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# A TRAIL in liver disease

*By Tim Fulmer, Senior Writer*

The molecular mechanisms underlying cholestatic liver disease, which is caused by disrupted bile flow, are relatively unknown.[1] A paper in the *Proceedings of the National Academy of Sciences* now points to a proapoptotic pathway in the liver involving death receptor 5 and tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) that potentially could be targeted to help treat the disease or prevent progression to liver failure.

At least two companies are developing antibodies that agonize death receptor 5 (DR5) to treat cancer. However, researchers polled by *SciBX* warned that targeting the pathway to interfere with apoptosis in the liver requires caution, and they suggested other approaches to restore bile acid homeostasis and to combat fibrosis associated with the disease.

Autoimmunity and bacterial infection are two potential causes of bile duct deterioration that can lead to cholestasis (bile duct blockage). Although cholestatic liver disease is less frequent than disease resulting from alcohol abuse or chronic hepatitis infection, a direct consequence of cholestasis is the accumulation of toxic levels of bile acid in the liver. Over time, this can lead to liver fibrosis, cirrhosis and end-stage liver disease.[2,3]

Two of the best studied cholestatic liver diseases are primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Urso ursodiol, a bile acid derivative marketed by **Axcan Pharma Inc.** in North America, is the only drug approved to treat PBC. There are no approved drugs for PSC, which is also associated with significant risk for cholangiocarcinoma or cancer of the bile ducts.

In the *PNAS* paper, researchers from Japan and Australia reported that activating a death receptor–dependent apoptotic pathway in mouse liver induced bile duct inflammation and blockage, suggesting the pathway might play an important role in cholestatic liver disease.[4]

In wild-type mice, intraperitoneal administration of antibodies that target and agonize DR5 elicited severe jaundice and increased serum levels of bilirubin and alkaline phosphatase—two established biomarkers of liver dysfunction—compared with administration of control IgG antibody.

Histological examination of mice that received the anti-DR5 mAb revealed hardening of the extra-hepatic bile duct from fibrosis and inflammatory cell infiltration.

In murine ligation models of cholestatic liver disease, knocking out DR5 or its ligand TRAIL delayed onset of hepatocyte damage and inflammation compared with what was seen in wild-type controls. In the same models, TRAIL and DR5 knockout also led to significantly better survival rates than those of wild-type counterparts ($p<0.001$).

Finally, an examination of serum samples from PSC patients revealed that TRAIL expression on cholangiocytes was substantially higher than that seen on cholangiocytes in healthy controls ($p<0.05$). Cholangiocytes are the epithelial cells of the bile duct.

The authors proposed, "TRAIL/DR5-mediated signaling may be involved in the pathogenesis and pathological features of chronic cholestatic diseases in human beings, particularly in the diseases with progressive destruction of the bile ducts."

One potential approach to targeting TRAIL and DR5 would be to use a soluble DR5 that binds TRAIL before it can interact with DR5 on liver cells. Indeed, a soluble DR5 decoy receptor has previously been shown to inhibit TRAIL-mediated apoptosis in multiple cancer cell lines.[5]

However, because TRAIL can bind other receptors besides DR5, and because the binding interactions between TRAIL and DR5 are quite complex, this approach would require further *in vivo* testing, said Shelia Violette, VP of research at **Stromedix Inc.**, a developer of fibrosis therapeutics.

Violette told *SciBX* that an alternative strategy might be to develop an antagonist anti-DR5 mAb that does not trigger DR5 oligomerization and subsequent apoptosis, as potentially occurs with agonist anti-DR5 antibodies like the one used in the paper.

At least two biotechs are developing antibodies that agonize death receptors. Lexatumumab (HGS-ETR2), an antibody from **Human Genome Sciences Inc.** that agonizes DR5 (TRAIL-R2), is in Phase I testing for cancer. The company's mapatumumab (HGS-ETR1) antibody that agonizes DR4 (TRAIL-R1) is in Phase II testing for cancer.

AMG 655, an mAb from **Amgen Inc.** that agonizes DR5, is in Phase Ib/II trials for multiple cancers.

