The New-Generation Pan-Peroxisome Proliferator-Activated Receptor Agonist IVA337 Protects the Liver From Metabolic Disorders and Fibrosis

Guillaume Wettstein,[1] Jean-Michel Luccarini,[1] Laurence Poekes,[2] Patrick Faye,[1] Francine Kupkowski,[1] Vanessa Adarbes,[1] Evelyne Defrêne,[1] Céline Estivalet,[1] Xavier Gawronski,[1] Ingrid Jantzen,[1] Alain Philippot,[1] Julien Tessier,[1] Pascale Tuyaa-Boustugue,[1] Fiona Oakley,[3] Derek A. Mann,[3] Isabelle Leclercq,[2] Sven Francque,[4] Irena Konstantinova,[1] Pierre Broqua,[1] and Jean-Louis Junien[1]

IVA337 is a pan-peroxisome proliferator-activated receptor (PPAR) agonist with moderate and well-balanced activity on the three PPAR isoforms (α, γ, δ). PPARs are regulators of lipid metabolism, inflammation, insulin resistance, and fibrogenesis. Different single or dual PPAR agonists have been investigated for their therapeutic potential in nonalcoholic steatohepatitis (NASH), a chronic liver condition in which steatosis coexists with necroinflammation, potentially leading to liver fibrosis and cirrhosis. Clinical results have demonstrated variable improvements of histologically assessed hepatic lesions depending on the profile of the tested drug, suggesting that concomitant activation of the three PPAR isoforms would translate into a more substantial therapeutic outcome in patients with NASH. We investigated the effects of IVA337 on several preclinical models reproducing the main metabolic and hepatic features associated with NASH. These models comprised a diet-induced obesity model (high-fat/high-sucrose diet); a methionine- and choline-deficient diet; the foz/foz model; the CCl_4-induced liver fibrosis model (prophylactic and therapeutic) and human primary hepatic stellate cells. IVA337 normalized insulin sensitivity while controlling body weight gain, adiposity index, and serum triglyceride increases; it decreased liver steatosis, inflammation, and ballooning. IVA337 demonstrated preventive and curative effects on fibrosis in the CCl_4 model and inhibited proliferation and activation of human hepatic stellate cells, the key cells driving liver fibrogenesis in NASH. Moreover, IVA337 inhibited the expression of (pro)fibrotic and inflammasome genes while increasing the expression of β-oxidation-related and fatty acid desaturation-related genes in both the methionine- and choline-deficient diet and the foz/foz model. For all models, IVA337 displayed an antifibrotic efficacy superior to selective PPARα, PPARδ, or PPARγ agonists. *Conclusion*: The therapeutic potential of IVA337 for the treatment of patients with NASH is supported by our data. (*Hepatology Communications* 2017;1:524–537)

Introduction

Nonalcoholic steatohepatitis (NASH) is a highly prevalent, multifactorial, and multistep disease associated with increasing risk of cardiovascular mortality and severe liver conditions, such as cirrhosis and hepatocellular carcinoma.[1,2] NASH is now becoming a leading cause of liver transplantation in developed countries. Although not fully understood, it is widely accepted that insulin resistance and steatosis play key roles in the pathogenesis of the disease. Because lifestyle change provides limited

Abbreviations: α-SMA, alpha smooth muscle actin; CPT, carnitine palmitoyltransferase; EC_{50}, 50% effective concentration; Emax, maximal effect; HF/HS, high fat/high sucrose; HFD, high-fat diet; hHSC, human hepatic stellate cell; PPAR, peroxisome proliferator-activated receptor; HSC, hepatic stellate cell; IL, interleukin; MCD, methionine-choline-deficient; MMP, matrix metalloproteinase; MUFA, monounsaturated fatty acid; NAS, nonalcoholic fatty liver disease activity score; ND, normal diet; NF-κB, nuclear factor kappa B; NLRP3, NOD-like receptor family, pyrin domain containing 3; PDGF, platelet-derived growth factor; po, per os; PPAR, peroxisome proliferator-activated receptor; SCD1, stearoyl-coenzyme A desaturase-1; TGF-β, transforming growth factor beta; TGF-βR, transforming growth factor beta receptor; TIMP, tissue inhibitor of metalloproteinase.

Received March 17, 2017; accepted May 19, 2017.
Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1057/suppinfo.
Supported by Inventiva S.A.

improvement and because of lack of approved medication, discovering new efficacious therapies is of high interest.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear receptors that function as master regulators in adipose tissue and the liver. They overall control insulin sensitivity, glucose, and lipid metabolism as well as inflammation and fibrogenesis.[3,4] The PPARγ isoform is highly expressed in adipose tissue; its activation promotes adipocyte differentiation, increases glucose uptake and triglyceride storage (hence reducing free fatty acid flux to the liver), and increases secretion of the anti-inflammatory cytokine adiponectin.[5,6] The PPARα isoform, which is highly expressed in hepatocytes, controls fatty acid transport and β-oxidation and dampens the inflammatory response.[7,8] The PPARδ isoform (also known as PPARβ) contributes to the regulation of glucose and lipid metabolism while exerting anti-inflammatory properties in the liver by skewing M2 polarization of Küpffer cells.[9-11] PPARγ and PPARδ are expressed at various levels in hepatic stellate cells (HSCs), a driver of liver fibrosis; PPARγ is key in keeping HSCs in a quiescent nonfibrogenic state.[12,13]

A protective role of PPAR agonists has been demonstrated in preclinical models of nonalcoholic fatty liver disease/NASH as well as in patients with NASH. The selective PPARα agonist Wy14,643 improved steatosis, inflammation, and fibrosis in mice receiving a methionine- and choline-deficient (MCD) diet and improved metabolic disorders, steatosis, and ballooning in high-fat diet (HFD) fed foz/foz mice.[14,15] In patients, the PPARα agonist fenofibrate had limited efficacy on NASH but a significant effect on hepatocyte ballooning.[16] Some but not all PPARδ agonists have had beneficial effects in preclinical models of NASH.[17,18] Elafibranor (GFT505), which combines PPARα and PPARδ activation, improved metabolic disorders and reduced the severity of steatohepatitis and fibrosis in several animal models and in patients with NASH.[19] Selective PPARγ activation by pioglitazone or rosiglitazone improved insulin resistance and reduced steatosis, inflammation, and fibrosis in animal models and in patients with NASH.[20,21] Taken together, these results indicate that activation of each of the three PPAR isoforms individually provides therapeutic benefit to patients with NASH. Combining PPARα, PPARδ, and PPARγ activation may therefore bring an innovative and efficacious therapeutic approach by targeting a larger array of disturbances that contribute to the development and progression of NASH.

IVA337 is a next-generation pan-PPAR agonist designed to produce moderate and well-balanced activation of the three PPAR isoforms. This unique agonist profile translates into an excellent efficacy and safety profile with no hemodilution, heart weight gain, or creatinine increase (manuscript under preparation) in preclinical models as well as in clinical phase 1 and 2a studies in patients with type 2 diabetes (manuscript under preparation). The aim of the present study was to assess the effects of IVA337 in preclinical models

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep4.1057

Potential conflict of interest: Dr. Wettstein, Mr. Luccarini, Mr. Faye, Mrs. Kupkowski, Mrs. Adarbes, Mrs. Defrêne, Mrs. Estivalet, Mr. Gawronski, Mrs. Jantzen, Mr. Philippot, Mr. Tessier, Mrs. Tuyaa-Boustugue, Dr. Konstantinova, Dr. Broqua, and Dr. Junien are Inventiva employees

ARTICLE INFORMATION:

From the [1]Inventiva, Daix, France; [2]Pôle d'Hépato-gastro-entérologie, Université Catholique de Louvain, Bruxelles, Belgium; [3]Medical School, Newcastle University, Newcastle, United Kingdom; [4]Antwerp University Hospital, Gastroenterology and Hepatology, Antwerp, Belgium.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Guillaume Wettstein, Ph.D.
Inventiva
50 rue de Dijon
Daix, France
E-mail: guillaume.wettstein@inventivapharma.com
Tel.: + 33(0)-380-447-571

reflecting the most important pathologic processes and phenotypic characteristics of NASH from insulin resistance, steatosis, inflammation, and ballooning to fibrosis. The effect of IVA337 on the proliferation and activation of human HSCs *in vitro* was also investigated.

Materials and Methods

ANIMAL MODELS

All experiments were performed in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care accreditation of our animal facilities.

High-Fat/High-Sucrose Diet

C57Bl6/J mice (4 weeks of age; approximately 20 g) received a diet enriched with 34.9% fat and 13% sucrose (D03062301; Research Diets) or a normal diet (ND) for 8 weeks. Mice were then randomized according to their body weight, serum glucose, and insulin levels to receive either the vehicle or IVA337 at 3, 10, or 30 mg/kg body weight (n = 10 per group) administrated per os (po) once a day together with a high-fat (HF)/high-sucrose (HS) diet for 4 weeks.

