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# Strategies and Endpoints of Antifibrotic Drug Trials

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## Abstract

There is an urgent need to develop antifibrotic therapies for chronic liver disease, and to clarify which endpoints in antifibrotic trials will be acceptable to regulatory agencies. AASLD sponsored an endpoints conference to help accelerate the efficient testing of antifibrotic agents and to develop recommendations on clinical trial design for liver fibrosis. In this review we summarize the salient and novel elements of this conference and provide directions for future clinical trial design. The paper follows the structure of the conference and is organized into five areas: I) Antifibrotic trial design; II) Preclinical proof of concept studies; III) Pharmacologic targets: rationale and lessons to learn; IV) Rational drug design and development; V) Consensus and recommendations on design of clinical trials in liver fibrosis. Expert overviews and collaborative discussions helped to summarize the key unmet needs and directions for the future, including: 1) Greater clarification of at-risk populations and study groups; 2) Standardization of all elements of drug discovery and testing; 3) Standardization of clinical trial approaches; 4) Accelerated development of improved non-invasive markers; 5) Need for exploration of potential off-target toxicities of future antifibrotic drugs.

### Keywords

antifibrotic; biomarker; cirrhosis; end point; fibrosis; imaging; liver; serum; treatment

There is an intensified focus on the development of antifibrotic therapies for chronic liver disease for at least three reasons: 1) Our understanding of the pathogenesis of hepatic

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fibrosis progression and regression has advanced significantly, with the identification of important therapeutic targets and promising drugs in pre-clinical models; 2) The public health impact of fatty liver disease, which will soon replace chronic HBV and HCV infection as the leading cause of cirrhosis (1); 3) novel surrogates to assess fibrosis content and progression may soon permit short-term clinical studies in smaller, select populations of patients.

Due to these convergent forces, stakeholders from academia, regulatory agencies, and the biotech and pharmaceutical industries seek clarity and consensus on rational design of clinical trials testing candidate antifibrotic drugs and identification of relevant endpoints. In response to these challenges, the AASLD sponsored an endpoints conference to help accelerate the efficient testing of antifibrotic agents. Conference participants represented a range of expertise and perspectives, which together yielded important insights and recommendations. Herein we summarize the salient and novel elements of this conference and attempt to provide directions for future clinical trial design.

This review is intended to integrate the important advances that were highlighted at the conference rather than to than transcribe specific lectures or provide a general overview of the field. For the latter, readers are referred to several recent review articles (2, 3).

This review follows the structure of the conference and is organized into five areas: I) Antifibrotic trial design; II) Preclinical proof of concept studies; III) Pharmacologic targets: rationale and lessons to learn; IV) Rational drug design and development; V) Consensus and recommendations on design of clinical trials in liver fibrosis.

## I. Antifibrotic trial design

(speakers: Arun Sanyal, Scott L. Friedman, Keyur Patel, Massimo Pinzani, Claude B. Sirlin, and Detlef Schuppan).

There is an immediate need to clarify which endpoints in antifibrotic trials will be acceptable to regulatory agencies including the US Food and Drug administration (FDA) and the European Medicines Agency (EMA). The FDA offers two potential pathways for approval, either of which must ultimately show that an intervention improves how a patient “feels, functions, or survives". In antifibrotic drug trials this translates into prevention of cirrhosis associated with a risk of decompensation, development of HCC, or death. The regular approval pathway requires evidence of a clinically meaningful benefit, whereas an alternative pathway (“subpart H”) can be based on the use of surrogate endpoints that are “reasonably likely” to reflect changes in clinically meaningful outcomes. At present it remains to be clarified which purely fibrosis-related endpoints will be meaningful predictors of clinical outcomes and what quantity of data related to clinical outcomes paralleled by evaluation of surrogate markers will be required.

Antifibrotic trials in chronic liver diseases present unique challenges if conducted in patients who are not cirrhotic, because clinical events that could be used as trial endpoints are rare, and studies will largely need to rely upon non-invasive surrogates. Current clinical trials with potential antifibrotics are primarily based on liver biopsy to assess fibrosis progression.

