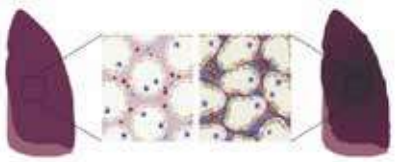
aragen.com

aragen

Preclinical Evaluation of new Antifibrotics in NASH-Induced Fibrosis Models

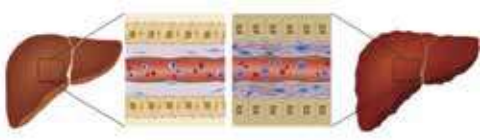
Author: Nagendra Ningaraj, PHD, MBA, CCRP
Sr. Director, Scientific Affairs
(Sources: Public information and Case studies from Aragen)

Normal Fibrosis

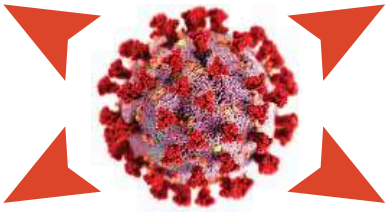


LUNG
BLM-Induced: Young
BLM-Induced: Aged Mice
Interstitial Lung Disease
Silica-Induced

Normal Fibrosis

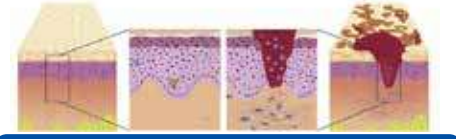


LIVER
CCl4, TAA
NASH fibrosis: CDAHFD,
WD+CCl4, AMLN Diet
DDC induced biliary fibrosis



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. 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. Aragen scientists have developed a mouse corona virus model to study lung fibrosis in BSL II set up, along with other 17 fibrosis models, including lung, liver, kidney, scleroderma, NASH-induced Fibrosis and Biliary Fibrosis. Our scientists have extensive experience and expertise with preclinical IPF rodent models for testing new antifibrotic drugs. For more information and to contact us, please visit: Fibrosis - Aragen Life Sciences

Key words: Interstitial lung diseases (ILD), Idiopathic Pulmonary fibrosis (IPF), Human Nonalcoholic Steatohepatitis (NASH), 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). Fatty liver disease caused by excess fat cells in it leads to inflammation in the liver and progressive liver damage referred as the nonalcoholic steatohepatitis (NASH) (2). NASH resembles hepatitis caused by alcohol use and most often associated with being overweight, high blood lipids and high blood sugar. NASH is a rapidly growing and global health problem compounded by the current absence of specific treatments. A major limiting factor in the development of new NASH therapies is the absence of models that capture the unique cellular structure of the liver microenvironment and recapitulate the complexities of non-alcoholic fatty liver disease (NAFLD) progression to NASH (3). 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 NASH disease progression for developing new Antifibrotics

Mechanism of pathogenesis of NASH is complex (4). The term NASH was coined in 80s to describe a new syndrome occurring in often diabetic and obese patients and females who had a liver biopsy. Although this clinical syndrome is somewhat better understood, no drug therapy is available for these patients. Patients with NASH present with obesity, diabetes, hyperlipidemia, hypertension, and, in some instances, other metabolic abnormalities such as polycystic ovary disease. Secondary NASH may be caused by drugs such as tamoxifen, certain industrial toxins, and rapid weight loss. Studies have shown that NASH induces inflammation, fibrosis, or necrosis in chronically ill patients for non-alcoholic steatohepatitis to develop. Increased cytokine activity, oxidative stress, and mitochondrial dysfunction trigger NASH. Researchers now better understand the clinical features, potential mechanisms of NASH and potential therapeutic interventions. This success is attributed to the application of physiological relevant stimuli, and a readout close to the clinical end point observed in patient-derived tissues (5). These developments resulted in rigorous effort for developing new and effective treatment strategies.

How Aragen is different from other CROs?

Aragen is developing current models including, rodent models, ex vivo models, and in vitro models of fibrosis for drug discovery efforts. In consultation with clients and partners, our scientists use 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.

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 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 Nonalcoholic Steatohepatitis, is warranted.

NASH-Fibrosis Models

Replicating human NASH in animal models is not possible due to variable interplay between environmental factors and genetic determinants observed in humans. Nevertheless, preclinical animal models are essential to understand NASH pathophysiology and test new antifibrotics. Among several mouse models of NASH, models based on overnutrition with adipose restriction/inflammation and metabolic complications, particularly insulin resistance, may be most useful to investigate critical etiopathogenic factors (5).