MCD Diet

C57Bl6/J mice (6 weeks of age; approximately 25 g) received an MCD diet together with either vehicle (methylcellulose 1% + poloxamer 0.1%) or IVA337 (10 or 30 mg/kg) po once a day (n = 10 per group) for 3 weeks.

foz/foz Model

Six-week-old Alms1 mutant foz/foz mice were fed an HFD (60 kcal% fat; D12492; Research Diets) or an ND for 6 weeks. A group of mice were killed to examine their pathologic status; the remaining mice were randomized to receive the HFD alone (n = 10) or with IVA337 at 75 mg/kg of diet (n = 10) or 200 mg/kg of diet (n = 12) for another 6 weeks. The ND group (n = 8) stayed on the ND regimen for another 6 weeks.

CCl_4-Induced Fibrosis

In a prophylactic setup, C57Bl6/J mice (6 weeks of age; approximately 25 g) received 100 μL of either sunflower seed oil or CCl_4 (3.5 mL/kg diluted in sunflower seed oil) intraperitoneally twice a week for 3 weeks. Rosiglitazone (PPARγ agonist; 5 mg/kg) or IVA337 (30 mg/kg) were administered po once daily on top of CCl_4 for 3 weeks (n = 8 per group). In a therapeutic design, mice received CCl_4 for 3 weeks to initiate liver fibrosis. Treatment, i.e., vehicle, IVA337 (10 or 30 mg/kg), rosiglitazone (5 mg/kg), fenofibrate (PPARα agonist; 100 mg/kg), or GW501516 (PPARδ agonist; 10 mg/kg), was then administrated per daily gavage along with CCl_4 for an additional 3 weeks (n = 8 per group).

IN VITRO EXPERIMENTS

Activation Assay

Human primary HSCs (hHSCs; #5300; ScienCell) were seeded on plastic six-well plates for 7 days in complete medium with either dimethyl sulfoxide 0.1% or a compound (IVA337, 3 μM; rosiglitazone, 3 μM; fenofibrate, 30 μM; or GW501516, 3 μM). hHSC activation was evaluated with western blot by measuring the expression of α-smooth muscle actin (α-SMA).

Proliferation Assay

hHSCs were seeded in 96-well plates for 24 hours, then serum starved for 24 hours. They were challenged with platelet-derived growth factor (PDGF; 10 ng/mL) with or without a tested compound for 24 hours at various concentrations (3 nM to 30 μM, with a semi-log scale) in triplicates. 5-Ethynyl-2′-deoxyuridine was incorporated for 17 hours, after which cells were fixed with 4% formaldehyde; immunocytochemistry staining for 5-ethynyl-2′-deoxyuridine was then performed.

Statistical Analysis

Two groups were compared using a *t* test. Experiments with more than two groups were analyzed using one-way analysis of variance followed by Dunnett's test.

Results

IVA337 ACTIVATES THE THREE PPAR ISOFORMS WITH MODERATE AND BALANCED ACTIVITY IN THE TRANSACTIVATION ASSAY

In the transactivation assay (see Supporting Information), IVA337 acts as a pan-PPAR agonist with moderate and balanced activity on the three PPAR isoforms.

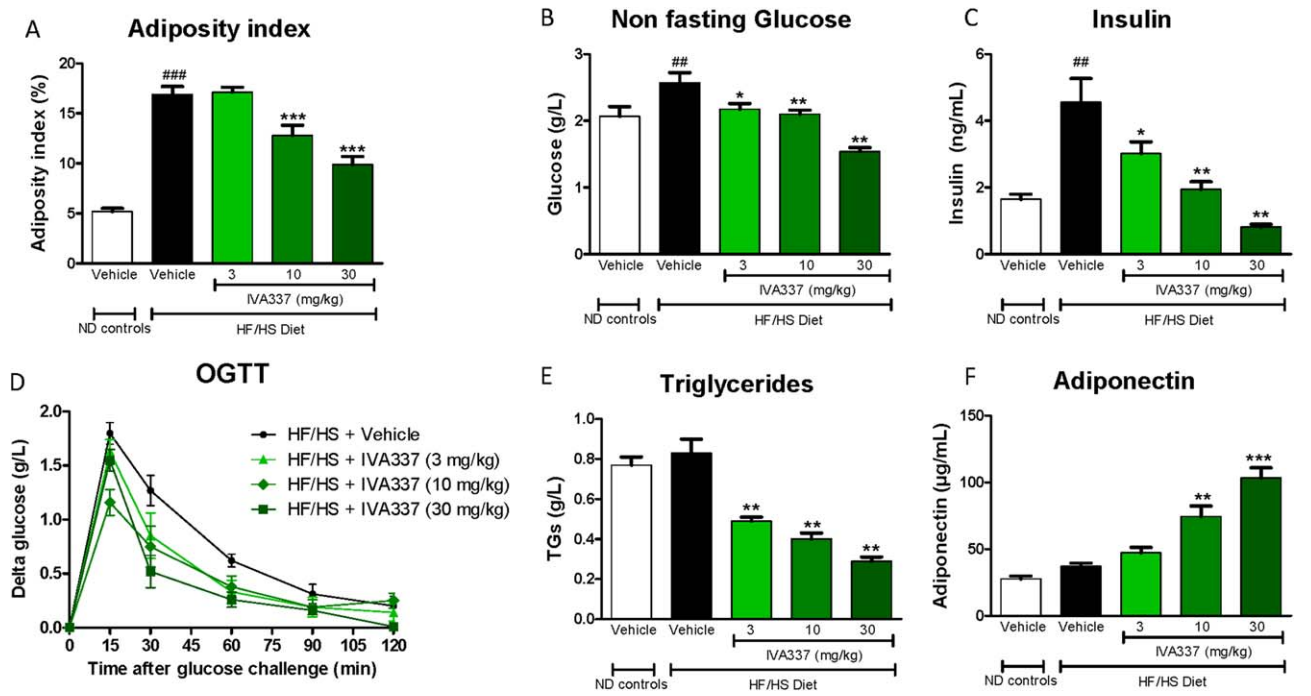

FIG. 1. IVA337 dose dependently decreases adiposity index and normalizes glucose and insulinemia in a diet-induced obesity model. (A) The adipose index (total WAT/body weight) was calculated in mice under a chow diet (ND controls) and mice under an HF/HS diet treated or not with IVA337 at 3, 10, and 30 mg/kg (n = 10 per group). (B-E) Plasma analyses were performed at sacrifice for nonfasting glucose, insulin, triglycerides, and adiponectin levels. (F) An OGTT was carried out at 5 weeks. Data represented as mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ versus ND controls; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus HF/HS diet + vehicle. Abbreviations: OGTT, oral glucose tolerance test; WAT, white adipose tissue.

IVA337 50% effective concentration (EC_{50}) levels for the human PPARs (hPPARs) were 1.63E-06 M for PPARα, 8.49E-07 M for PPARδ, and 2.28E-07 M for PPARγ. IVA337 EC_{50} levels for the rodent PPARs were 3.78E-07 M for PPARα, 1.55E-06 M for PPARδ, and 2.23E-07 M for PPARγ. The maximal effect (Emax) reached 100% for both hPPARα and hPPARδ and 80% for hPPARγ when compared to fenofibrate, GW501516, and rosiglitazone, respectively.

IVA337 DECREASES BODY WEIGHT GAIN AND INSULIN RESISTANCE INDUCED BY AN HF/HS DIET

The HF/HS model was used to evaluate the effect of IVA337 on insulin resistance and other parameters linked to metabolic syndrome. Compared to the ND, mice fed for 12 weeks with the HF/HS diet had an increased body weight (55%; $P < 0.001$) (Supporting Fig. S1A), adiposity index (225%; $P < 0.001$), nonfasting glucose (24%; $P < 0.01$), and circulating insulin levels (176%, $P < 0.01$) (Fig. 1A-C). IVA337 dose dependently reduced body weight gain (–37% at 30 mg/kg; $P < 0.05$) and adiposity index increase (–60% at 30 mg/kg; $P < 0.001$) (Supporting Fig. S1A; Fig. 1A). IVA337 also normalized insulinemia and nonfasting glucose and reduced circulating leptin levels (Fig. 1B,C; Supporting Fig. S1B). During an oral glucose tolerance test, IVA337 dose dependently improved glucose tolerance (Fig. 1D). IVA337 decreased circulating triglycerides, elevated serum ketone bodies (Supporting Fig. S1C), and increased circulating adiponectin, demonstrating PPARα and PPARγ target engagement (Fig. 1E,F).