However, liver biopsy is prone to sampling variability; mitigating this problem necessitates large numbers of well-stratified patients, and a long duration of treatment, which are significant obstacles (4, 5).

Patient selection and optimal stratification are key factors determining the success of a proof-of-concept trial. Subjects should be at least at an intermediate stage of fibrosis (e.g., Metavir stage 2-3),in order to detect dynamic changes. Appropriate stratification (etiology, age, gender, alcohol use, metabolic syndrome, surrogates of hepatic inflammation and genetic risk factors) should ideally be included. In contrast to HCV, in NASH the development of an aggregate genetic risk score to predict disease progression has been elusive, because the disease is highly heterogeneous and no single risk algorithm reflects the different pathways.

One critical determinant of clinical deterioration in patients with advanced fibrosis is the hepatic venous pressure gradient (HVPG), with HVPG > 10 mm indicating an increased risk of clinical decompensation (6). Thus, trials could be stratified according to this key benchmark, and endpoints might include the percentage of patients who transition into this high-risk group or regress to lower values while on therapy compared to placebo. In patients with more advanced fibrotic disease, MELD reflects decompensation risk. However, treatment of patients with advanced disease may be difficult due to suboptimal therapeutic response.

For patients with earlier stage disease who are not at immediate risk for clinical decompensation, there is an urgent need to establish reliable biomarkers that reflect clinically meaningful benefit. Serum markers are attractive because of the ease of access and ability to sample regularly. Serum markers of fibrosis can be broadly characterized as indirect markers (e.g., transaminases, platelets, bilirubin, growth factors), and direct markers that represent molecules from the fibrotic tissue (e.g., procollagen/collagen peptides, matrix glycoproteins, proteoglycans/glycosaminoglycans, and fibrogenic mediators like TGF-β1) (7-9). Combined tests include both indirect and direct markers. Current marker panels include Fibrotest, APRI (indirect), Fibrospect, European Liver Fibrosis test ELF (direct), HepaScore, and Fibrometer (combined). The diagnostic performance of these markers, however, can vary greatly among different studies with a wide range of sensitivity, partly due to selection bias (7, 8, 10). All have area under the receiver operating curve (AUROC) scores of ~0.8 in differentiating between absent or mild fibrosis (Metavir stage F0-1) and significant to severe fibrosis (stage F2-F4), but none accurately reflects intermediate fibrosis stages (7, 11). Reliance on liver biopsy as a “gold standard” for fibrosis marker validation is problematic due its significant sampling variability (7-10). For example, fibrosis assessment in two independent biopsies from the same patient differs by at least one stage in ~25%, ~40% and ~60% in subjects with chronic HCV, NASH and biliary fibrosis, respectively. Thus in a real-world scenario of chronic HCV patients (whose biopsies display the lowest sampling variability), even an exceedingly good marker panel that would predict “real” F0-F1 vs. F2-F4 with 99% accuracy, fibrosis may go undetected, with an AUROC ~0.85 (12). This also applies to the ability to detect minor but clinically relevant changes in fibrosis in early and especially intermediate stages (7, 11, 12). It is likely that several of the current serum fibrosis markers may reflect hepatic matrix turnover and fibrogenesis rather than the

amount of deposited connective tissue. More longitudinal studies are needed to evaluate this issue and compare these biomarkers not only to other diagnostic tools (e.g. liver biopsy or transient elastography) but, more importantly, to clinical outcomes (7, 13-15).

Liver stiffness measurement by elastography is a broadly validated non-invasive tool for assessing fibrosis content and predicting the decompensation risk and clinical outcomes in cirrhotics (16, 17). Transient elastography (Fibroscan) has recently been approved by the FDA as a test for cirrhosis. Since stiffness decreases in response to successful antiviral therapy for chronic HCV (16), Fibroscan may be useful not only to stratify patients prior to antifibrotic treatment, but also to track hepatic inflammation and fibrosis, the two major determinants of liver stiffness.