Some mice models that show inflammatory infiltrate and pattern of liver fibrosis observed in human NASH are good models for pharmacologic testing. On the other hand, mice that readily develop obesity with liver disease similar to human NASH serve as a good preclinical NASH model (6-8). These mice develop significant fibrosis due to specific genetic strains or mutations that cause consumption of diet enriched with fat, modest amounts of cholesterol, and/or simple sugars ("Western diet"). In addition, there are purely dietary models, such as high-fat/high-cholesterol, Western diet, and choline-deficient, amino acid-defined serve well as preclinical fibrosis models. NASH-related fibrosis in NASH models should show steatohepatitis and some focal bridging fibrosis, and etiopathogenesis. Our scientists will work with clients and partners to discuss a minimum set of requirements that investigators and drug companies should consider optimizing pharmacologic therapies for NASH.

Case study 1: The NASH–CDAHFD animal model for preclinical testing of new antifibrotics

Our scientists have created a customized, client-Specific study design in a mouse model for CDAHFD- induced NASH. The highlight of this model is as follows.

Study animals: C57BL/6 mice

Disease induction: Choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD) consisting of 60 kcal% fat and 0.1% methionine by weight

Option of test article administration: PO, IP, IV, IM, SC, nebulization, and osmotic pumps

Treatment regimen: Therapeutic or Prophylactic

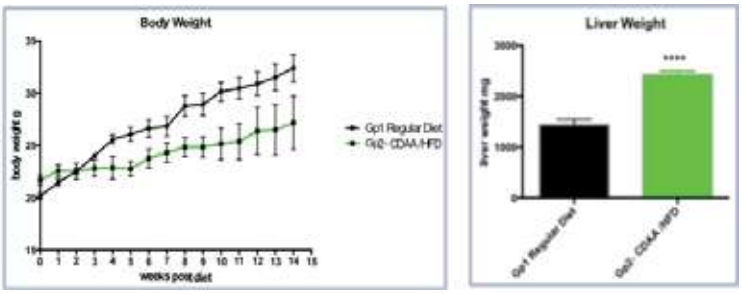
Reference: Model adapted from Matsumoto et al. (9).

Standard Readouts: Body weight, daily activity, survival, liver enzymes, liver histology: H&E and picrosirius red (PSR) staining

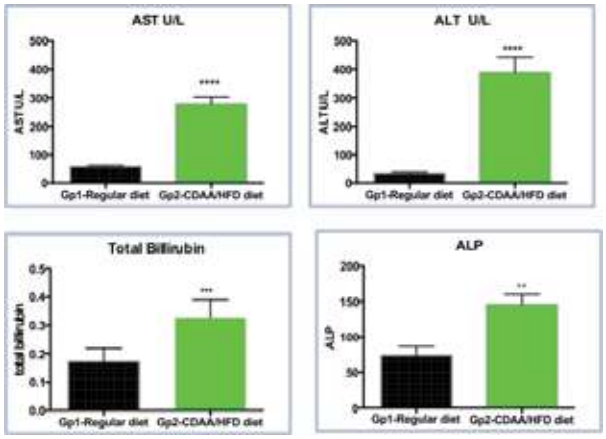
Fibrosis Readouts: Hepatic hydroxyproline content, serum/plasma biomarkers, liver FibroPanelTM gene expression, abdominal fat collection, liver histology: H&E and picrosirius red (PSR) staining.

Duration of Diet intake: Normal chow- 20 weeks; CDAHFD: 12 or 20 weeks.

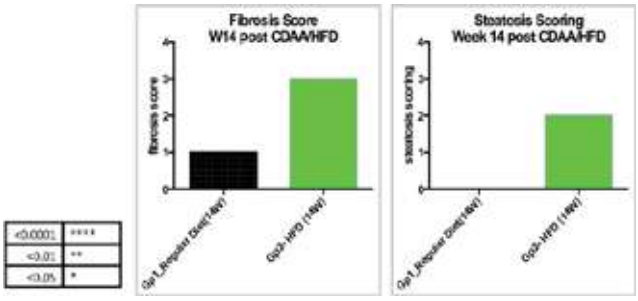
Readouts show increase in body and liver weights of mice



