrosis Model

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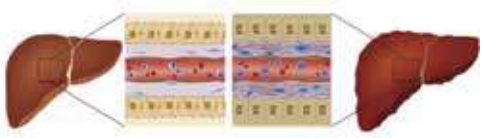
(Sources: Public information and Case studies from Aragen)

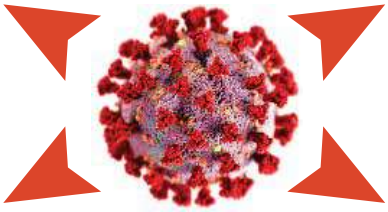
**Normal** **Fibrosis**

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

**Normal** **Fibrosis**

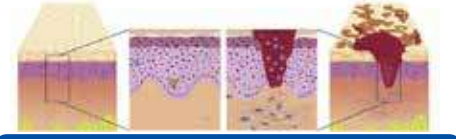


**LIVER**
CCl4, TAA
NASH fibrosis: CDAHFD,
WD+CCl4, AMLN Diet
DDC induced biliary fibrosis



**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. 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. Nonalcoholic steatohepatitis (NASH) is inflammation of your liver caused by excess fat cells in it, called as fatty liver disease. 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 antifibrotic drugs. Recent reports implicate SARS-CoV 2 infection in causing lung fibrosis through multiple signaling pathways and TGF-β activation. Aragen has 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. Aragen scientists have extensive experience and expertise with preclinical IPF rodent, including Corona mouse Virus-Induced Fibrosis model for testing new antifibrotic drugs. For more information, visit: Fibrosis - Aragen Life Sciences

Key words: Interstitial lung diseases (ILD), Idiopathic Pulmonary fibrosis (IPF), SARS-CoV2-induced kidney, lung fibrosis, 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). Approximately, 50,000 new ILD cases are diagnosed annually in the US. Main factors causing IPF, include airborne contaminants, radiation treatments, some medications, genetics, and autoimmune diseases (2). 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. New antifibrotics need to be tested through IND-enabling preclinical models for eventual clinical evaluation in humans.

Recent reports implicate SARS-CoV infection in causing lung fibrosis (3), kidney fibrosis (4), NASH (5) through multiple signaling pathways and TGF-β activation. 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. We 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 screening of new vaccines and antivirals, including few that are already in various phase of clinical trials. Aragen will continue to support your Antifibrotics development effort by providing reliable and effective preclinical services in this important disease area.

## Understanding mechanism of SARS CoV2 induced Fibrosis for developing new Antifibrotics

Mechanism of organ fibrosis pathogenesis caused by SARS CoV2 infection is complex (4). COVID-19 causes acute exacerbations of IPF higher mortality. Several studies have shown that SARS-CoV-2 directly infects lung (5) and kidney cells leading to lung and kidney failures. It was shown that COVID-19 infection increased tubule-interstitial kidney fibrosis in patient autopsy samples. Clinical results suggest that SARS-CoV-2 can directly infect kidney cells and induce cell injury with subsequent fibrosis. These data could explain both acute kidney injury in COVID-19 patients and the development of chronic kidney disease in long COVID infection (6). These developments resulted in rigorous effort for developing new and effective treatment strategies to attenuate fibrosis.

## How Aragen is different from other CROs?

Aragen is working on the current models including, rodent models, *ex vivo* models, and *in vitro* models of fibrosis for future 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 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. For more visit: Fibrosis - Aragen Life Sciences

