Efruxifermin treatment significantly reduced markers of collagen synthesis and fibrosis and normalized levels of Pro-C3 in patients with liver fibrosis associated with NASH

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BACKGROUND

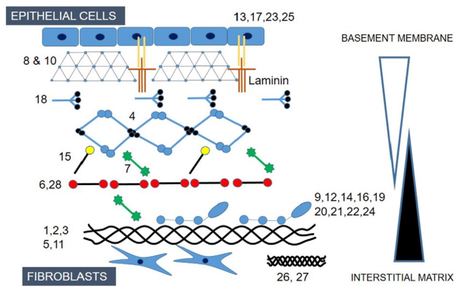
Progression of liver fibrosis is accompanied by an increase in collagen, in particular types I and III, in the interstitial extracellular matrix (ECM). Diverse collagens in the ECM network may play roles in pathologic tissue fibrosis (Fig. 1). Biomarkers of collagen synthesis may be prognostic tools in the clinic and may also serve to monitor response to anti-fibrotic therapies.

While liver biopsy is the current gold-standard for evaluating liver fibrosis, the procedure samples a small portion of the liver, and histopathological interpretation is complicated by high inter- and intra-reader variability. Digital pathology methods have emerged as an approach to reduce variability and increase reproducibility in the reading of histology slides, while also quantifying additional histological characteristics of liver tissue.

Efruxifermin (EFX) is a long-acting Fc-FGF21 analogue being developed as a potential therapy for patients with fibrosis due to non-alcoholic steatohepatitis (NASH). In the phase 2a BALANCED study (NCT03976401) of patients with biopsy-confirmed NASH (F1-3), 16-week treatment with EFX significantly reduced liver fat content and improved markers of liver injury, fibrosis, and lipid and glucose metabolism while demonstrating an acceptable safety and tolerability profile[1]. An additional cohort of patients with compensated cirrhosis due to NASH was recruited, and treatment with EFX was safe and well-tolerated with promising preliminary anti-fibrotic effects.

To corroborate and begin to understand the antifibrotic effects observed in the BALANCED study, we analyzed the effects of EFX on liver histology as scored by digital pathology, and on markers of ECM remodeling.

Figure 1. Distribution and primary structure of collagens.
From Karsdal et al, (2017)[2]



METHODS

Figure 2. BALANCED Main Study Design

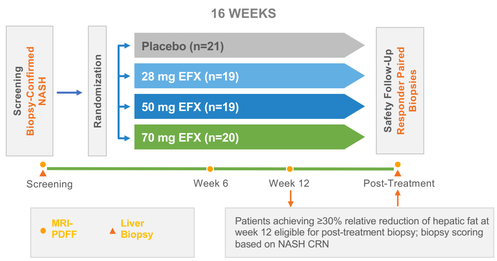
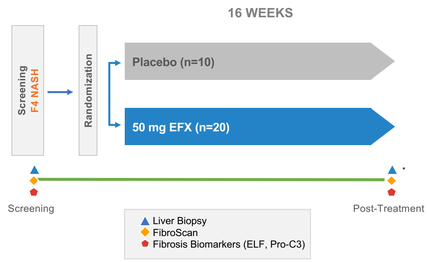


Figure 3. BALANCED Cohort C Design



* Protocol amended to include voluntary end-of-treatment biopsies

80 patients with biopsy-confirmed F1-F3 NASH were randomized in the BALANCED Main Study (Fig. 1), of whom 79 received at least one dose of study drug. Week 12 MRI-PDFF data were available for 68 patients. Of these, 50 (comprising 48 EFX-treated and two placebo patients) showed a ≥30% relative reduction of liver fat and were therefore eligible for an end-of-study liver biopsy. Of these, 40 EFX-treated and two placebo patients received an end-of-treatment biopsy.