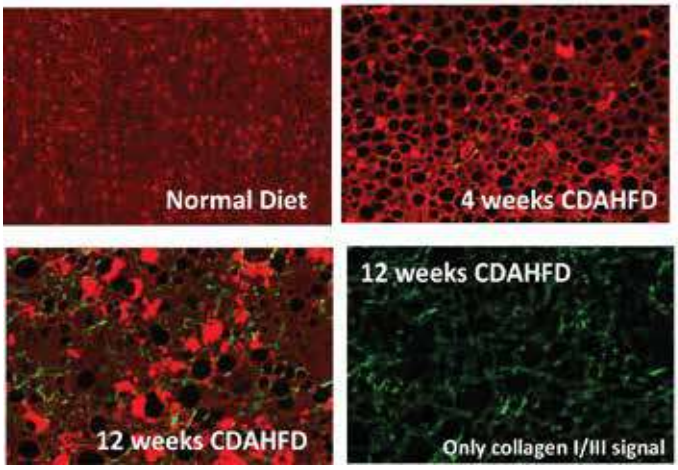
Liver enzyme data demonstrates increases in CDAHFD mice

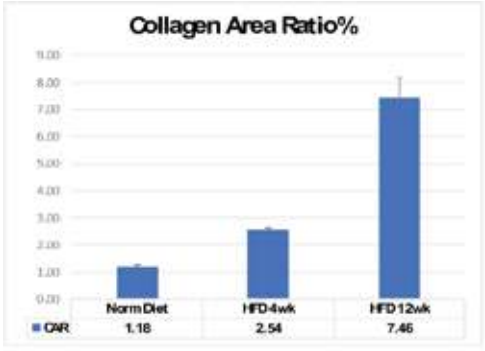


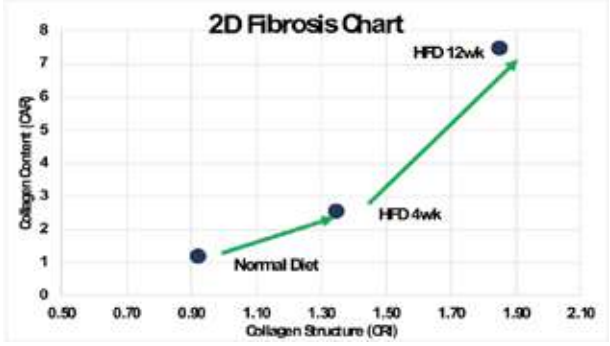
Liver Histology scoring for Fibrosis and Steatosis increases in CDAHFD mice



Representative Second Harmonic generation images from normal Diet & CDAHFD control mice