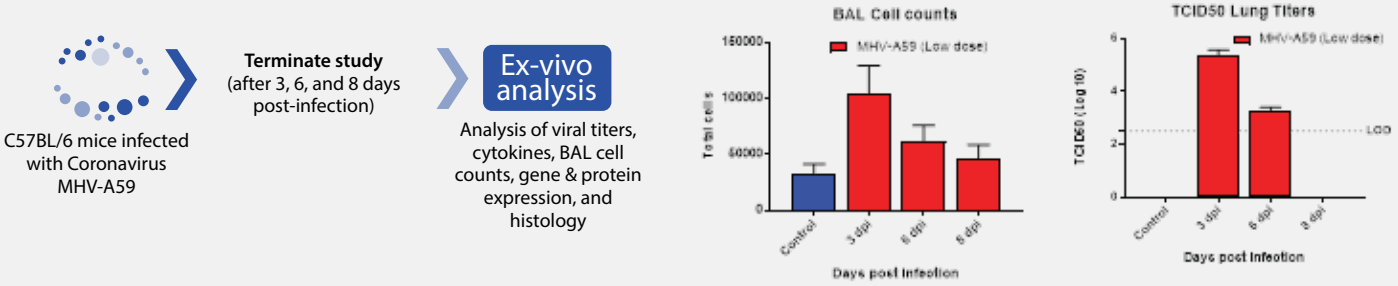
A very small number of efficacious compounds against fibrosis move to clinical trials, although several compounds show efficacy against pulmonary fibrosis in animal models. Hence relevant preclinical animal models are key to

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, Corona mouse Virus-Induced Fibrosis, and *ex vivo* (precision-cut lung slices) or *in vitro* models to assist your high-throughput drug discovery or validation of drug effects.

Over the years, numerous agents have been shown to inhibit fibrosis in this model. 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. The use of alternative and more robust animal models, which better reflect human SARS-CoV-2 -Induced Fibrosis as a surrogate model that can be studied in BSL-II set up, is warranted.

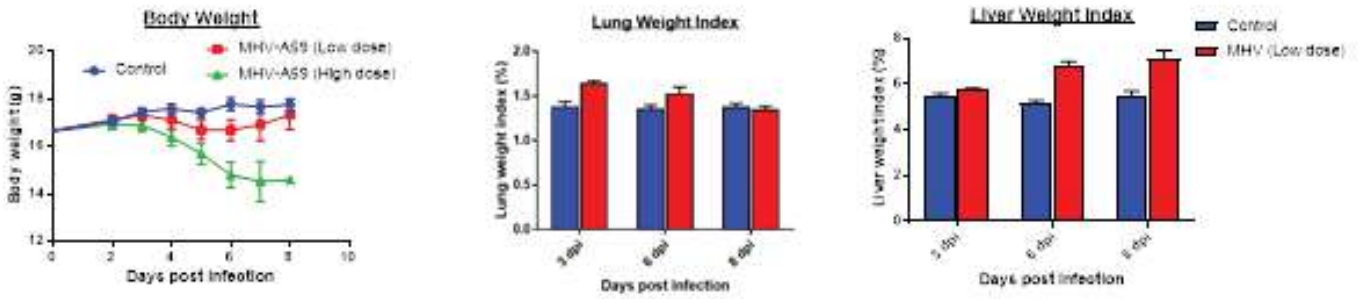
## Case study: Mouse Coronavirus Model (MHV-A59) development

Recent reports implicate SARS-CoV infection in causing lung fibrosis through multiple signaling pathways and TGF-β activation (5,6). Aragen has developed a mouse corona virus model to study lung fibrosis, which most SARS-CoV-2 infected patients develop. This model can be studied with BSL-2 Containment to evaluate vaccines and antivirals for treating respiratory infection with Fibrotic effects.