IVA337 PREVENTS STEATOHEPATITIS INDUCED BY AN MCD DIET

We used the MCD diet model to evaluate the effect of IVA337 on liver steatosis and inflammation. IVA337 prevented steatosis (–98% at 30 mg/kg; $P <$

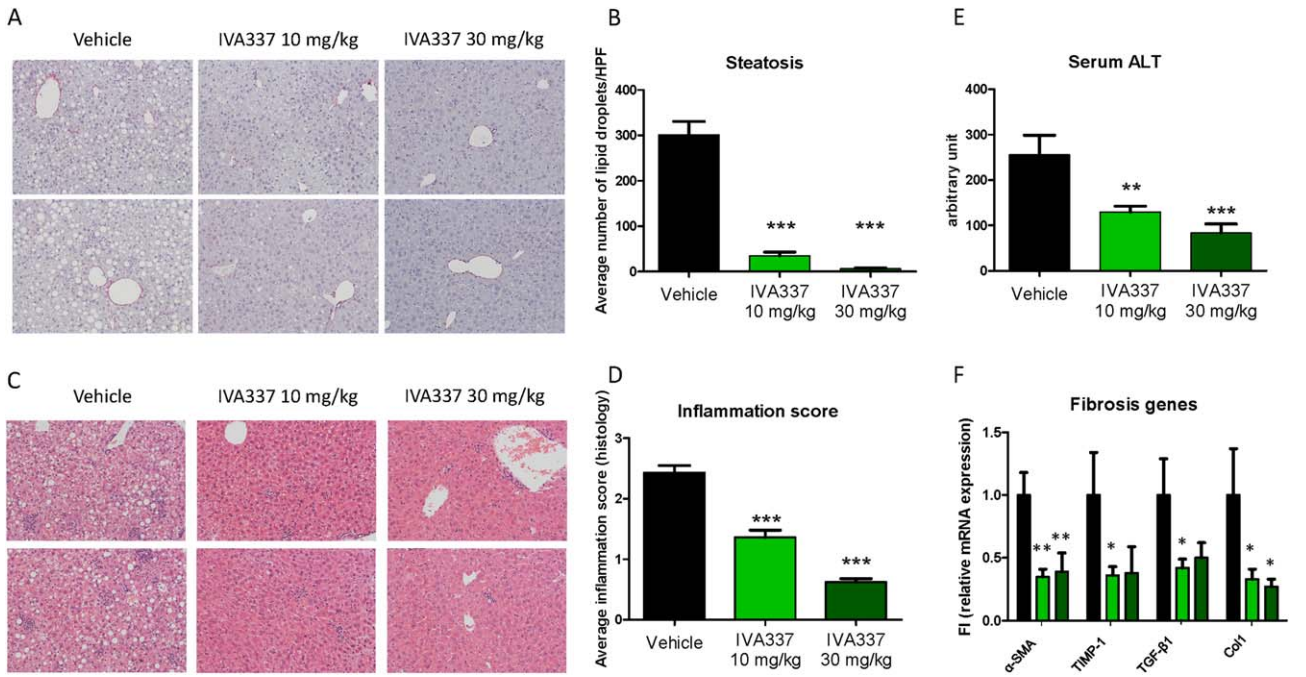

FIG. 2. IVA337 improves steatosis, inflammation, fibrosis, and ALT in the MCD model. (A,B) Steatosis and (C,D) inflammation were histologically measured at magnification ×20 in mice under an MCD diet for 3 weeks receiving or not IVA337 at 10 and 30 mg/kg (n = 10 per group). (E) ALT level was measured in the blood, and (F) the expression of fibrotic genes was evaluated by RT-qPCR in the liver. Data represented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus MCD diet + vehicle. Abbreviations: ALT, alanine aminotransferase; col1, collagen type I; RT-qPCR, real time polymerase chain reaction.

0.001) (Fig. 2A,B) and inflammation (–75% at 30 mg/kg; $P < 0.001$) as measured histologically by lipid droplet count or lobular inflammation foci count, respectively (Fig. 2C,D). IVA337 also significantly reduced plasma alanine aminotransferase levels (Fig. 2E). Consistent with the results obtained in the HF/HS model, IVA337 decreased serum as well as liver triglyceride levels (Supporting Fig. S2A,B). IVA337 also inhibited the induction of profibrotic and fibrotic genes, such as transforming growth factor beta (TGF-β1), α-SMA, tissue inhibitor of metalloproteinase 1 (TIMP1) and collagen 1 in MCD livers (Fig. 2F).

IVA337 REDUCES STEATOSIS, INFLAMMATION, BALLOONING, AND FIBROTIC GENE EXPRESSION IN THE foz/foz MODEL

The effect of IVA337 was investigated in the Alsm1 mutant foz/foz mice fed an HFD, a model closely reproducing the natural history of NASH in humans. IVA337 was mixed into the HFD; a pharmacokinetic study indicated that a concentration of IVA337 at 75 or 200 mg in 1 kg of diet gave the same drug exposure as IVA337 at 10 and 30 mg/kg of body weight, respectively, given by daily gavage (data not shown). After 6 weeks of the HFD, foz/foz mice developed obesity and insulin resistance (Fig. 3A,B). Mice fed an HFD and treated with IVA337 quickly and fully normalized blood glucose levels in less than a week (Fig. 3B) with food intake being similar between the HFD groups with or without IVA337 (Supporting Fig. S3A). IVA337 at 30 mg/kg completely restored glucose tolerance to the level measured in chow-fed mice (Supporting Fig. S3B,C). IVA337 also normalized fasting glycemia, insulin, and the homeostasis model assessment index after 6 weeks of treatment (Supporting Fig. S3D-F). Similar to the other models, IVA337 treatment significantly increased adiponectin levels (Fig. 3C). Histologic examination of the liver indicated that IVA337 dose dependently reduced steatosis, ballooning, and inflammatory foci induced by the HFD (Fig. 3D-F). According to these three parameters, all mice in the HFD control group presented with a nonalcoholic fatty liver disease activity score (NAS) superior to 5 (mean = 6.6), which is considered to be definitive of NASH. IVA337 dose dependently and significantly decreased the number

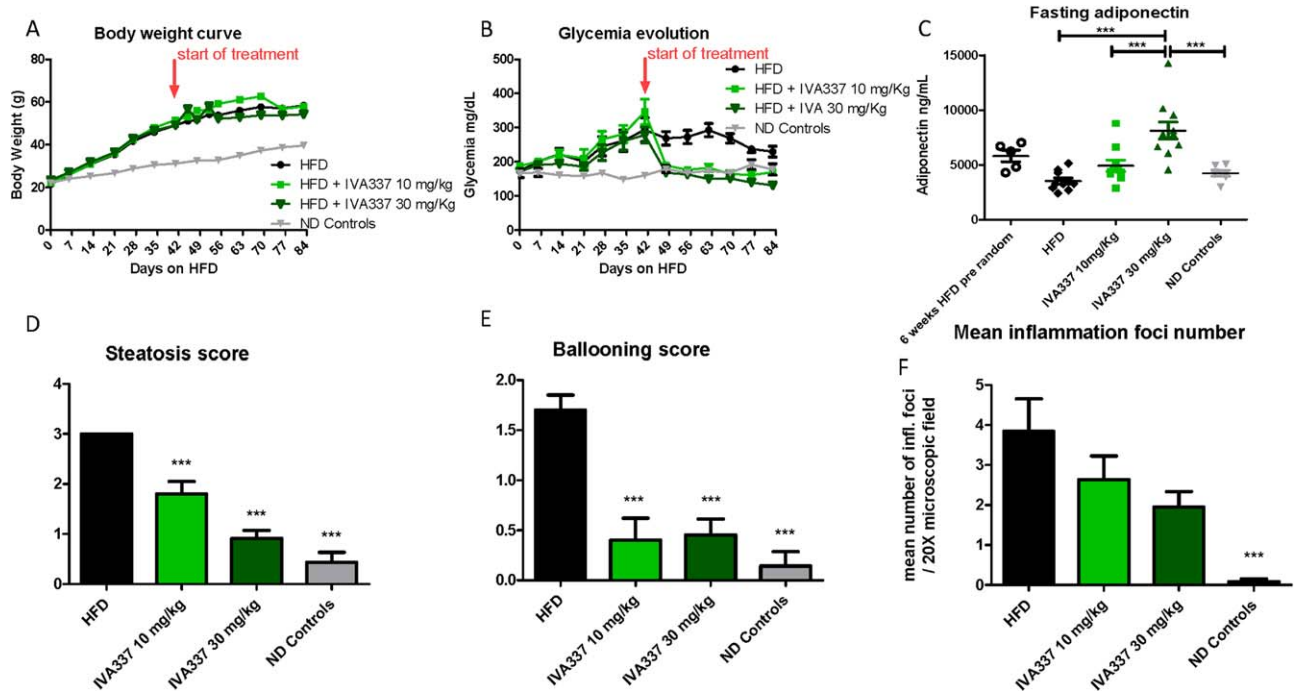

FIG. 3. IVA337 normalizes hyperglycemia and reduces steatosis, ballooning, and inflammation in the foz/foz model. During the 12-week experiment, mice under ND (n = 8), HFD (n = 10), HFD + IVA337 at 10 mg/kg (n = 10), or HFD + IVA337 at 30 mg/kg (n = 12) were followed for (A) body weight and (B) glycemia evaluation once a week. After sacrifice, (C) circulating adiponectin was measured and (D-F) histologic analyses at magnification ×20 of the liver were performed to quantify steatosis, ballooning, and inflammation foci. Data represented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus HFD + vehicle.

of mice classified as definite NASH. At the highest dose, only one mouse had an NAS equal to 5, while the other 10 had an NAS < 5 (mean = 2.8; Supporting Fig. S4A, B). Although no fibrosis was observed histologically, IVA337 reduced the expression of fibrotic genes (α-SMA, collagen 3, TGF-β2, TGF-β3, TGF-β receptor [TGF-βRI and RII], TIMP1, TIMP2, and matrix metalloproteinase 2 [MMP2]) induced by the HFD regimen (Supporting Fig. S5A-C) and reduced macrophage recruitment within the liver (Supporting Fig. S5D,E). IVA337 had no effect on body weight, liver, white adipose tissue, or heart weight (Supporting Fig. S6A-F).