Use of MR technologies offers many theoretical advantages over other non-invasive methodologies, because in addition to stiffness, additional features of the liver can be assessed including function, texture, relaxometry and diffusion parameters (17). Moreover, compared to transient bedside elastography, MR elastography (MRE) permits assessment of the whole liver. In at least one trial presented at the conference, MRE was more accurate than bedside elastography to predict histological fibrosis (unpublished). However, no MR technique has yet been developed in humans that can directly visualize fibrosis, although such methodologies are advancing in animal models (18). Importantly, unlike other technologies, performance features of MR have been standardized across institutions. At present, MRE offers high diagnostic accuracy in cross-sectional studies, but longitudinal studies and wider adoption of the technology are required to establish this modality as a legitimate surrogate endpoint.

Now that clear evidence of fibrosis reversibility has been established in humans following successful suppression or eradication of HBV and HCV (19, 20), there are new opportunities to determine which non-invasive markers and technologies most accurately reflect reduction of fibrosis. One cannot assume, however, that the imaging features of fibrosis regression will simply represent a reversal of the changes associated with progression. Nonetheless, these technologies can be explored, any one of which could emerge as a robust indicator of reversion. New “omics” methodologies are under development, including glycomics and proteomics (21), as well as detection of micro-RNAs and circulating microparticles (22). New imaging methodologies include infrared imaging of collagen using a fibrin-derived peptide (23), assessment of elastin content (24), binding activity to growth factor receptors (25), or quantitative imaging of fibrogenic liver cells (26). However, none is sufficiently mature to justify inclusion as a clinical trial marker yet. Because of this high unmet need for non-invasive tests of this type, biomarker development remains a high priority for FDA (27).

A separate approach is to develop non-invasive methods that quantify the functional reserve of liver rather than define its histology or fibrosis. Compared to conventional tests of liver function, such as indocyanine clearance, or antipyrine, caffeine or galactose elimination, which depend on liver perfusion or enzyme induction; a novel test which is based on the excretion capacity for bile acids (dual cholate clearance -HepQuant) appears to be a superior predictor of clinical outcomes in patients with chronic HCV (28). Further validation of

technologies like this that quantify latent functional reserve as a predictor of clinical outcomes in fibrosis (or fibrotic NASH); is an important priority.

## II. Preclinical proof-of-principle studies

(speakers: Jonathan A. Dranoff, Wajahat Z. Mehal, Robert F. Schwabe, Jonathan Fallowfield and Peter Olinga).

Translation from basic biological concepts to efficacious therapy requires continued basic research, but must be complemented by a focus on targets, methodology, and, importantly, models that are truly relevant to human disease pathogenesis.

### Biologically Relevant Cells and Pathways

While pathogenic and cellular targets of therapy are increasingly clarified, this has not led to simplification, with other relevant cell types to consider. The paradigm of myofibroblasts (MF) as the key fibrogenic cell is well established (29, 30). However, the origins of liver MF are diverse. Two distinct resident liver cell populations - hepatic stellate cells (HSC) and portal fibroblasts (PF)(31) – give rise to the majority of liver MF in the injured liver. Both cell types may strongly contribute to fibrosis (32, 33), but HSC represent the major MF source (34). There are also other potential sources, such as mesothelial cells near the liver capsule (35).

Non-fibrogenic cells that initiate signals directing MF function may also be valuable cellular targets for therapy. For example, cholangiocytes O'Hara, 2013 #26084} secrete a variety of pro-fibrotic signaling molecules, and hepatocytes may promote liver fibrosis via release of apoptotic fragments or reactive oxygen species (ROS) (36). Equally relevant may be "modifier cells", which signal in a context-dependent manner during injury or healing. Such cells include a range of inflammatory cells, including resident macrophages (Kupffer cells). Also, non-resident cells of the reticuloendothelial system (e.g., invading monocytes/ macrophages) - may control fibrosis progression or resolution depending on their maturation or phenotype (37).