In a Phase I trial of lexatumumab in patients with advanced solid malignancies, the highest dose of the antibody was associated with asymptomatic elevation of liver enzymes.[6]

Targeting components of the apoptotic pathway downstream from DR5 could be an effective strategy in liver disease, said Gregory Gores, professor of medicine and physiology and chair of the Division of Gastroenterology and Hepatology at the **Mayo Clinic**.

In murine bile duct ligation models, Gores and colleagues have shown that a broad-spectrum small molecule caspase inhibitor (IDN-6556) lowered hepatocyte apoptosis, liver injury and hepatic fibrogenesis compared with what was seen in saline-treated controls.[7]

**Pfizer Inc.** gained IDN-6556 when it acquired Idun Pharmaceuticals Inc. in 2005. At that time, the compound was in two Phase II trials in liver transplantation and HCV. According to Pfizer's 2008 pipeline report, the compound (renamed PF-3491390) is in Phase II testing in liver fibrosis.

However, Scott Friedman, professor of medicine and chief of the Division of Liver Diseases at **Mount Sinai School of Medicine**, warned that the general strategy of inhibiting apoptosis in cholestatic liver disease might inadvertently promote cancer.

"Caspase inhibitors may block apoptosis in liver cells. However, by so doing, they might also increase the likelihood that cancer appears, so caution is warranted as we move forward with these agents," he said.

"This particularly applies to patients with primary sclerosing cholangitis, which is consistently associated with increased risk for bile duct cancer. Consequently, we have to be careful with how we use this strategy in some forms of cholestatic liver disease."

### Timing is everything

Even if more animal data confirm that TRAIL and DR5 interactions underlie cholestatic liver disease, the question remains how early in the disease treatment should begin. Once major bile duct damage has occurred, potential therapies like anti-TRAIL antibodies may be too late to the party.

Additional studies in mouse models and larger animals should provide a better understanding of how and when it is most effective to inhibit bile duct damage, said Mark Smyth, a principal investigator on the *PNAS* paper and head of the Cancer Immunology Program at **Peter MacCallum Cancer Centre**.

At Stromedix, Violette said there is a clear rationale for considering the company's STX-100 mAb in cholestatic and fibrotic liver diseases, such as PSC and biliary atresia, because its target, integrin $\alpha_v\beta_6$, is upregulated in fibrotic liver disease as a result of biliary injury.

For example, in separate studies of murine bile duct ligation models, a small molecule inhibitor of integrin $\alpha_v\beta_6$ and an anti–integrin $\alpha_v\beta_6$ antibody lowered progression of biliary fibrosis.[8,9]

STX-100 is in a Phase I safety trial in healthy volunteers, with plans to begin a Phase IIa trial next year in patients with chronic allograft nephropathy (CAN), Violette said. Stromedix in-licensed the compound from **Biogen Idec Inc.**

**Intercept Pharmaceuticals Inc.** is looking at targeting stages of cholestatic liver disease that generally occur prior to cirrhosis. "Our strategy in testing farnesoid X receptor agonists in cholestatic diseases like primary biliary cirrhosis has been to exploit their hepato-protective regulation of bile homeostasis," said Mark Pruzanski, president and CEO.

Bile acids regulate their own biosynthesis and transport by binding to and activating the farnesoid X receptor (FXR). Activation of FXR initiates transcription of genes that decrease the concentration of bile acids within hepatocytes.

"The rationale for agonizing the farnesoid X receptor is twofold—first, to downregulate transcription factors that drive bile synthesis, and second, to upregulate bile transporters in hepatocytes and thus help cells clear out excess accumulated bile acids that can cause toxicity," Pruzanski said.

Intercept's FXR agonist INT-747, a semisynthetic bile acid derivative, is in two ongoing Phase II trials to treat primary biliary cirrhosis in precirrhotic patients—one trial as a monotherapy and the other in combination with ursodiol. The primary endpoint of the trials is a reduction in serum alkaline phosphatase.

Rebecca Pruss, CSO of **Trophos S.A.**, noted that other tumor necrosis factor (TNF) superfamily ligands besides TRAIL might also have to be targeted to adequately treat liver disease.