IVA337 ACTS POSITIVELY ON THE EXPRESSION OF GENES CONTROLLING β-OXIDATION, LIPOTOXICITY, INFLAMMASOME, AND INFLAMMATION IN THE MCD AND foz/foz MODELS

In both the MCD and foz/foz mice, which are two mechanistically distinct animal models, IVA337 strongly and dose dependently induced stearoyl-coenzyme A desaturase-1 (SCD1), a gene controlling monounsaturation of free fatty acid and the activation of which would decrease lipotoxicity. IVA337 induced carnitine palmitoyltransferase (CPT)1b and CPT2, genes controlling β-oxidation (Fig. 4B,E), and decreased the expression of the inflammasome genes NOD-like receptor family, pyrin domain containing 3 (NLRP3), apoptosis-associated speck-like protein containing a CARD, caspase1, interleukin (IL)-1β, and IL18 (Fig. 4A,D) as well as the inflammatory genes C-C chemokine receptor type 2, chemokine (C-C motif) ligand 5, and nuclear factor kappa B1 (NF-κB1) (Fig. 4C,F).

IVA337 PREVENTS AND REVERSES CCl_4-INDUCED LIVER FIBROSIS

In a preventive design, IVA337 at 30 mg/kg inhibited CCl_4-induced collagen deposition (83% decrease compared to the CCl_4 vehicle; $P <$ 0.01), reduced plasma triglyceride, and increased plasma adiponectin

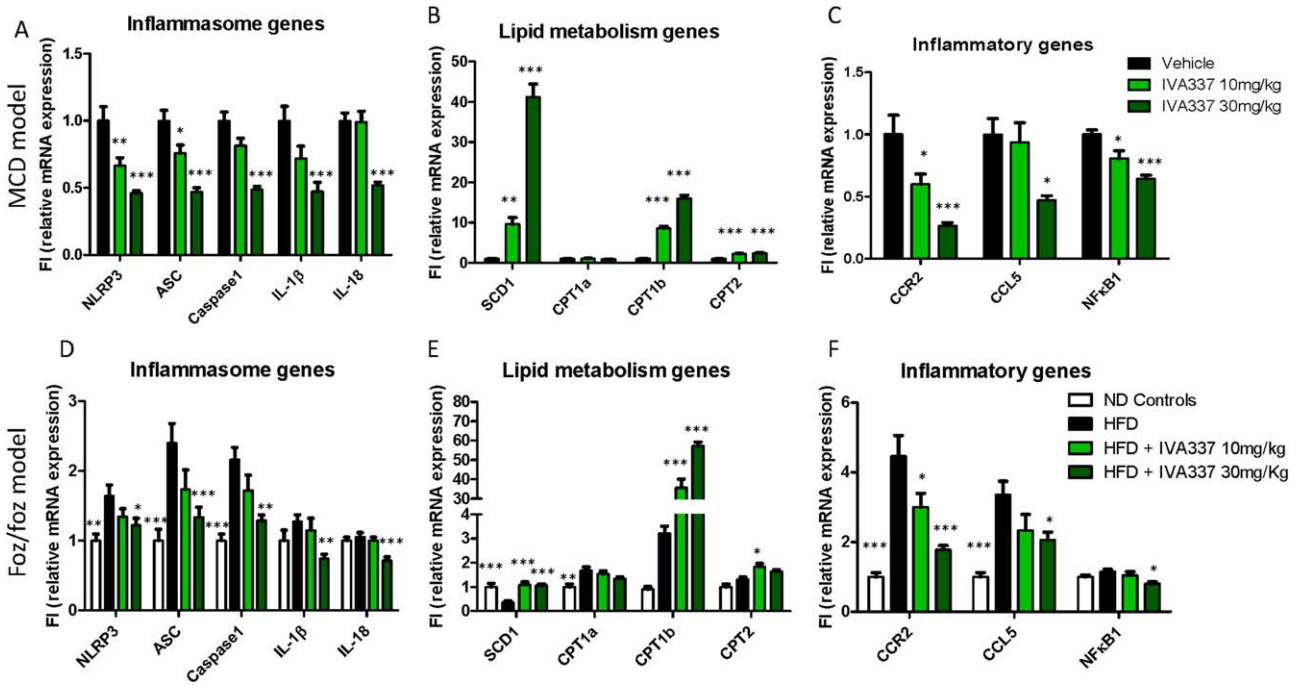

FIG. 4. IVA337 inhibits Inflammasome-related genes and NF-κB expression and induces lipid metabolism-related gene expression in the MCD and foz/foz models. Gene analyses were performed on the liver of mice from (A-C) the MCD model (n = 10 per group) and (D-E) the foz/foz model (n = 8-12 per group). (A,D) Liver inflammasome-related genes, (B,E) lipid metabolism genes, and (C,F) inflammatory genes. Data represented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus MCD + vehicle (A-C) or HFD + vehicle (D-F). Abbreviations: ASC, apoptosis-associated speck-like protein containing a CARD; CCL5, chemokine (C-C motif) ligand 5; CCR2, C-C chemokine receptor type 2; mRNA, messenger RNA.

(Supporting Fig. S7A-C). IVA337 also inhibited the expression of the key fibrotic genes TGF-β1, collagen 1, and fibronectin, whereas rosiglitazone (5 mg/kg) had a limited efficacy on collagen deposition and fibrotic gene expression (Supporting Figs. S7A and S8A-E).

IVA337 was next investigated in a curative setting. CCl_4 treatment increased liver collagen deposition (measured by hydroxyproline content) to 175% ($P < 0.001$) and 210% ($P < 0.01$) of control levels after 3 and 6 weeks, respectively. CCl_4 also induced a thickening and increased number of fibrotic septa (Fig. 5A). CCl_4-induced fibrosis was associated with inflammation demonstrated by the increased RNA expression of F4/80, a marker of macrophages (Table 1). IVA337 treatment at 30 mg/kg after 3 weeks of CCl_4 prevented further fibrosis progression (Fig. 5B,C) whether measured by hydoxyproline content or PicroSirius Red morphometry. The histologic examination also demonstrated a decrease in the number of collagen septa (Fig. 5A). This effect on collagen deposition was accompanied by a repression of fibrogenic genes, such as TGF-β1, TGF-β2, TGF-β3, fibronectin, collagen I, MMP2, MMP9, and F4/80 at the doses of 10 and 30 mg/kg (Table 1).

REVERSION OF CCl_4-INDUCED LIVER FIBROSIS BY IVA337: COMPARISON WITH SINGLE PPAR AGONISTS

The effect of IVA337 on liver fibrosis was compared to three selective PPAR agonists, fenofibrate (PPARα), GW501516 (PPARδ), and rosiglitazone (PPARγ), administered for the last 3 weeks of a 6-week CCl_4 regimen. At the tested doses, the three compounds are selective for their respective PPAR isoform. Fenofibrate and rosiglitazone but not GW501516 produced histologic improvements with smaller fibrotic septa and a significant reduction of hydoxyproline content (Fig. 6A,B). Only IVA337 and fenofibrate demonstrated antifibrotic efficacy by Sirius Red morphometry (Fig. 6C). IVA337 and fenofibrate decreased alanine aminotransferase and serum