### Bidirectionality of Liver Fibrosis

There are notable differences between the features of fibrosis regression in animal models and in human liver disease. Whereas fibrosis regression in animals has been well documented for decades, evidence in humans has emerged more recently as treatments have improved (19, 20). Specifically, treating HCV is yielding growing populations of patients with sustained virological responses (SVR) (i.e. cure) and evidence of fibrosis resolution, even in cirrhotics. Key mechanisms that explain fibrosis regression in vivo include monocyte/macrophage polarization from a fibrogenic to a fibrolytic phenotype (37-39) and reversion of HSC to an inactive phenotype resembling, but distinct from, quiescent HSC (38). However, such HSC are primed for re-activation should re-traumatization occur (39). Several factors regulate the dynamics between matrix deposition and clearance, such as the balance between putatively fibrolytic matrix metalloproteinases (MMPs) and their inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) . Another key factor is the cross-linking of fibrillar collagen, which increases its resistance to degradation, which is

mainly mediated by lysyl oxidase-2 (LOXL2), which has emerged as an attractive therapeutic target for inhibition (40). Taken together, these findings support the idea that rational treatments may focus not only on prevention or inhibition of fibrosis progression, but also on resolution.

### Fibrosis Models

The lack of single, highly relevant animal models for human hepatic fibrosis is a persistent shortcoming that must be addressed adequately by the research community (41). While parenchymal toxins such as carbon tetrachloride,, biliary surgery inducing common bile duct ligation or nutritional interventions like the methionine choline deficient diet are highly effective in establishing advanced fibrosis in rodents, they do not faithfully represent key elements of human disease. Standardization, systematic analysis and optimal combinations of divergent rodent models would greatly facilitate testing of antifibrotic compounds and enable direct comparisons of the efficacy of the candidate drugs (26). The area of greatest unmet need is animal models of NASH, as no models to date faithfully reflect all the pathophysiologic and histologic features of the disease in humans (42).

## III. Pharmacologic targets: rationale and lessons to learn

(speakers: Thomas A. Wynn, Kumar Sharma, Neil C. Henderson, Sophie Lotersztajn, Frank Tacke, Natalie J. Torok, and Frank A. Anania)

Successful therapeutic approaches should aim at halting core profibrotic and/or enhancing fibrolytic pathways. Core pathways are likely to be evolutionarily conserved regulating central events in fibrosis/fibrolysis across different organs (43). Targeting these may be efficient but at the expense of possible off-target effects (e.g. hepatocyte injury, interference with regeneration). On the other hand, some of these pathways should only be highly active only in the fibrotic tissue.

In recent years there has been marked interest in developing antifibrotic agents. The following does not to provide a full list, but rather highlights some novel targets that show promise or are already in clinical trials.

### Targeting fibrogenic events

TGFβ1 has a fundamental role in fibrogenesis in all organs (44). While systemic inhibition of TGFβ1 signaling can enhance inflammation and result in untoward effects on liver parenchymal and progenitor cells, targeting specific steps in TGFβ1 activation may be of benefit. In this context αv integrins are important determinants of liver, lung and kidney fibrogenesis (45). Integrins are heterodimeric cellular receptors that mediate cell-matrix and cell-cell interactions; specifically, the integrins αvβ6 and avβ8 facilitate TGFβ1 release and its activation from the latent form (46). Inhibition of αvβ6 could be a highly effective and targeted antifibrotic approach, since it is only expressed on proliferating cholangiocytes (47). Clinical trials are planned using antibodies to avβ6 and small molecule inhibitors to specific αv integrins. A downstream target that amplifies TGFβ1 signaling is connective tissue growth factor (CTGF) . Targeting CTGF with a monoclonal antibody (FG-3019) has

shown promise in animal models of pulmonary fibrosis and currently is being tested in clinical trials (48).

The fate of MF is also a key determinant of the severity and reversibility of fibrosis. Deactivation by inhibiting the cannabinoid receptor 1 (CB1) potently attenuated experimental fibrosis (49). While the first-generation CB1 antagonist rimonabant was withdrawn because of its potential to induce depression, novel peripheral-acting CB1 antagonists have been developed (50). Conversely, increased CB2 signaling is associated with reduced inflammation and improved fibrosis (51), but also with enhanced insulin resistance and steatosis in obese mice (52).

