Editorial

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FXR agonists for NASH: How are they different and what difference do they make?

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Six years ago, the release of interim data from the FLINT trial investigating a single dose of obeticholic acid (OCA) in a biopsy-controlled 72-week study of patients with non-alcoholic steatohepatitis (NASH) led to a frenzy of research into novel FXR agonists.[1] This study was remarkable in many ways: i) it was the first to demonstrate that an investigational drug can improve histopathology in terms of non-alcoholic fatty liver disease activity score (NAS) score and fibrosis, thus laying out a potential path for drug approval, and ii) it showed that a farnesoid X receptor (FXR) agonist can be so effective that even interim data were sufficient to show significance. Although the results from this phase II study with OCA in NASH could be largely reproduced in the subsequent REGENERATE phase III trial[2] the FDA still has not approved OCA for NASH, officially because of concerns about the safety of this drug. So, what are the published liabilities of OCA?

OCA has consistently shown an increase in pruritus in patients with primary biliary cholangitis as well as in those with NASH. Moreover, OCA decreased HDL-c and increased LDL-c in various trials, which poses a potential atherosclerotic risk. It was not clear whether these effects were drug (OCA) or target (FXR) specific. But other FXR agonists of different chemotypes, both steroidal and non-steroidal, have demonstrated that pruritus and the worsening of the HDL-c/LDL-c ratio are manifestations of FXR agonism, since all FXR agonists caused these effects in different human trials (see Table 1; Fig. 1).

Now, data have been published in this journal from a phase IIa study of another FXR agonist, MET-409,[3] different in structure from bile acids but also different from the isoxazole, carboxylic acid-bearing FXR agonists such as tropifexor, cilofexor, Px-102/104 or TERN-101. The authors make the point that MET-409, because of its substantially different chemical nature (a derivative of fexaramine without a carboxylic acid) results in a different gene expression pattern and that this results in an improved clinical profile. True or not?

This is difficult to judge because the data available from clinical-stage FXR agonists in NASH cannot be compared one to one simply because the trial protocols differ from each other in various aspects. Table 1 lists publicly available data on various surrogate markers and histopathology of clinical stage FXR agonists in NASH, but this compilation has to be viewed with many caveats. It is one thing to demonstrate potent liver fat reduction by MRI-proton density fat fraction (MRI-PDFF) in a short-term trial, but it is a different challenge to translate this into reduction of fibrosis or NASH resolution without major adverse effects. MET-409 clearly shows potent liver fat reduction, more than all other FXR agonists in a direct comparison, but there are some warning signals seen by transient increases of alanine aminotransferase (ALT). The steady increases of fibroblast growth factor-19 (FGF-19) over time are a potential indicator of drug accumulation. This might explain the very potent liver fat reduction but could also favor the worsening of the HDL-c/LDL-c ratio, the liver toxicity signals and the high pruritus incidence, at least with the higher dose. Plasma levels of the drug are not indicative, here, since FXR needs to be hit in the intestine and in the liver and there are various examples of asymmetric plasma/tissue ratios for FXR-targeting drugs. On a positive note, it can be assumed that lowering the dose of MET-409 will likely ameliorate these side effects, however, the dose that optimally balances adverse and beneficial effects needs to be demonstrated in longer term studies.

Two factors have been discussed as being decisive for the therapeutic index, *i.e.* the balance between beneficial effects on hepatic fat, stiffness or histopathology and potential adverse effects. One is the chemotype of the FXR agonist, and the other its intestinal *vs.* liver agonism. Arguably agonists of different chemotypes induce different conformations upon binding to FXR which translates into a different transcriptional repertoire. The latter is a well-known phenomenon with nuclear receptors such as FXR.[4] Harrison *et al.* performed RNA sequencing gene expression analysis on mouse liver after administering MET-409 or a "carboxylic acid-containing FXR agonist". Unfortunately, they just show a principal component analysis where both compounds elicit a different gene expression pattern, but they do not provide further details with regards to which genes are affected nor do they disclose the exact structures of the compounds under investigation. As early as 2003, Downes *et al.*[5] compared fexaramine, the template of MET-409, with GW4064 (the mother compound of the isoxazole structural class including PX20606, tropifexor, cilofexor and TERN-101[6]) and with the natural ligand