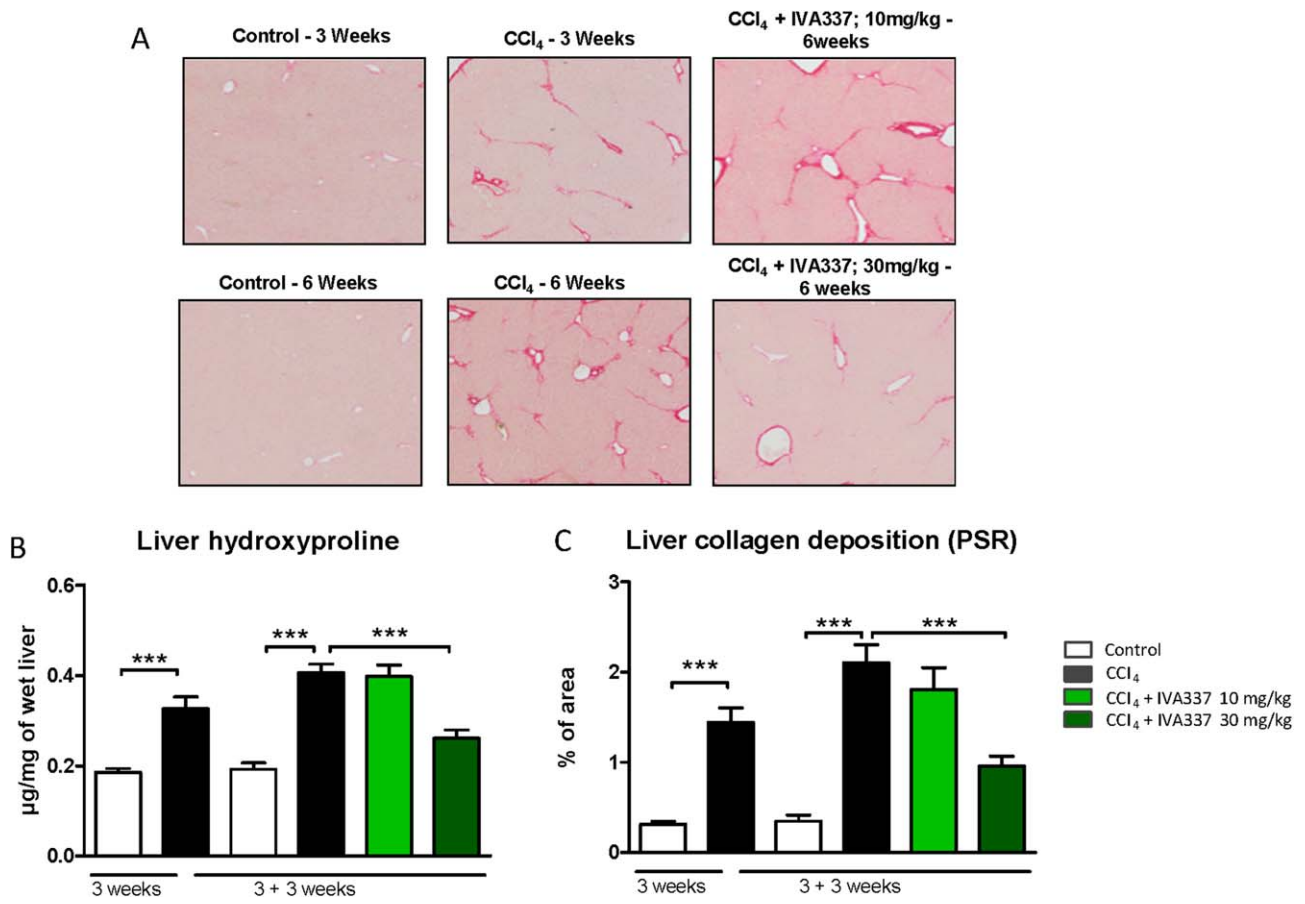

FIG. 5. IVA337 reverses fibrosis in a curative CCl_4-induced liver fibrosis model. (A) Liver histologic pictures (magnification × 20) from a 6-week CCl_4 study, (B) liver hydroxyproline content, and (C) PicroSirius Red analysis in mice treated or not with IVA337 10 and 30 mg/kg (n = 8 per group). Data represented as mean ± SEM. $^{***}P < 0.001$.

triglycerides, whereas expectedly, IVA337 and rosiglitazone increased adiponectin, demonstrating similar PPAR target engagement between IVA337 and fenofibrate on one hand and IVA337 and rosiglitazone on the other (Supporting Fig. S9A-C).

IVA337 INHIBITS PDGF-INDUCED PROLIFERATION, STIFFNESS-INDUCED ACTIVATION, AND TGF-β1-INDUCED OVEREXPRESSION OF FIBROTIC GENES IN hHSCs

We first investigated the effect of IVA337 and three selective PPAR agonists, fenofibric acid, GW501516, and rosiglitazone, on PDGF-induced proliferation of hHSCs. PDGF increased basal proliferation by more than 5-fold (Fig. 7A). IVA337 dose dependently and completely inhibited PDGF-induced hHSC proliferation (Fig. 7A). In contrast, the selective PPAR agonists demonstrated only partial effects up to the highest concentrations (Fig. 7A). We then studied the effects of the PPAR agonists on hHSC activation. After 7 days in culture, α-SMA expression was highly increased, demonstrating activation (Fig. 7B, upper western blot). Addition of 3 µM of IVA337 in the culture medium prevented an increase in α-SMA protein at day 7 (Fig. 7B, upper western blot). Rosiglitazone prevented overexpression of α-SMA to the same extent as IVA337. GW501516, but not fenofibric acid, prevented α-SMA overexpression with a lower potency than IVA337 and rosiglitazone (Fig. 6F, lower western blot). We finally tested the effects of the different PPAR agonists on TGF-β1-induced hHSC activation. As expected, TGF-β1 significantly induced

TABLE 1. EFFECT OF IVA337 ON HEPATIC GENE EXPRESSION IN A CURATIVE CCL_4 MODEL

3 + 3 weeks	TGF-β1	TGF-β2	TGF-β3	TGF-βRI	TGF-βRII	Col1	Fibronectin	MMP2	MMP9	F4/80
Control	1.00 ± 0.30	1.00 ± 0.21	1.00 ± 0.26	1.00 ± 0.21	1.00 ± 0.39	1.00 ± 0.28	1.00 ± 0.20	1.00 ± 0.22	1.00 ± 0.77	1.00 ± 0.22
CCl_4	1.59 ± 0.18¶	3.88 ± 0.67¶	4.40 ± 0.54¶	0.98 ± 0.21	1.38 ± 0.15§	12.33 ± 3.29¶	1.16 ± 0.15	16.80 ± 3.49¶	1.94 ± 0.68‖	1.72 ± 0.69‖
CCl_4 + IVA337 10 mg/kg	1.25 ± 0.17*	2.97 ± 0.87*	2.90 ± 0.52‡	0.82 ± 0.12	0.98 ± 0.14*	8.58 ± 2.09†	0.65 ± 0.08‡	9.57 ± 3.38‡	1.27 ± 0.48	1.46 ± 0.23
CCl_4 + IVA337 30 mg/kg	1.24 ± 0.16*	2.85 ± 0.53*	3.05 ± 0.47‡	0.69 ± 0.12†	0.94 ± 0.29*	9.25 ± 1.48*	0.51 ± 0.04‡	9.53 ± 2.16‡	0.82 ± 0.24‡	1.06 ± 0.47†

Note: In the curative model of CCl_4-induced liver fibrosis, TGF-β1, TGF-β2, TGF-β3, TGF-βRI, TGF-βRII, collagen1, fibronectin, MMP2, MMP9, and F4/80 gene expression were analyzed (n = 8 per group). The results are expressed as fold induction with respect to the control group. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ versus CCl_4 + vehicle. §$P < 0.05$; ‖$P < 0.01$; ¶$P < 0.001$ versus control.
Abbreviation: col1, collagen 1.

α-SMA, connective tissue growth factor, collagen 1α1, and plasminogen activator inhibitor 1 messenger RNA expression. IVA337 treatment totally abrogated this effect, but none of the three selective PPAR agonists prevented the induction of fibrotic genes by TGF-β1 (Fig. 7C-F).

Discussion

Several studies support the contribution of specific PPAR isoforms in the pathogenesis of steatohepatitis in animal models. PPARα- as well as PPARγ-deficient mice are more sensitive to the development of steatohepatitis under an MCD than wild-type mice.[21,22] Using selective PPAR agonists, it was reported that Wy-14,643 (a PPARα agonist), GW501516 (a PPARδ agonist), and PPARγ activation by pioglitazone prevent and/or reverse MCD-induced steatohepatitis.[21-25] In the foz/foz model, a protective effect on steatohepatitis was observed with PPARα agonist treatment.[15]

Liver necroinflammation is considered an important driver of disease progression. All three PPARs potentially control inflammation. PPARα, besides primarily governing fatty acid uptake, catabolism, and repressing gluconeogenesis, dampens the pro-inflammatory transcription factor NF-kB. PPARδ promotes anti-inflammatory M2 polarization of immune cells, among which are resident macrophages.[7] PPARγ through adipogenic effects, adiponectin induction, and inhibition of NF-kB activity potently decreases adipose and systemic inflammation. Achieving anti-inflammatory effects is desirable not only for control over metabolic syndrome but also to restrain liver inflammation and thereby interrupt liver disease perpetuation and progression. Lipotoxic-saturated fatty acids activate the inflammasome pathway in hepatocytes and the subsequent release of pro-inflammatory cytokines, such as IL-1β and IL-18.[26,27] NLRP3 is significantly upregulated in patients with NASH,[28] and inhibition or knockdown of the inflammasome components reduces insulin resistance, steatosis, and fibrosis.[29-31] Lee et al. showed that the PPARδ ligand GW501516 inhibits the activation of the inflammasome pathway *in vivo* and *in vitro* in HepG2 cells.[32] PPARs in various cell types in the liver and extrahepatic tissues, such as adipose tissue, contribute to decreased inflammation in metabolic disorders and steatohepatitis.