Significant efforts have been made in understanding fibrogenesis in NASH (2, 53). NASH progression is intimately linked to insulin resistance/type 2 diabetes, associated with lipotoxic hepatocyte death and intestinal dysbiosis, providing rational targets for antiinflammatory and antifibrotic therapy (54). Apart from lifestyle changes, current therapeutic strategies include improving insulin signaling or ameliorating hepatocyte oxidative stress (e.g. resveratrol, ClinicalTrials.gov, NCT01464801), farnesoid X receptor receptor (FXR) agonists like obeticholic acid, combined peroxisome proliferator activated receptor (PPAR)α/δ agonists, fibrosis-specific inhibitors of hedgehog signaling (53, 55, 56), or manipulation of the altered gut microbiota using probiotics or microbiota transfer (57).

Strategies that reduce redox injury could also improve liver function and fibrosis. However, the use of anti-oxidants has been disappointing, owing to differences between animal models and human disease, the inability of many agents to reach the relevant cellular compartments, and the stage and cell-specific regulation of oxidant and anti-oxidant pathways. Recently NADPH oxidases (NOXs) have emerged as drug targets. Activation of NOX 1, 2 and 4 play a major role in HSC activation (58-60), and NOX4 can induce apoptosis in hepatocytes (60). NOX1/NOX4 targeting using GKT137831 attenuated fibrosis in the $CCl_4$ (61), bile duct ligation models (60), in lung fibrosis (62) and in diabetic kidney disease. A phase II trial is underway in diabetic kidney fibrosis (clinicalTrials.gov NCT02010242).

## Targeting fibrosis reversal

Recent animal studies have revealed that during experimental fibrosis regression up to half of the MF undergo senescence and apoptosis, whereas the rest acquire a quiescent phenotype (32-34). The factors governing the inactivation of MF are under investigation. For example, PPARγ plays a role in the re-establishment of the quiescent phenotype (39) while matrix stiffness (63) and cross-linking is currently addressed by LOXL2 inhibition (40) (ClinicalTrials.gov, NCT01452308).

Recruitment and activation of monocytes/macrophages is central to fibrogenesis and fibrosis regression in rodents (64). While targeting macrophage recruitment/polarization would be attractive approaches, the functional heterogeneity of macrophage subpopulations in humans has not yet been adequately characterized. Thus no clear links can be made yet from animal studies to human disease and the macrophage subsets may be dependent on the etiology of the liver disease. One rational attempt is the use of chemokine antagonists whose role in fibrogenesis seems to be preserved among species. By preventing the early recruitment of

profibrotic mononuclear cells by CCL2 inhibition intrahepatic macrophages shift toward the "restorative" subset, accelerating fibrosis regression (65).

### Combination approaches

Because liver fibrosis and reversal are dynamic processes, inhibition of a single pathway may not result in sustained effects. Stage-specific combination therapies that target the core pathways, the ECM, and/or specific cell types may be necessary. Special attention should be directed towards possible off-target and toxic effects, e.g., to liver parenchymal cells or extrahepatic tissues. It can be anticipated that in the future it is possible that, as in cancer therapy, antifibrotics will be prescribed using a personalized approach that includes causal treatment of the primary disease and a tailored therapy based on its grade and stage, and the liver synthetic function.

## IV. Rational drug design and development

(speakers: Klaas Poelstra, Don C. Rockey, David A. Brenner, Veronica Miller, Gregory T. Everson, and Averell Sherker)

Effective targeting to activated HSC can be achieved by coupling potential antifibrotics to small molecular ligands for the platelet derived growth factor β receptor (PDGFRβ) or the insulin like growth factor II receptor. An example in clinical development is a dimeric PDGFRβ binding peptide with an attached interferon-γ that effectively attenuates fibrosis in rodent models while limiting the cytokine's side effects (66).