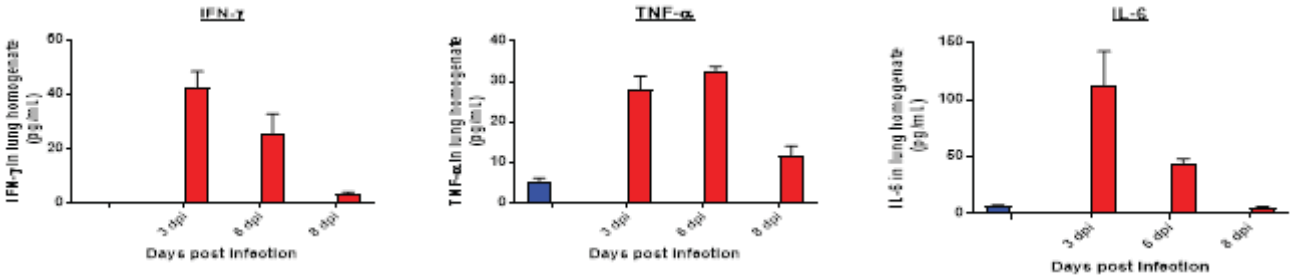



**Results:** We measured MHV-A59 viral load in lungs and in BAL cell counts (dpi) post infection with MHV-A59 (days 3-8) and analyzed 29 cytokines/chemokines. Viral load (TCID50) increased after 3 days post infection decreased on 6th and 8th day. Similar trend in BALF cell counts was seen after 3rd, 6th and 8th days. In this model, we observed that IL7 was significantly induced by MHV infection, which is like SARS-CoV-2-induced upregulation of critical cytokines often seen in COVID patients. Therefore, this mouse model serves as a surrogate SARS-CoV-2 model that can be studied in BSLII set up unlike BSLIII requirements for studies involving SARS CoV-2 models.

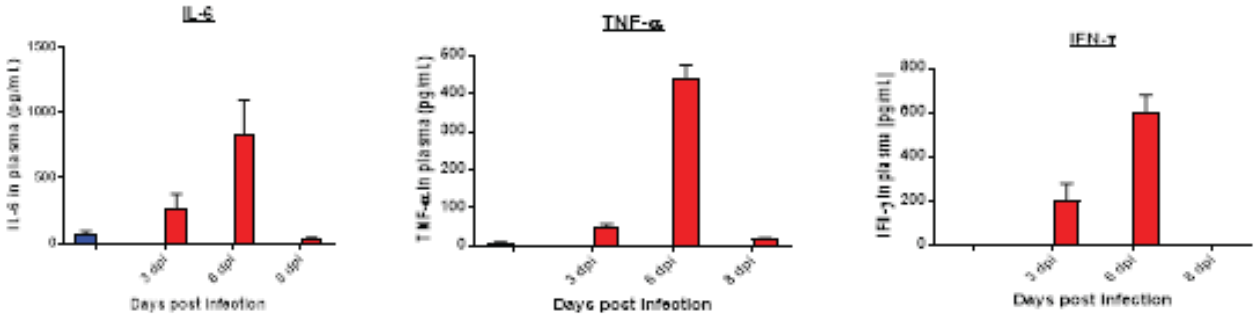
### Changes in Body, Lung, Liver weights post MHA-A59 infection in mice



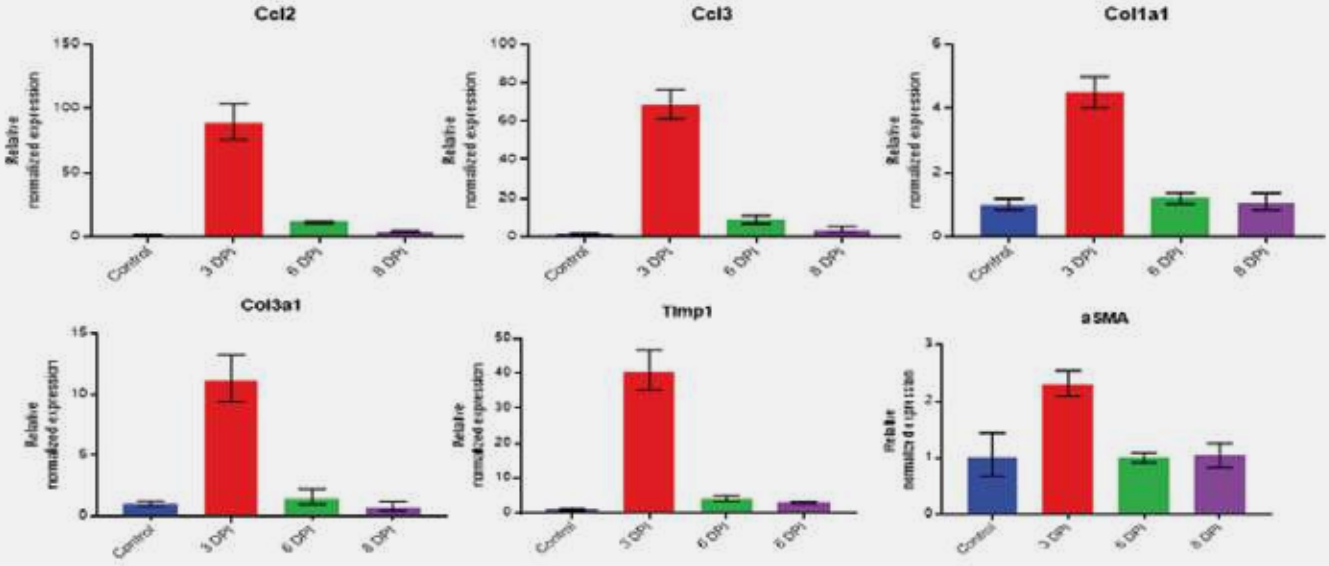
## Cytokine profile changes seen in mice lung homogenates post MHA-159 infection