An additional cohort of 30 patients with compensated cirrhosis due to NASH were randomized (Cohort C) 2:1 to 50 mg EFX or placebo. In response to requests from patients and investigators, the protocol was amended to include optional end-of-treatment biopsies, which were collected for 5 patients receiving placebo and 12 patients receiving EFX.

Biomarkers of collagen synthesis (Pro-C3, P3NP, P1NP, Pro-C4, and Pro-C6) and collagen degradation (C3M, C4M) were measured in all F1-F3 patients in the Main Study, with Pro-C3, P1NP, P3NP also measured in patients with compensated cirrhosis.

Normalization thresholds of Pro-C3 were based on the published thresholds for increased likelihood of ≥F2 NASH (12.6 µg/L) and the upper bound of the 90% confidence interval of healthy values (9.2 µg/L)[3].

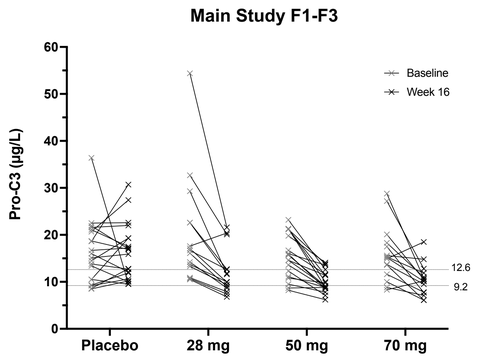
Cohort C paired biopsy slides were scanned, and analyzed using PathAI's (Boston, MA) digital pathology algorithm.

RESULTS

Table 1. Baseline Demographics and Characteristics in the BALANCED Main Study and Cohort C

Baseline characteristics (Mean unless noted)	Main Study (F1-F3)				Cohort C (F4)	
	Placebo (N=21)	EFX 28mg (N=19)	EFX 50mg (N=20)	EFX 70mg (N=20)	Placebo (n=10)	EFX 50mg (n=20)
Age (Years)	52	50	53	53	57.1	61.1
Sex (n, Male/Female)	6/15	9/10	10/10	9/11	7/3	4/16
Weight (kg)	99.6	108.2	103.6	103.1	119.1	97.9
Liver Fat Content (%, MRI-PDFF)	19.3	21.4	18.3	19.4	ND	ND
ALT(U/L)	50.7	62.5	53.4	56.8	32.7	31.7
AST(U/L)	38.6	41.1	35.4	44.6	28.9	31.4
HbA1c (%)	6.5	6.2	6.4	6.2	6.5	6.1
% Type 2 Diabetes	67	37	50	50	50	50
Triglycerides (mg/dL)	208.3	176.3	176.5	180.0	121.7	134.6
ELF Score	9.4	9.5	9.5	9.6	9.7	10.4
Pro-C3 (µg/L)	16.1	19.2	16.2	17.2	22.6	25.6
Liver Stiffness (kPa)	11.9	12.5	11.3	12.4	25.8	22.1

Figure 4. EFX significantly reduced Pro-C3 over 16 weeks in patients with F1-F3 fibrosis in the BALANCED Main Study.



Placebo patient with largest decrease in Pro-C3 lost >10% BW

Table 2. EFX normalized Pro-C3 levels in patients with F1-F3 NASH in the BALANCED Main Study. "Decreased across threshold" means that baseline value is above the threshold, and week 16 value is below the threshold.

	Baseline <12.6ng/mL	Week 16 <12.6ng/mL	Decreased across threshold
Placebo	7/21 (33%)	7/19 (37%)	3/14 (21%)
28mg	5/19 (26%)	10/15 (67%)	7/12 (58%)
50mg	6/19 (32%)	12/16 (75%)	6/10 (60%)
70mg	4/20 (20%)	12/15 (80%)	8/11 (73%)

	Baseline ≤9.2ng/mL	Week 16 ≤9.2ng/mL	Decreased across threshold
Placebo	3/21 (14%)	0/19 (0%)	0/18 (0%)
28mg	0/19 (0%)	5/15 (33%)	5/15 (33%)
50mg	2/19 (11%)	8/16 (50%)	6/14 (43%)
70mg	2/20 (10%)	5/15 (33%)	4/13 (31%)

12.6 ng/mL: threshold for increased likelihood of ≥F2 NASH
9.2 ng/mL: upper 90% confidence interval of healthy values[2]

Figure 5. EFX significantly reduced Pro-C3 over 16 weeks in patients with (A) moderate-to-severe (F2/F3) fibrosis or (B) F4 compensated cirrhosis.