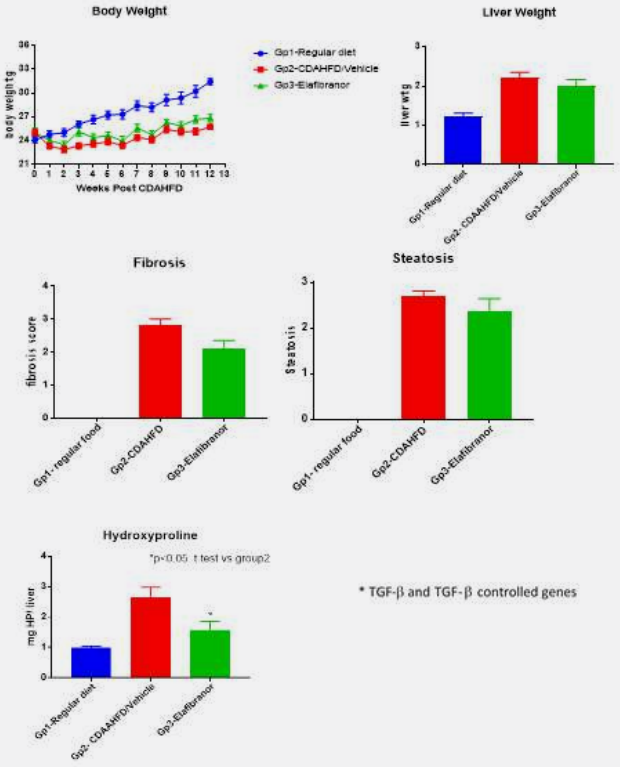


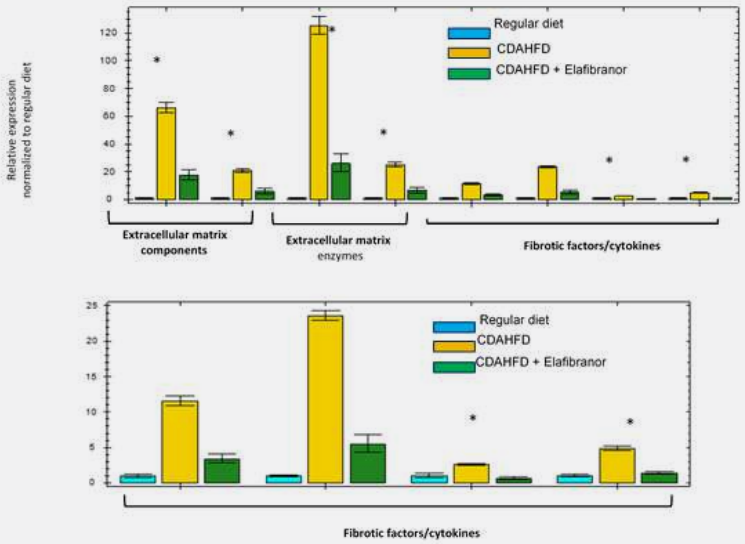




Case Study 2: Treatment of CDAHFD-induced NASH mice with Elafibranor

Elafibranor treatment reduced body and liver weights, liver histology, scoring for Fibrosis and Steatosis in treated mice. Hydroxyproline (HP) data indicates the reduction of HP content in Elafibranor treated mice. In addition, FibroPanelTM gene expression data demonstrated the effect of Elafibranor treatment on CDAHFD-induced NASH mice.



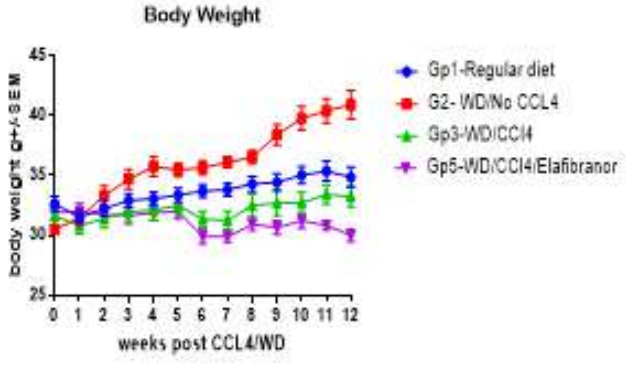


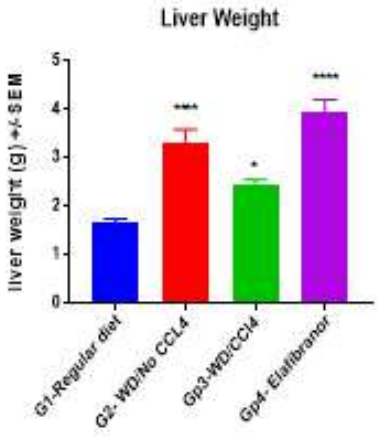
Case study 3: Non-Alcoholic Steatohepatitis Model

A customized, client-specific study design was developed in mice as part of the Western Diet + CCl4 -induced model (6). Disease was induced in C57BL/6 mice by feeding Western diet and weekly administration of Carbon Tetrachloride (CCl4). Test article provided by clients/ partners are administered wither via PO, or, or IP, or IV, or IM, or SC, or nebulization, and or osmotic pumps. The treatment regimen includes therapeutic or prophylactic.

Standard readouts are body weight, daily activity, survival, serum liver enzymes, liver histology: H&E and picrosirius red (PSR) staining. Fibrotic Readouts are hepatic hydroxyproline content, serum/plasma biomarkers, liver FibroPanelTM gene expression, abdominal fat collection, liver histology: H&E and picrosirius red (PSR) staining. Feeding schedule consists of Normal Chow -24 weeks or WD + CCl4- 6, 12, 24 weeks. Results of the above study are summarized in these graphs and pictures shown below.