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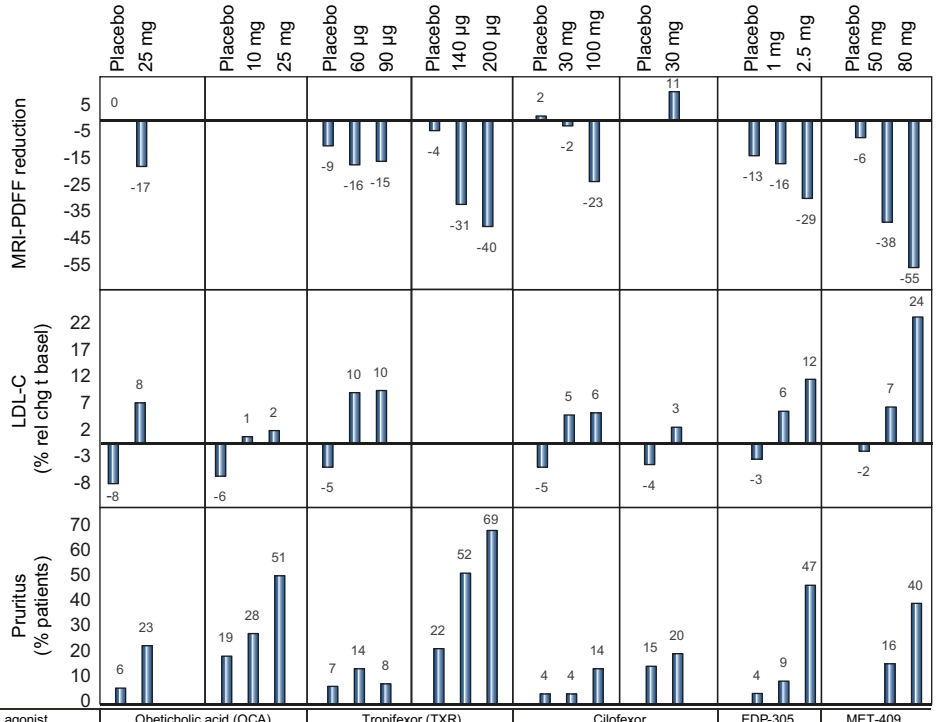