Even if TRAIL and DR5 signaling is found to be an initiating event leading ultimately to liver failure, "hepatocyte injury is likely due to a combination of death signaling mechanisms activated by TNF and FasL [Fas ligand] as well as TRAIL," she said. "Therefore, all of these pathways may need to be addressed in treating more common types of hepatic toxicity due to bile duct occlusion, hepatitis C and nonalcoholic steatohepatitis."

Trophos' TRO19622, a small molecule with a cholesterol-like structure that interacts with the mitochondrial permeability transition pore (MPTP), is in a Phase IIa trial to treat nonalcoholic steatohepatitis (NASH).

Indeed, targeting inflammation upstream of fibrosis and cirrhosis could potentially limit the need for antifibrotic therapeutics.

"We know that an inflammatory stage typically occurs prior to onset of liver fibrosis. Moreover, even though this stage is asymptomatic in most patients, it could nevertheless be detectable during routine blood tests as an abnormal elevation of liver enzymes," said Friedman. "In such instances, administering an efficacious anti-inflammatory could potentially block or at least slow onset of fibrosis. Perhaps this wouldn't rule out the use of antifibrotic agents, but it could improve their efficacy in some form of a combination therapy."

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> ***—Rebecca Pruss, Trophos S.A.***

### Next steps

Smyth said another useful next step to better understand the role of TRAIL and DR5 signaling in cholestatic liver disease would be to identify potential mutations and polymorphisms in the relevant genes in patients with PSC.

Gores wanted to see additional proof that the models used in the *PNAS* paper were actually developing PSC. "At the moment, there are no good animal models of PSC," he said. "The mice used in the paper could thus potentially help fill this gap. However, I would like to see some additional readouts—in particular, a longer observation period to confirm that the animals develop biliary cirrhosis and proof of cholangiographic features of PSC."

Cholangiography is a way of imaging the bile duct, most commonly using X-rays or MRI.

In PSC and PBC, Pruss said, "autoimmunity may lead to the production of agonist DR5 autoantibodies, and this possibility should be explored in order to block these antibodies from activating DR5" and triggering apoptosis.

A second possibility, she said, is that "factors such as cytokines or bile acids may contribute to increased TRAIL expression in cholangiocytes." Thus, inhibiting these factors rather than targeting TRAIL or DR5 directly could offer other therapeutic strategies.

Violette emphasized the importance of additional *in vivo* work to determine the differential effects of TRAIL and DR5 blockade on cell types within the liver.

DR5 is also expressed on hepatic stellate cells, for which inducing apoptosis could be beneficial to the treatment of fibrosis. This is directly opposite to what's seen in the *PNAS* paper with cholangiocytes, where apoptosis can trigger biliary injury and subsequent fibrosis. Thus, Violette said, additional *in vivo* studies in liver fibrosis models may be useful to better understand the consequences of TRAIL and DR5 blockade in the liver.

Friedman suggested that similar next steps could even include humans.

"High-resolution confocal microscopy of immunostained tissue from patient liver explants or biopsies would be important to determine the relative expression of DR5 on hepatic stellate cells and cholangiocytes," he said. "These expression levels could then potentially guide the choice of a proper dosing regimen for antagonists of TRAIL/DR5 apoptotic signaling, since stellate cells may also be sensitive to these agents."

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## COMPANIES AND INSTITUTIONS MENTIONED

**Amgen Inc.** (NASDAQ:AMGN), Thousand Oaks, Calif.
**Axcan Pharma Inc.** (TSX:AXP; AXCA), Mont St. Hilaire, Quebec, Canada
**Biogen Idec Inc.** (NASDAQ:BIIB), Cambridge, Mass.
**Human Genome Sciences Inc.** (NASDAQ:HGSI), Rockville, Md.
**Intercept Pharmaceuticals Inc.**, New York, N.Y.
**Mayo Clinic**, Rochester, Minn.
**Mount Sinai School of Medicine**, New York, N.Y.
**Peter MacCallum Cancer Centre**, Melbourne, Australia
**Pfizer Inc.** (NYSE:PFE), New York, N.Y.
**Stromedix Inc.**, Cambridge, Mass.
**Trophos S.A.**, Marseille, France