To evaluate the effect of concomitant activation of PPARα, PPARδ, and PPARγ on the metabolic and

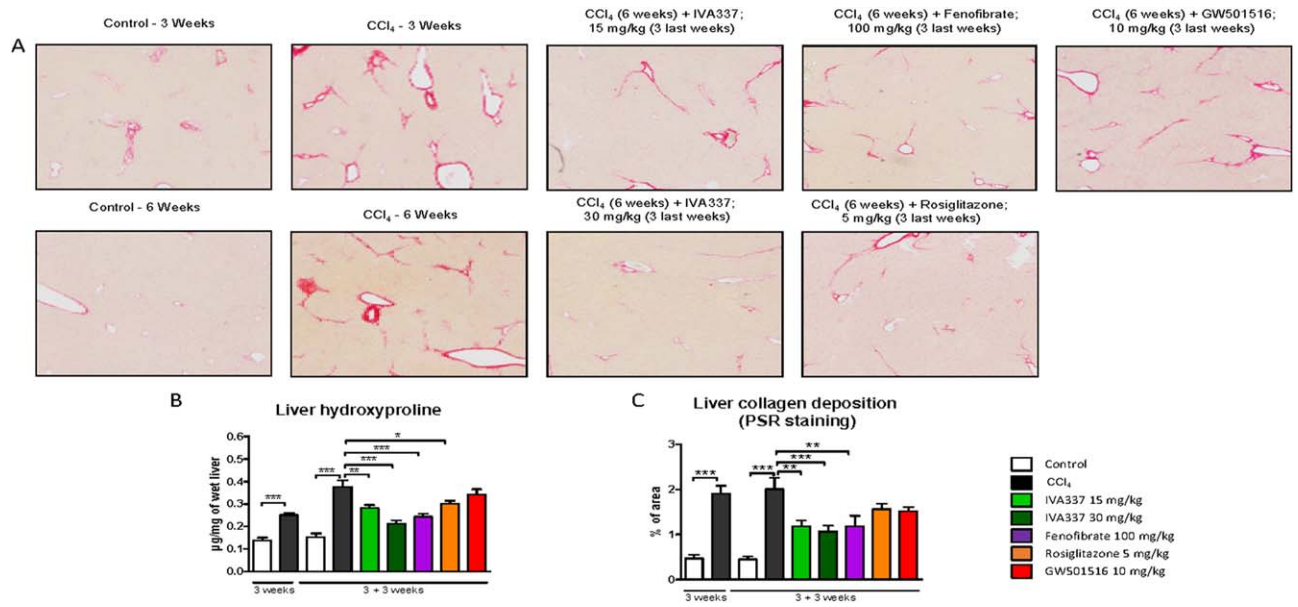

FIG. 6. IVA337 reversion of fibrosis in the CCl_4 model, comparison with selective PPAR agonists. (A) Liver histologic pictures from a 6-week CCl_4 study, (B) liver hydroxyproline content, and (C) PicroSirius Red analysis in mice treated or not with IVA337 (15 and 30 mg/kg), rosiglitazone (5 mg/kg), GW501516 (10 mg/kg) or fenofibrate (100 mg/kg) (n = 8 per group). Data represented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Abbreviation: PSR, PicroSirius Red.

liver injury processes relevant to NASH pathophysiology, we studied the effect of IVA337 on an HF/HS model, an MCD model, the *Alms1*-deficient foz/foz model, and the CCl_4 model. In addition, we looked at PPAR-related gene expression and investigated the effects of IVA337 and selective agonists of each isoform on activation and proliferation of hHSCs, key drivers of liver fibrosis in NASH.

As shown in the transactivation assay, IVA337 is a pan-PPAR agonist with balanced and moderate activity on the three PPAR isoforms. The efficacy of the molecule reaches 100% for hPPARα and hPPARβ/δ and 80% for hPPARγ. The potency of IVA337 for PPARα and PPARγ is in the same range as that of fenofibrate (EC_{50}, 2 µM; PPARα) and pioglitazone (EC_{50}, 0.3 µM; PPARγ), two clinically used and well-tolerated PPAR agonists with a good efficacy/safety ratio.[33] The balanced activity of IVA337 is further supported by preclinical and clinical results that show target engagement for the different PPARs, and pharmacological active doses are all in a similar dose range.

In the 3 weeks with the MCD model, IVA337 completely prevented steatosis and to a large extent the necroinflammatory changes. Similarly in the HFD foz/foz model in which steatohepatitis occurs as a complication of severe obesity and insulin resistance, IVA337 also largely attenuated steatosis and ballooning and reduced macrophage recruitment and fibrotic gene expression. Although we did not provide the specific mechanism of action that explains the positive effect of IVA337 on NASH features, the gene analysis performed on the MCD and foz/foz models provides an indication of the implications of the different PPAR isoforms. We highlight that IVA337 increased the expression of CPT1b and CPT2 genes, which have been widely documented to be direct target genes of PPARα and to participate in the transport to and oxidation of fatty acids in the mitochondria, metabolizing fat into energy.[34,35] Activation of this pathway would reduce lipid accumulation and also counteract the *de novo* lipogenesis contributing to inhibition of steatosis in the hepatocytes. The expression of SCD1, which catalyzes the desaturation of saturated free fatty acids, is also enhanced with IVA337 treatment. Using pioglitazone, Borengasser et al.[36] demonstrated that this gene is a downstream gene of PPARγ. The increase in SCD1 expression should lead to an increase in monounsaturated fatty acids (MUFAs) that are less toxic than saturated fatty acids. Interestingly, MUFA feeding prevents MCD-induced injury.[37] SCD1 inhibitors are currently tested in NASH because inhibition of SCD1 leads to a decrease in steatosis. $SCD1^{-/-}$ mice under an

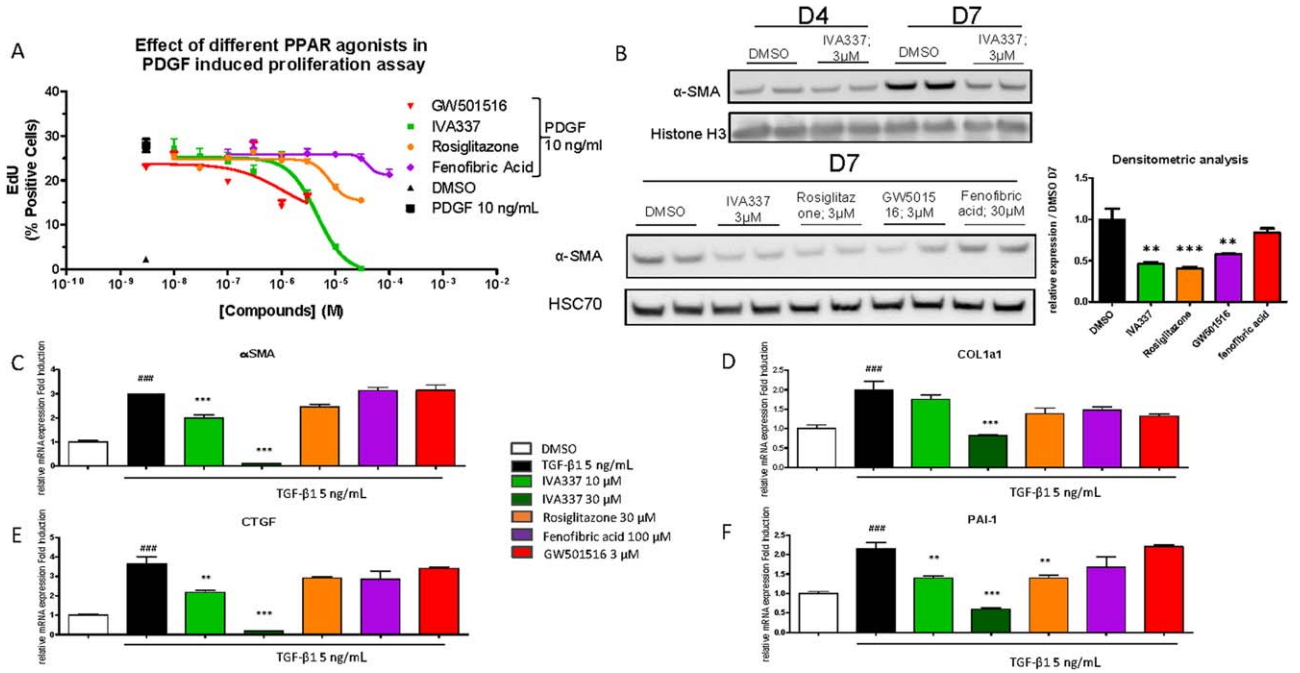

FIG. 7. IVA337 inhibits PDGF-induced proliferation, stiffness-induced activation, and TGF-β1-induced profibrotic gene expression; comparison with selective PPAR agonists. (A) Effects of IVA337, rosiglitazone, GW501516, and fenofibrate in a dose range (3 nM to 30 µM, with a semi-log dilution scale) on PDGF (10 ng/mL)-induced hHSC proliferation. (B) hHSCs plated on plastic differentiated into myofibroblasts after 7 days in culture. Effect of IVA337 (3 µM), rosiglitazone (3 µM), GW501516 (3 µM), and fenofibric acid (30 µM) were evaluated. (C-F) TGF-β1-induced profibrotic gene expression after 24 hours. Data represented as mean ± SEM. $^{***}P < 0.001$ versus DMSO; $^{**}P < 0.01$, $^{***}P < 0.001$ versus TGF-β1. Abbreviations: col1α1, collagen type Iα1; CTGF, connective tissue growth factor; DMSO, dimethyl sulfoxide; EdU, 5-ethynyl-2′-deoxyuridine; mRNA, messenger RNA; PAI-1, plasminogen activator inhibitor 1.