The close communication between MF and sinusoidal endothelial cells underscores the importance of vascular mediators in fibrosis (67, 68). Thus, agents that induce vasoconstriction (such as endothelin-1 via the ETA receptor, angiotensin II, prostaglandin F2) not only promote portal hypertension, but also cause fibrogenic activation of HSC. In contrast, vasorelaxants (such as nitric oxide, relaxin, prostacyclins) have antifibrotic effects. Several clinical studies have attempted to address this by using angiotensin receptor 1 blockers, but this area needs further study.

Efforts are being developed to block MF activity in its steps from initiation to perpetuation and proliferation, and finally to induce their inactivation and promote matrix dissolution. Based on our improved understanding of these processes, many agents with proven safety for indications other than fibrosis; are emerging. Blockade of IL-17 or the lysophosphatidic acid receptor 1 (69, 70) can prevent initiation. Prominent antiproliferative drugs, also via repurposing of existing drugs, may include inhibitors of PDGFβR (71), or more general tyrosine kinase inhibitors, such as sorafenib or nintedanib (72, 73).

A new organizational initiative was introduced at the meeting aimed at accelerating the path to antifibrotic drug development by convening a forum modeled on prior efforts that advanced drug development for HIV and HCV (74, 75). This 'collaborative liver forum' intends to identify unmet needs, standardize ongoing efforts at biomarker discovery, and ultimately accelerate trial design and drug approval. This initiative is supported by

representatives from patient groups, scientists, clinicians, biotech/pharmaceutical companies and regulatory authorities.

## V. Conclusions and Recommendations

The meeting brought together a broad range of stakeholders in the field representing academia, NIH, pharmaceutical/biotech industry, non-profit organizations, and regulatory agencies. Collaborative discussions helped crystallize key unmet needs and directions for the future which are listed here:

1. Because liver fibrosis (especially NASH) is a heterogeneous condition, with intervals of progression and regression, greater clarification of at-risk populations is required to more accurately identify patients suitable for clinical trials who are most likely to benefit from effective therapies. This will require genetic, serological, functional, and/or imaging modalities to facilitate stratification and follow-up. Proof-of-principle phase 2 trials should enroll study groups that are as homogeneous and well characterized as possible.
2. Standardization of all elements of drug discovery and testing/validation of biomarkers is a high priority. Analysis of drugs in preclinical models must utilize proven and standardized methodologies so that results can be more easily compared between candidate drugs and biomarkers.
3. Similarly, clinical trials must be standardized, with iterative improvements based on lessons learned. Improved cooperation between all stakeholders is a likely outcome of this endpoints meeting, exploiting organizational models that previously were successful in accelerating drug development for HIV and HCV. Clinical trials must also incorporate assessment of quality of life metrics, and there is a need for more standardized patient outcome reporting tools that are liver disease-specific. Other parameters requiring standardization include disease and subgroup definitions, patient outcomes, and biomarker technologies and their interpretation.
4. Accelerated development of improved non-invasive markers is a critical unmet need and a high priority. This will enable the integration of surrogate biomarkers into future clinical trial design measured in addition to, or instead of clinical endpoints. With respect to current imaging methodologies, more comprehensive comparison of MR technologies to bedside transient elastography is needed. These technologies must be complemented by wider validation of functional tests, which will be more sensitive and earlier indicators of a meaningful therapeutic response than imaging tests or biopsy.
5. Trials of novel therapeutic agents must explore off-target toxicities both within liver and in other organs. Such efforts could uncover novel therapeutic targets or inform drug dosing to minimize long-term risk.

The previous 30 years have successfully uncovered key elements of the pathogenesis of hepatic inflammation and fibrosis, and have led to a clearer understanding of fibrosis dynamics in human disease. One can view this period, therefore, as the end of a crucial early

phase of hepatic fibrosis investigation, which has set the stage for an exciting new era that will culminate in effective drugs to prevent the development of end-stage disease in patients with chronic fibrosing liver injury and may hold a promise for reversal of advanced fibrosis.

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