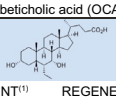
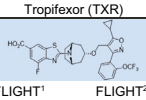
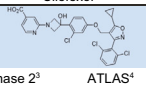
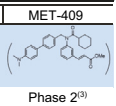
FXR agonist	Obeticholic acid (OCA)		Tropifexor (TXR)		Cilofexor		EDP-305	MET-409
Structure								
Study Name/Phase	FLINT[(1)]	REGENERATE[(2)]	FLIGHT[1] Part A, B	FLIGHT[2] Part C	Phase 2[3]	ATLAS[4] Phase 2B	ARGON-1[5] Phase 2	Phase 2[(3)]
Duration of treatment	72 weeks	18 months	12 weeks	48 weeks	24 weeks	48 weeks	12 weeks	12 weeks
Placebo (PBO)	0 mg	0 mg	0 µg	0 µg	0 mg	0 mg	0 mg	0 mg
Dose 1	25 mg	10 mg	60 µg	140 µg	30 mg	30 mg	1.0 mg	50 mg
Dose 2	-	25 mg	90 µg	200 µg	100 mg	-	2.5 mg	80 mg
MRI-PDFF reduction (% to baseline)								
PBO	0.0	N/A	-9.3	-3.6	1.9	N/A	-13.0	-6.2
Dose 1	-17.0	N/A	-16.4	-31.3	-1.8	11.0	-16.0	-37.9
Dose 2	-	N/A	-15.1	-39.5	-22.7	-	-29.0	-54.9
ALT (% of change from base line)								
PBO	-22.0	-19.6	-14.6	-8.4	-5.7	-8.0	-19.6	-4.4
Dose 1	-45.8	-31.5	-20.4	-31.6	-19.0	-25.0	-23.6	28.2
Dose 2	-	-44.9	-23.2	-32.5	-17.7	-	-35.1	-24.9
LDL-c (% of change from base line)								
PBO	-7.6	-6.2	-4.5	N/A	-4.5	-4.0*	-3.0	-1.5
Dose 1	7.6	1.2	9.5	N/A	5.3	3.0*	6.0	6.8
Dose 2	-	2.4	9.9	N/A	5.7	-	12.0	23.7
HDL-c (% of change from base line)								
PBO	2.7	-1.5	-4.4	N/A	-8.2	-3.0*	0.0*	2.6
Dose 1	-1.8	-4.0	-4.0	N/A	-2.7	-4.0*	-2.0*	-20.3
Dose 2	-	-10.4	-9.8	N/A	-6.4	-	-8.0*	-23.4
Pruritus (% of patients)								
PBO	6.3	19.0	7.0	22.0	4.0	15.0	4.2	0.0
Dose 1	23.4	28.0	14.0	52.0	4.0	20.0	9.1	16.0
Dose 2	-	51.0	8.0	69.0	14.0	-	47.2	40.0
% of Fibrosis reduction >1 stage								
PBO	19.0	12.0	N/A	21.0	N/A	11.0	N/A	N/A
Dose 1	35.0	18.0	N/A	26.0	N/A	12.0	N/A	N/A
Dose 2	-	23.0	N/A	25.0	N/A	-	N/A	N/A

Fig. 1. Available key data for FXR agonists in advanced clinical development in the indication NASH. Original significance indications are not provided here and should be checked in the original literature. n.a., not available; *Absolute changes in mg/dl; [1]Sanyal *et al.*, Poster SAT-357 at ILC April 10–14, 2019; note that the values in this table represent the means of the bipartite numbers in the poster; [2]Lucas *et al.*, Hepatology 2020; 72 (S1), oral abstract slides AASLD - The Liver Meeting 2020, 2020; [3]Patel *et al.* Hepatology; 2020, 72(1), 58-71; [4]Loomba *et al.*, Hepatology 73(2):625-643; [5]Ratziu *et al.*, oral abstract AS078 slides from The Digital ILC, August 28, 2020. Numbers in parentheses refer to the references of the editorial. Important Note: The data from this table for different clinical stage FXR agonists do not allow for direct comparisons since they come from different clinical studies using different protocols, different NASH populations with different characteristics, etc. FXR, farnesoid X receptor; NASH, non-alcoholic steatohepatitis.

chenodeoxycholic acid (CDCA). Their data identified a set of commonly FXR regulated genes but, importantly, also individually regulated ones. By determining the crystal structures of each of these FXR agonists in complex with the FXR ligand binding domain they could show that all 3 induce distinct FXR conformations likely resulting in different transcriptional repertoires.

But this does not exclude that "off-target" effects might also have an impact on the phenotypic outcome of pharmacotherapy. Dwivedi *et al.*[7] have demonstrated that GW4064 exerts part of its pharmacological response by acting as an agonist of estrogen-related receptor alpha. What is urgently needed is a broad and deep comparison of the gene expression profiles of all clinically

relevant FXR agonists in both wild-type and FXR knockout mice or cell lines. But for this we need public disclosure of MET-409's and EDP-305's chemical structures.