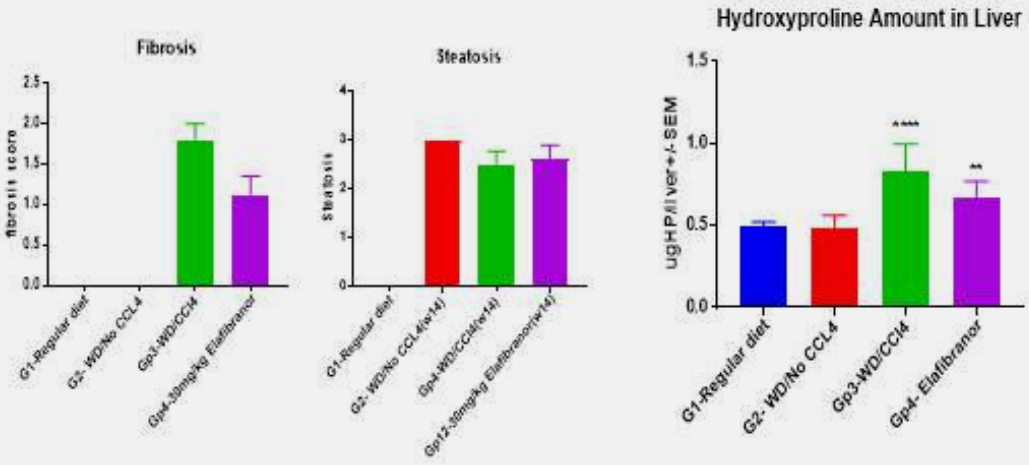
Effect of Elafibranor treatment on mice body and liver weights



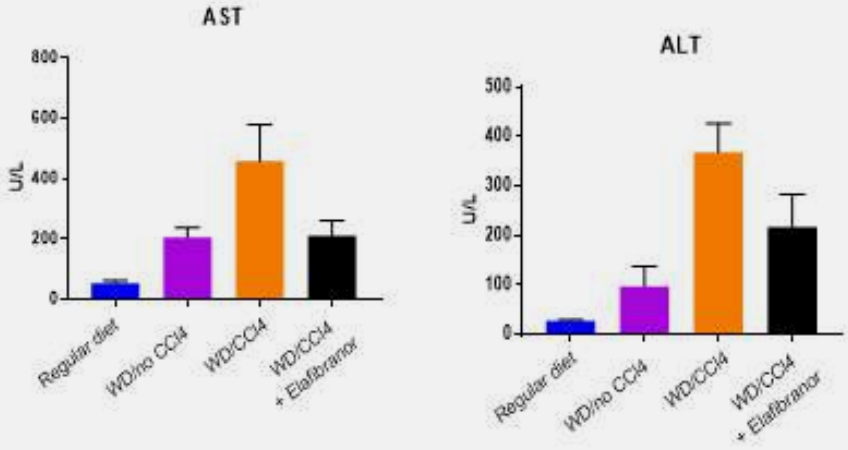


Liver Histology scoring for Fibrosis and Steatosis **effects in Elafibranor treated mice**

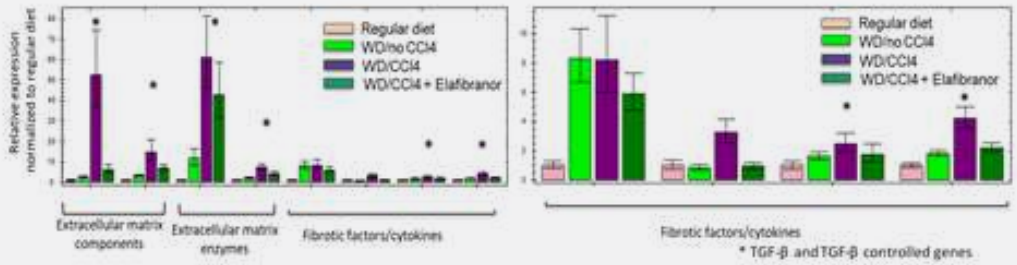
Hydroxyproline data indicates reduction of HP content in Elafibranor treated mice