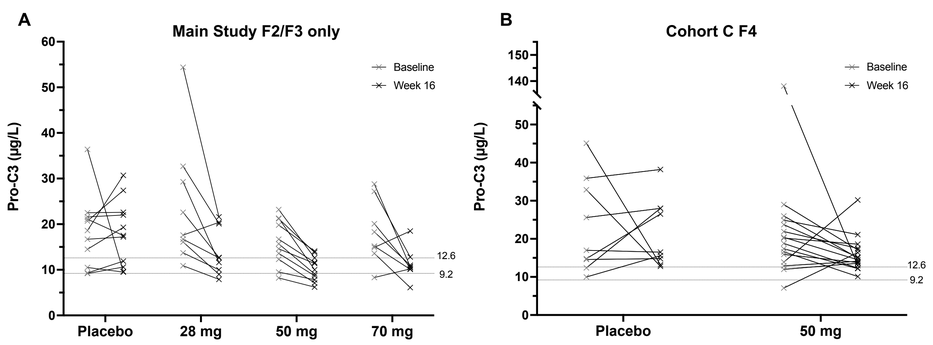
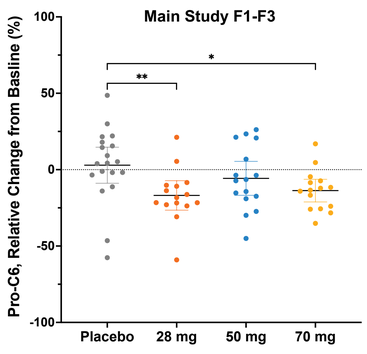
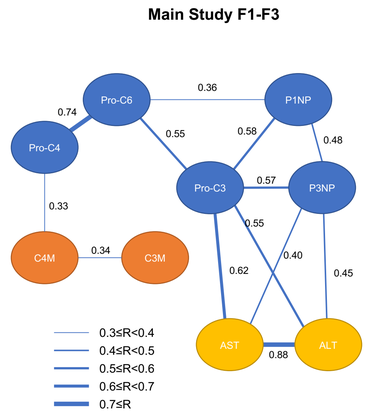


Figure 6. EFX reduced Pro-C6 (endotrophin) over 16 weeks in patients with F1-F3 fibrosis due to NASH in the BALANCED study.