MCD have decreased steatosis but have a marked increase in hepatocellular apoptosis, liver injury, and fibrosis compared with SCD1$^{+/+}$ mice. Finally, we demonstrated that IVA337 decreased the expression of the inflammasome components and downstream cytokine targets. This effect of IVA337 might be due to PPARδ because it was previously shown that PPARδ activation decreases the expression of inflammasome components (NLRP3, caspase1, and IL-1) when stimulated with palmitate (a saturated fatty acid) and lipopolysaccharides in hepatocytes.[32] This effect could also be due to the PPARγ effect on SCD1 because saturated fatty acids activate the inflammasome whereas MUFAs inhibit the inflammasome components.[26,38] Overall, these results indicate that activation of PPARα, PPARδ, and PPARγ in the hepatocytes would contribute to the antisteatotic and anti-inflammatory effect of IVA337 in the MCD and foz/foz models.

In addition to its effect on steatosis and necroinflammation, we also demonstrated that IVA337 has a potent antifibrotic effect. The fibrotic pathology was activated in the MCD as well as in the foz/foz model, although the 3-week (MCD) or 12-week (foz/foz) regimen applied was too short to observe fibrosis histologically. Treatment with IVA337 significantly decreased the expression of the key profibrotic genes, such as TGF-β1 and α-SMA. IVA337 prevented and interrupted progression of liver fibrosis in the CCl_4 model. In order to understand the relative contribution of the PPAR isoforms, we compared the effect of IVA337 to that of each of the three selective PPAR agonists, fenofibrate (PPARα), GW501516 (PPARδ), and rosiglitazone (PPARγ). In the CCl_4 therapeutic model, the rank order of antifibrotic efficacy was IVA337 $\geq$ fenofibrate $>$ rosiglitazone $>$ GW501516, with PPARγ and PPARδ agonists having a partial effect on fibrosis. Our results are consistent with published studies on the effect of selective PPAR agonists on liver fibrosis.[14,21,39] However, results with GW501516 in our study and in the literature differ from those obtained with KD3010, another PPARδ agonist that was shown to be very active on liver fibrosis

induced by CCl_4 or bile duct ligation.[39] This indicates that PPARδ-mediated inhibition of fibrosis is likely to be ligand dependent owing to different pharmacokinetic properties or recruitment of different coregulators.[39,40] *In vitro* studies support that IVA337 dose dependently and completely inhibits PDGF-induced HSC proliferation, while the single agonists only have a partial effect. Both IVA337 and rosiglitazone prevented myofibroblastic transformation of HSC on stiff support, while GW501516 had a partial effect, and fenofibrate was inactive. Surprisingly, none of the single agonists inhibited TGF-β1-induced fibrotic gene expression, yet it was completely blocked by IVA337. Our previous work supports that inhibition of TGF-β1-induced myofibroblast transdifferentiation by IVA337 is mediated through inhibition of phospho-SMAD2/3 expression.[41] Thus, IVA337 with pan-PPAR ligand-binding potency consistently inhibits hHSC proliferation, culture-mediated activation, and TGF-β1-driven profibrotic activation and prevents fibrosis and fibrosis progression *in vivo*. As none of the single agonists achieved such a level of control on the fibrotic process, the effect of IVA337 is likely to be explained by a cumulative effect of multiple PPAR targeting. This further strengthens the potential of IVA337 as an antifibrotic agent in patients with NASH.

IVA337, in addition to improving the main NASH parameters, also improved metabolic features relevant to NASH. Indeed, dysregulation of metabolism, such as insulin resistance and type 2 diabetes, is closely linked to the development of NASH. In the HF/HS model, fenofibrate (PPARα) is reported to prevent body and fat mass increase and fasting insulin increase but does not improve fasting glucose or glucose tolerance, while rosiglitazone (PPARγ) further increases body and fat mass versus diet-control animals and in contrast to fenofibrate restores glucose tolerance and decreases fasting glucose and insulin levels.[42] IVA337 almost normalized all these parameters; it also decreased plasma triglycerides and increased β oxidation. IVA337 also quickly normalized fasting glucose and insulin levels and fully restored glucose tolerance in obese and insulin-resistant HFD-fed foz/foz mice. This profile may reflect the complementary (lipid and glucose metabolism) as well as the opposing effects (on fat mass) of PPARα/δ and PPARγ activation. Besides PPARα, PPARδ activity likely contributes to the observed effect because PPARδ agonists reduce body weight gain and glucose and lipid abnormalities and increase liver fatty acid β oxidation.[43,44] Of note in this context, the PPARδ agonist GW0742 also corrected hypertension, vascular inflammatory and oxidative status, and endothelial dysfunction.[43] On the other hand, IVA337 increased circulating adiponectin, a canonical PPARγ target that contributes to decreasing inflammation and improving insulin resistance in the liver; the adverse effect of PPARγ activation in the adipose tissue is adipogenesis and fat mass gain. In patients, adiponectin inversely correlates with steatosis and steatohepatitis.[45] Together, this supports the conclusion that the effects of IVA337 on insulin sensitivity, body weight gain, and other metabolic disorders induced by the HF/HS diet or HF diet in foz/foz mice result from the concomitant activation of the three PPAR isoforms and that a pan-PPAR activation could potentially deliver a superior improvement of NASH-associated metabolic disorders compared to individual PPAR agonists.

In humans, PPAR targeting is beneficial for metabolic steatohepatitis. It has been shown that the PPARα expression level in the liver negatively correlates with the severity of NASH.[46] During a 48-week clinical trial in patients with biopsy-proven NASH, fenofibrate significantly decreased ballooning and improved metabolic parameters but not inflammation or steatosis.[16] Selective PPARδ agonists have not been investigated in patients with NASH, but in overweight subjects GW501516 and MBX-8025 improved metabolic parameters during a 2-week and 8-week duration trial, respectively.[17,18] More recently, the dual PPARα/δ agonist elafibranor (GFT505) achieved improvement in steatohepatitis without fibrosis worsening in patients with a NAS score $\geq$4 and decreased fibrosis in the subgroup of patients with NASH who responded to GFT505.[47] PPARγ activation by pioglitazone significantly improves steatosis, ballooning, and inflammation as well as metabolic markers in patients with NASH after 6 or 12 months of treatment.[48] A recent 18-month study in prediabetic and diabetic patients with biopsy-proven NASH demonstrated that pioglitazone was well tolerated without adverse effect and was associated with long-term metabolic and histologic improvement.[33] As selective targeting of each PPAR isoform confers some therapeutic benefit for patients with NASH, it is therefore expected that combined activation of the three PPAR isoforms might bring substantial advantage over specific and dual agents by interacting on different pathways in the NASH to fibrosis sequence.

In conclusion, this study demonstrates that IVA337, a safe, well-tolerated, moderate, and well-balanced pan-PPAR agonist, rapidly and powerfully

improves metabolic parameters and NASH histopathologic features, such as steatosis, ballooning, inflammation, and fibrosis, in animal models. As IVA337 concomitantly activates the three PPAR isoforms, it modulates various metabolic and pathologic pathways, culminating or adding up to control metabolic features and NASH pathology. According to these preclinical data and the clinical results reported with several single or dual PPAR agonists and IVA337's good safety profile (manuscript under preparation), IVA337 is considered to be a promising candidate for NASH treatment.