The other key aspect which is widely discussed as the basis of differences in clinical outcome is the issue of intestinal *vs.* liver activity of a given FXR agonist. Houten *et al.*[8] have provided insights into the natural level of FXR activation by generating an FXR promoter-driven luciferase reporter mouse. They observed that FXR is intensely activated in the intestine but only barely in the liver under normal physiological conditions. This suggests that potent liver activation of FXR is not part of the natural program of FXR diurnal activities, rather liver FXR seems to be activated upon a liver emergency situation such as cholestasis where intrahepatic bile levels reach critical levels. Then, FXR turns on a rescue program by inducing bile acid export pumps (BSEP, MRP2, MDR3[9]), bile acid conjugation enzymes,[10] enzymes involved in detoxification and the glutathione antioxidative defense system.[11] This prompted Fang *et al.* to suggest that fexaramine, as an intestinally biased FXR agonist (and the template for MET-409 according to Harrison *et al.*) is sufficient to elicit the desired metabolic changes but largely avoids the unwanted side effects.[12] However, there are also publications demonstrating that liver FXR activity is needed to unravel the full liver protective potential of FXR activation.[13,14]

In the debate over intestinal *vs.* hepatic FXR tropism it should not be forgotten that overly potent intestinal FXR activation triggers a supraphysiological induction of FGF-19 which is well described for its liver fat lowering and otherwise liver protective functions. But it is also well known as a key driver of hepatocyte proliferation increasing the risk of hepatocellular carcinoma formation. Human liver and colon cancers show high frequencies of gene abnormalities or amplifications in the FGF-19/FGFR4 axis which should prompt us to watch the degree of intestinal FXR activation very carefully.[15,16]

These fascinating pharmacological considerations have raised many clinically oriented questions. How do these compound-specific properties translate into clinical results? Which pathways are responsible for the changes in HDL-c and LDL-c, what is the driver of pruritus which was observed in all FXR agonist interventional studies? Can we expect to identify the molecular clues to define which genes are relevant for which effects in which tissues?

For this latter question the simple answer is: not in the near future! And this is because many of these adverse effects cannot be recapitulated in appropriate animal models. The HDL-c/LDL-c metabolism is substantially different between rodents and humans and even monkeys do not provide predictable results, as shown in a very nice translational study with an LXR agonist (LXR being the closest relative of FXR).[17] The HDL lowering by FXR agonists can partly be attributed to an upregulation of SR-BI, the scavenger receptor for HDL particles on hepatocytes, but there is also strong evidence of transintestinal cholesterol efflux induced by FXR agonist-dependent changes in the bile acid pool.[18,19] The increase in LDL-c is believed to be a result of a combination of Cyp7A1 downregulation, reduced LDL-receptor expression on hepatocytes, modulation of apolipoproteins-C and -E and of cholesteryl ester transfer protein (CETP), but which of these effects has the strongest impact is currently not known.

The pathogenesis of pruritus is even more cryptic. Various mechanisms for FXR-induced pruritus have been proposed: Autotaxin induction, activation of TGR-5 (the other GPCR-type bile acid receptor, activated directly by steroidal FXR agonists[20] or indirectly through changes in the bile acid pattern), and most recently another GPCR was found as a pruritogenic receptor.[21]

So, the curtain falls and all questions remain open? We need principal investigators to include more translational markers in clinical studies. These could be: cholesterol flux analysis, gene expression analyses and correlation with compound levels in target tissues, detailed bile acid analyses and correlation with incidence of pruritus, etc.

All in all, FXR agonists are quite effective in reducing different sequelae associated with NASH. FXR is designed to rescue the liver in critical conditions and emerging data have demonstrated beneficial effects of FXR agonism on portal hypertension, bacterial translocation or other dysfunctional processes coexisting with advanced liver disease.[22,23] This may turn out to be a major advantage when treating patients with cirrhosis, as clinical outcomes are key approvable endpoints in this population. We are now reasonably confident that the mode of action of FXR agonists will likely support a whole range of beneficial effects, both for patients before and at the cirrhotic stage. The major challenge will be to identify the optimal dose which alleviates concerning side effects while maintaining decisive histological and clinical efficacy, an equation that remains to be solved.

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Conflict of interest

The author declares that he is cofounder, shareholder and CEO of Phenex Pharmaceuticals AG, a company that has been involved in the identification of FXR agonists.

Please refer to the accompanying ICMJE disclosure forms for further details.

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Supplementary data

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Author names in bold designate shared co-first authorship

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