Changes in liver Enzyme of mice across groups

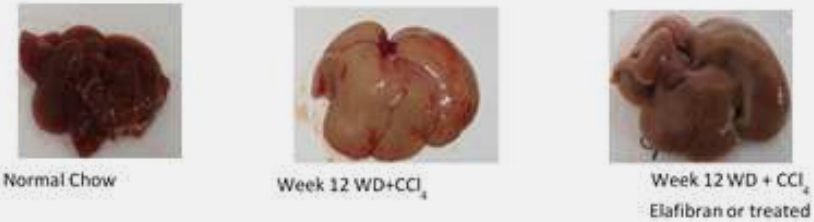


FibroPanel™ gene expression following Elafibranor **treatment**

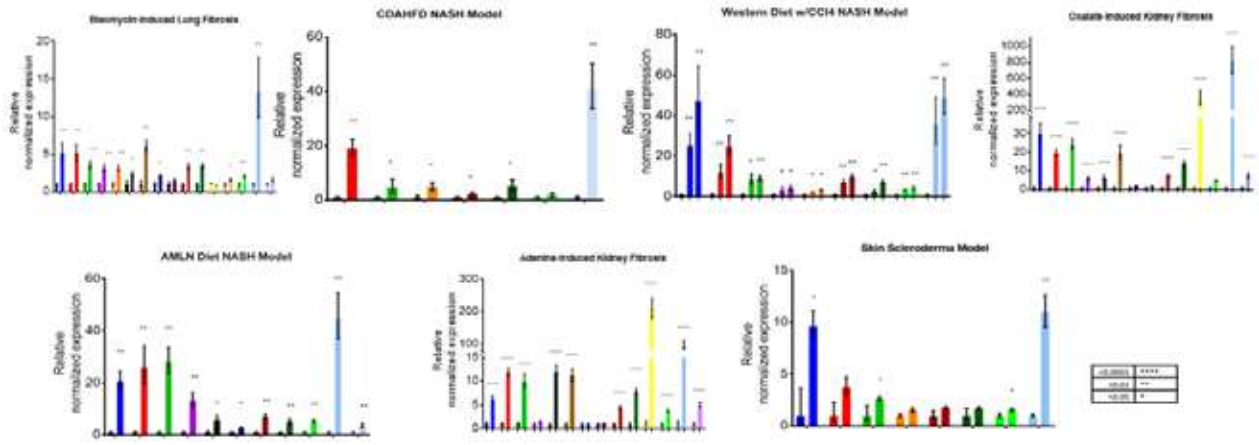


Elafibranor treatment improves liver morphology seen in histology **images**

Differences in vehicle and Elafibranor-treated mice **(dissected livers)**



FibroPanelTM shown here for known genes involved several models of fibrosis. Fibrosis -related genes measured here include TGF-β, TGF-β controlled genes, cytokines, and tissue remodeling genes in Bleomycin-induced lung, CDAHFD-induced, Western Diet + CCl4-induced, adenine-induced kidney, AMLN diet NASH, skin scleroderma, oxalate-induced kidney fibrosis models.



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 NASH-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

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.

References

1. Kaul B, Cotton V, Collard HR, V, Valenzuela C. Variability in Global Prevalence of Interstitial Lung Disease, Front. Med., 04 November 2021.
2. IPF Foundation | Ending IPF Through Research
3. 3. Freag MS, Namgung B, Reyna Fernandez ME, Gherardi E, Sengupta S, Jang HL. Human Nonalcoholic Steatohepatitis on a Chip. Hepatol Commun. 2020 Nov 29;5(2):217-233. doi: 10.1002/hep4.1647.
4. McClain CJ, Mokshagundam SP, Barve SS, Song Z, Hill DB, Chen T, Deaciuc I. Mechanisms of non-alcoholic steatohepatitis. Alcohol. 2004 Aug;34(1):67-79.
5. Farrell G, et al. Mouse Models of Nonalcoholic Steatohepatitis: Toward Optimization of Their Relevance to Human Nonalcoholic Steatohepatitis. Hepatology. 2019 May;69(5):2241-2257).
6. Tsuchida, Takuma et al. "A simple diet- and chemical-induced murine NASH model with rapid progression of steatohepatitis, fibrosis and liver cancer." Journal of hepatology vol. 69,2 (2018): 385-395.
7. Gerckens, M., Schorpp, K., Pelizza, F., Wögrath, M., et al. (2021). Phenotypic drug screening in a human fibrosis model identified a novel class of antifibrotic therapeutics. Science advances, 7(52).
8. Yanagihara T, Chong SG, Vierhout M, Hirota JA, Ask K, Kolb M. Current models of pulmonary fibrosis for future drug discovery efforts. Expert Opin Drug Discov. 2020 Aug;15(8):931-941.
9. Matsumoto, Masahiko et al. "An improved mouse model that rapidly develops fibrosis in non-alcoholic steatohepatitis." International journal of experimental pathology vol. 94,2 (2013): 93-103.