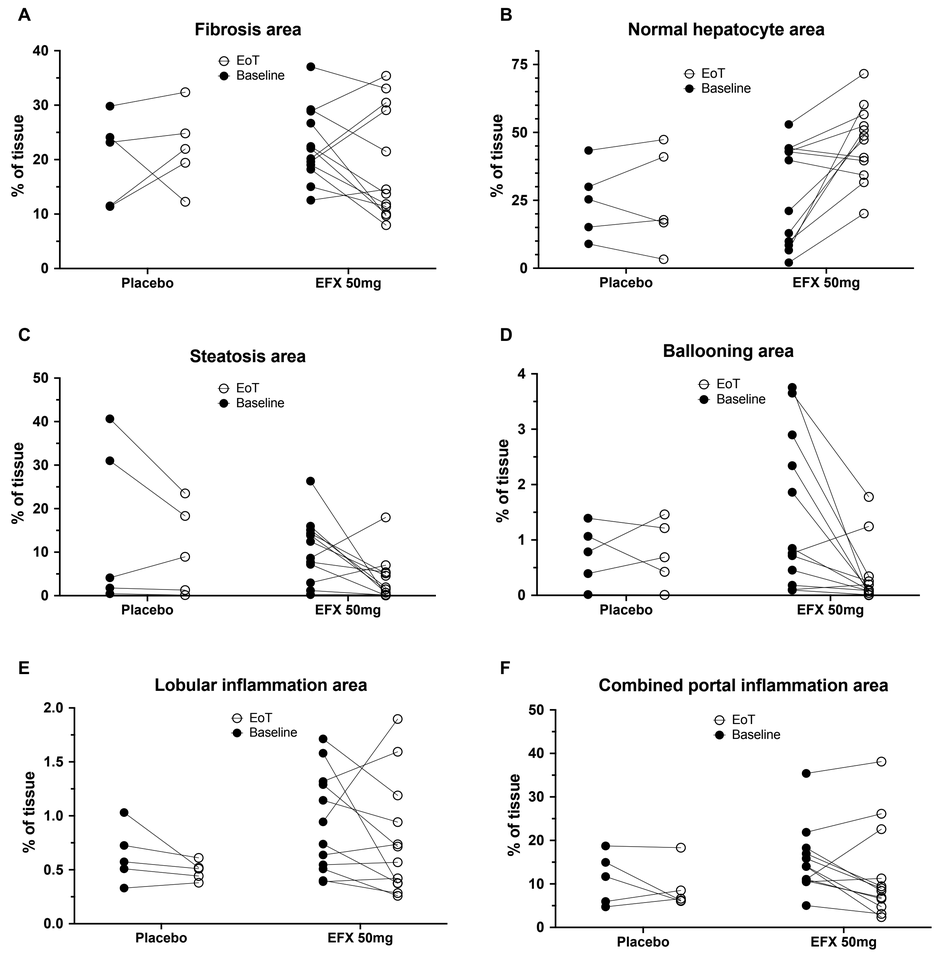
* p<0.05, Kruskal-Wallis test with Dunn's post-test

Figure 7. Network map of Spearman correlation coefficients for percent change from baseline to week 16* across multiple markers of liver injury and collagen turnover (*i.e.*, synthesis and degradation).



* week 12 for P3NP

Figure 8. PathAI-scored proportion of tissue area assigned to (A) collagen/fibrosis, (B) normal hepatocytes, (C) steatosis, (D) hepatocellular ballooning, (E) lobular inflammation, or (F) portal inflammation for biopsies obtained from 5 placebo and 12 EFX-treated patients with cirrhosis (Cohort C). Scoring of non-steatotic tissue areas does not account for the impact of large reductions in steatosis area.



CONCLUSIONS

- EFX normalized Pro-C3, marker of type-III collagen synthesis and soft tissue fibrogenesis, well into the normal range in about one-third of patients with moderate-to-severe fibrosis due to NASH, and significantly reduced Pro-C3 in patients with cirrhosis due to NASH
- EFX reduced Pro-C6/endotrophin, a biomarker of type-VI collagen synthesis associated with microfibrillar collagen structures, and under development for predicting cardiovascular and renal outcomes
- Changes in markers of type-I, type-III, type-IV, and type-VI collagen correlated changes in liver injury markers, and appeared to be reduced in a coordinated way by EFX in patients with NASH fibrosis, suggesting a concerted restructuring of the ECM
- EFX appeared to exert a broad effect on different collagen structures in the ECM, including basement membrane (type-IV), fibrillar (type-III), and microfibrillar (type-VI) collagen
- Digital pathology scoring by PathAI suggests improvements across multiple key characteristics of liver histopathology in patients with cirrhosis due to NASH, including area of fibrosis/collagen and normal or ballooned hepatocytes
- EFX treatment demonstrated anti-fibrotic activity across conventional histopathology scoring, collagen biomarker analysis, and digital pathology scoring of paired biopsies

REFERENCES

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2. Karsdal *et al*. (2017) *Drug Deliver Rev* 121:43–56
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