REFERENCES

1) Vanni E, Bugianesi E, Kotronen A, De Minicis S, Yki-Järvinen H, Svegliati-Baroni G. From the metabolic syndrome to NAFLD or vice versa? Dig Liver Dis 2010;42:320-330.
2) Harrison S. The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. Am J Gastroenterol 2003; 98:2042-2047.
3) Poulsen LI, Siersbæk M, Mandrup S. PPARs: fatty acid sensors controlling metabolism. Semin Cell Dev Biol 2012;23:631-639.
4) Feige JN, Gelman L, Michalik L, Desvergne B, Wahli W. From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions. Prog Lipid Res 2006;45:120-159.
5) Grygiel-Górniak B. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications -- a review. Nutr J 2014;13:17.
6) Lalloyer F, Staels B. Fibrates, glitazones and peroxisome proliferator-activated receptors. Arterioscler Thromb Vasc Biol 2010;30:894-899.
7) Lefebvre P, Chinetti G, Fruchart JC, Staels B. Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis. J Clin Invest 2006;116:571-580.
8) **Zambon A**, **Gervois P**, Pauletto P, Fruchart JC, Staels B. Modulation of hepatic inflammatory risk markers of cardiovascular diseases by PPAR-alpha activators: clinical and experimental evidence. Arterioscler Thromb Vasc Biol 2006;26:977-986.
9) **Lee CH**, **Olson P**, Hevener A, Mehl I, Chong L-W, Olefsky JM, et al. PPARdelta regulates glucose metabolism and insulin sensitivity. Proc Natl Acad Sci U S A 2006;103:3444-3449.
10) **Tailleux A**, **Wouters K**, Staels B. Roles of PPARs in NAFLD: potential therapeutic targets. Biochim Biophys Acta 2012;1821:809-818.
11) **Odegaard JI**, **Ricardo-Gonzalez RR**, Red Eagle A, Vats D, Morel CR, Goforth MH, et al. Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. Cell Metab 2008;7:496-507.
12) Hazra S, Xiong S, Wang J, Rippe RA, Krishna V, Chatterjee K, et al. Peroxisome proliferator-activated receptor gamma induces a phenotypic switch from activated to quiescent hepatic stellate cells. J Biol Chem 2004;279:11392-11401.
13) Marra F, Efsen E, Romanelli RG, Caligiuri A, Pastacaldi S, Batignani G, et al. Ligands of peroxisome proliferator-activated receptor gamma modulate profibrogenic and proinflammatory actions in hepatic stellate cells. Gastroenterology 2000;119:466-478.
14) Ip E, Farrell G, Hall P, Robertson G, Leclercq I. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. Hepatology 2004;39: 1286-1296.
15) Larter CZ, Yeh MM, Van Rooyen DM, Brooling J, Ghatora K, Farrell GC. Peroxisome proliferator-activated receptor-alpha agonist, Wy-14,643, improves metabolic indices, steatosis and ballooning in diabetic mice with non-alcoholic steatohepatitis. J Gastroenterol Hepatol 2012;27:341-350.
16) Fernández Miranda C, Pérez Carreras M, Colina F, López-Alonso G, Vargas C, Solís-Herruzo JA. A pilot trial of fenofibrate for the treatment of non-alcoholic fatty liver disease. Dig Liver Dis 2008;40:200-205.
17) **Riserus U**, **Sprecher D**, Johnson T, Olson E, Hirschberg S, Liu A, et al. Activation of peroxisome proliferator-activated receptor (PPAR)delta promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. Diabetes 2008;57:332-339.
18) Bays HE, Schwartz S, Littlejohn T 3rd, Kerzner B, Krauss RM, Karpf DB, et al. MBX-8025, a novel peroxisome proliferator receptor-delta agonist: lipid and other metabolic effects in dyslipidemic overweight patients treated with and without atorvastatin. J Clin Endocrinol Metab 2011;96:2889-2897.
19) Staels B, Rubenstrunk A, Noel B, Rigou G, Delataille P, Millatt LJ, et al. Hepatoprotective effects of the dual peroxisome proliferator-activated receptor alpha/delta agonist, GFT505, in rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. Hepatology 2013;58:1941-1952.
20) **Yu J**, **Zhang S**, Chu ES, Go MY, Lau RH, Zhao J, et al. Peroxisome proliferator-activated receptors gamma reverses hepatic nutritional fibrosis in mice and suppresses activation of hepatic stellate cells in vitro. Int J Biochem Cell Biol 2010;42: 948-957.
21) Ip E, Farrell GC, Robertson G, Hall P, Kirsch R, Leclercq I. Central role of PPARalpha-dependent hepatic lipid turnover in dietary steatohepatitis in mice. Hepatology 2003;38:123-132.
22) Wu CW, Chu ES, Lam CN, Cheng AS, Lee CW, Wong VW, et al. PPARgamma is essential for protection against nonalcoholic steatohepatitis. Gene Ther 2010;17:790-798.
23) Nagasawa T, Inada Y, Nakano S, Tamura T, Takahashi T, Maruyama K, et al. Effects of bezafibrate, PPAR pan-agonist, and GW501516, PPARdelta agonist, on development of steatohepatitis in mice fed a methionine- and choline-deficient diet. Eur J Pharmacol 2006;536:182-191.
24) **Hsiao PJ**, **Hsieh TJ**, Kuo KK, Hung WW, Tsai KB, Yang CH, et al. Pioglitazone retrieves hepatic antioxidant DNA repair in a mice model of high fat diet. BMC Mol Biol 2008;9:82.
25) Leclercq IA, Lebrun VA, Stärkel P, Horsmans YJ. Intrahepatic insulin resistance in a murine model of steatohepatitis: effect of PPARγgamma agonist pioglitazone. Lab Invest 2007;87:56-65.
26) L'homme L, Esser N, Riva L, Scheen A, Paquot N, Piette J, et al. Unsaturated fatty acids prevent activation of NLRP3 inflammasome in human monocytes/macrophages. J Lipid Res 2013;54:2998-3008.
27) Wan X, Xu C, Yu C, Li Y. Role of NLRP3 inflammasome in the progression of NAFLD to NASH. Can J Gastroenterol Hepatol 2016;2016:6489012.
28) Csak T, Ganz M, Pespisa J, Kodys K, Dolganiuc A, Szabo G. Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. Hepatology. 2011;54:133-144.
29) Wree A, McGeough MD, Peña CA, Schlattjan M, Li H, Inzaugarat ME, et al. NLRP3 inflammasome activation is required for fibrosis development in NAFLD. J Mol Med (Berl) 2014;92:1069-1082.

30) **Stienstra R**, **van Diepen JA**, Tack CJ, Zaki MH, Van de Veerdonk FL, Perera D, et al. Inflammasome is a central player in the induction of obesity and insulin resistance. Proc Natl Acad Sci U S A 2011;108:15324-15329.
31) Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 2011;17:179-188.
32) Lee HJ, Yeon JE, Ko EJ, Yoon EL, Suh SJ, Kang K, et al. Peroxisome proliferator-activated receptor-delta agonist ameliorated inflammasome activation in nonalcoholic fatty liver disease. World J Gastroenterol 2015;21:12787-12799.
33) Cusi K, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, et al. Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: a randomized trial. Ann Intern Med 2016;165:305-315.
34) Glosli H, Gudbrandsen OA, Mullen AJ, Halvorsen B, Røst TH, Wergedahl H, et al. Down-regulated expression of PPARalpha target genes, reduced fatty acid oxidation and altered fatty acid composition in the liver of mice transgenic for hTNFalpha. Biochim Biophys Acta 2005;1734:235-246.
35) Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. J Clin Invest 1999;103:1489-1498.
36) **Yao-Borengasser A**, **Rassouli N**, Varma V, Bodles AM, Rasouli N, Unal R, et al. Stearoyl-coenzyme A desaturase 1 gene expression increases after pioglitazone treatment and is associated with peroxisomal proliferator-activated receptor-gamma responsiveness. J Clin Endocrinol Metab 2008;93:4431-4439.
37) Li ZZ, Berk M, McIntyre TM, Feldstein AE. Hepatic lipid partitioning and liver damage in nonalcoholic fatty liver disease: role of stearoyl-CoA desaturase. J Biol Chem 2009;284:5637-5644.
38) Finucane OM, Lyons CL, Murphy AM, Reynolds CM, Klinger R, Healy NP, et al. Monounsaturated fatty acid-enriched high-fat diets impede adipose NLRP3 inflammasome-mediated IL-1β secretion and insulin resistance despite obesity. Diabetes 2015;64:2116-2128.
39) Iwaisako K, Haimerl M, Paik Y-H, Taura K, Kodama Y, Sirlin C, et al. Protection from liver fibrosis by a peroxisome proliferator-activated receptor delta agonist. Proc Natl Acad Sci U S A 2012;109:E1369-E1376.
40) Camp HS, Li O, Wise SC, Hong YH, Frankowski CL, Shen X, et al. Differential activation of peroxisome proliferator-activated receptor-gamma by troglitazone and rosiglitazone. Diabetes 2000;49:539-547.
41) Ruzehaji N, Frantz C, Ponsoye M, Avouac J, Pezet S, Guilbert T, et al. Pan PPAR agonist IVA337 is effective in prevention and treatment of experimental skin fibrosis. Ann Rheum Dis 2016;75:2175-2183.
42) Fernandes Santos C, Carneiro RE, de Souza Mendonca L, Aguila MB, Mandarim-de-Lacerda CA. Pan-PPAR agonist beneficial effects in overweight mice fed a high-fat high-sucrose diet. Nutrition 2009;25:818-827.
43) **Toral M**, **Gómez-Guzmán M**, Jiménez R, Romero M, Zarzuelo MJ, Utrilla MP, et al. Chronic peroxisome proliferator-activated receptorβ/δ agonist GW0742 prevents hypertension, vascular inflammatory and oxidative status, and endothelial dysfunction in diet-induced obesity. J Hypertens 2015;33:1831-1844.
44) Barroso E, Rodríguez-Calvo R, Serrano-Marco L, Astudillo AM, Balsinde J, Palomer X, et al. The PPARβ/δ activator GW501516 prevents the down-regulation of AMPK caused by a high-fat diet in liver and amplifies the PGC-1α-Lipin 1-PPARα pathway leading to increased fatty acid oxidation. Endocrinology 2011;152:1848-1859.
45) Kaser S, Moschen A, Cayon A, Kaser A, Crespo J, Pons-Romero F, et al. Adiponectin and its receptors in non-alcoholic steatohepatitis. Gut 2005;54:117-121.
46) **Francque S**, **Verrijken A**, Caron S, Prawitt J, Paumelle R, Derudas B, et al. PPARα gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis. J Hepatol 2015;63:164-173.
47) Ratziu V, Harrison S, Francque S, Bedossa P, Lehert P, Serfaty L, et al. Elafibranor, an agonist of the peroxisome proliferator-activated receptor-alpha and -delta, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. Gastroenterology 2016;150:1147-1159.
48) Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010;362:1675-1685.

Author names in bold designate shared co-first authorship.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1057/suppinfo.