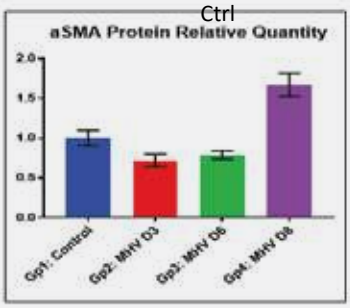

## Cytokine profile changes seen in mice lung homogenates post MHA-159 infection



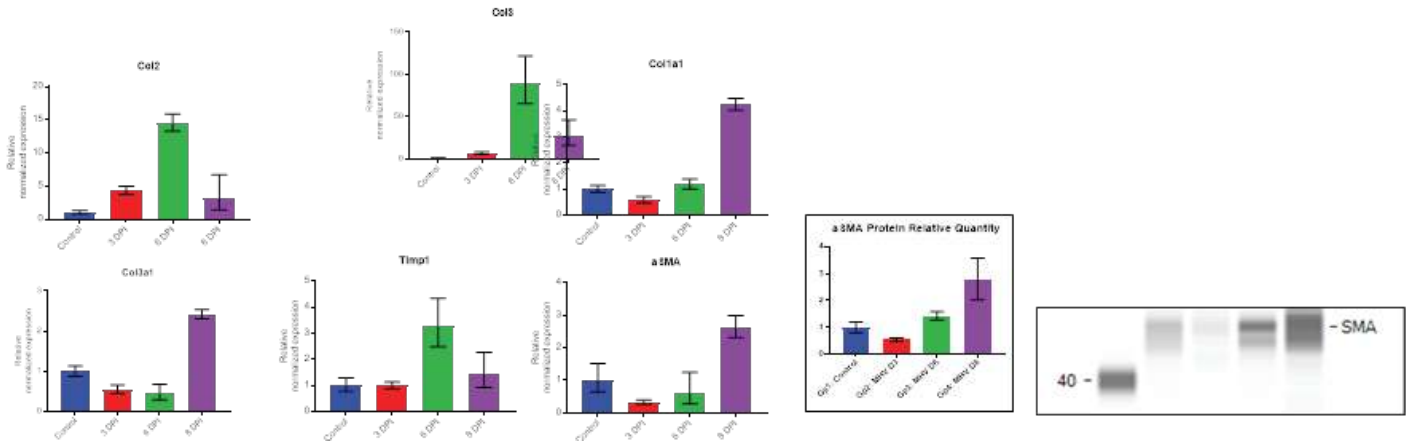

## Fibrosis -specific gene Expression in Lung Tissue




## αSMA Protein Expression in Lung Tissue:




**Fibrosis -specific gene Expression in Liver Tissue**




## 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 IPF.

The mouse corona virus model serves as a surrogate for SARS-CoV-2 model that can be studied in BSL-II set up unlike BSLIII requirements for studies involving SARS CoV-2 models. 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.

## 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.

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