Characterization of the Patterns of Resolution of Histopathology After Efruxifermin Treatment of Patients with NASH Fibrosis (F2/3) for 24 Weeks

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BACKGROUND

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). Findings from our 24-week Phase 2b (HARMONY) study confirmed those from the 16-week phase 2a (BALANCED) study of patients with biopsy-confirmed NASH (F1-3): 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[1,2,3]. EFX treatment was associated with rapid improvements in liver histology and regression of fibrosis and resolution of NASH.

As in the Phase 2a study[4], the rapid histological improvements and changes in collagen features in the Phase 2b study prompted several post-hoc quantitative and qualitative evaluations to further characterize the changes, and features of regression in biopsies from the Phase 2b study.

AIMS

These post-hoc, histopathology analyses aim to characterize changes in liver in participants in the Phase 2b study.

Quantitative analyses evaluated (1) steatosis-activity-fibrosis (SAF) and (2) SAF-Activity (SAF-A) scores.

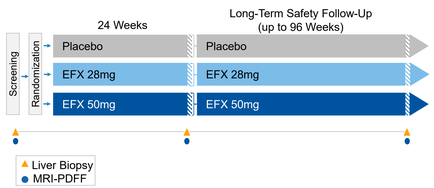
Two qualitative analyses were also undertaken:

(1) Evaluation of exploratory features associated with fibrosis regression

(2) Comparison of paired biopsies to determine if post-treatment biopsies improved, worsened or stayed the same relative to pre-treatment.

METHODS

Figure 1. HARMONY Main Study Design



HARMONY is an ongoing, randomized, placebo-controlled phase 2b study, evaluating EFX 28 and 50 mg, dosed subcutaneously (SC), once weekly (QW).[3] Participants with biopsy-confirmed F2–F3 NASH (n=128) were randomized (1:1:1) to groups that received 28 mg or 50 mg EFX or placebo, SC QW; 126 received at least 1 dose of study drug; 113 participants underwent liver biopsy at week 24 (primary endpoint). Biopsies were scored independently by 2 NASH-CRN-trained pathologists, blinded to groups and biopsy sequence.

Additional post-hoc histopathology evaluations were conducted to further characterize the effect of EFX on the liver.

Table 1. Baseline Demographics

Baseline characteristics (Mean unless otherwise noted)	Placebo (N=43)	EFX 28mg (N=42)	EFX 50mg (N=43)
Age (Years)	55	57	52
Sex (% Female)	63	69	53
Weight (kg)	108	104	103
Fibrosis Stage (%F2/%F3)	30/70	36/64	37/63
Liver Fat Content (%, MRI-PDFF)	17.1	18.5	17.5
ALT (U/L)	62	50	63
AST (U/L)	57	42	52
HbA1c (%)	6.8	6.8	6.8
% Type 2 Diabetes	65	76	70
Triglycerides (mg/dL)	170	158	154
ELF Score	9.8	9.7	9.8
FAST Score	0.68	0.61	0.67
Pro-C3 (µg/L)	16.5	15.3	18.4
Liver Stiffness by VCTE (Fibroscan) (kPa)	15	14	18
NAS	5.4	5.1	5.6

RESULTS

Consensus Approach for Histologic Evaluation of Primary and Secondary Endpoints

- Biopsies independently scored by two pathologists, with a third pathologist available to adjudicate in absence of consensus
- Pathologists underwent protocol-specific training to align on NASH-CRN scoring interpretation
- Pathologists blinded to subject, treatment, and sequence
- No paired biopsy reads (i.e., pre- and on-treatment biopsies not read side-by-side)
- This method was used for primary efficacy analysis and post-hoc analysis unless otherwise specified

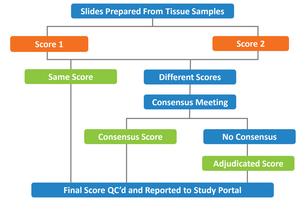


Table 2. Primary and Secondary Endpoints (NASH CRN)

Endpoint	Placebo N = 41	EFX 28 mg N = 38	EFX 50 mg N = 34
Fibrosis improvement without worsening of NASH [a]	8 (19.5%)	15 (39.5%)*	14 (41.2%)*
NASH resolution without worsening of fibrosis[b]	6 (14.6%)	18 (47.4%)**	26 (76.5%)***
Fibrosis improvement and resolution of NASH	2 (4.9%)	11 (28.9%)**	14 (41.2%)***
Resolution of ballooning [d, nt]	10 (24.4%)	20 (52.6%)	29 (85.3%)
NASH Resolution without worsening of fibrosis plus a ≥2-point reduction in overall NAS score [c, nt]	3 (7.3%)	18 (47.3%)	25 (73.5%)
Improvement in NAS by ≥2 without worsening of fibrosis [nt]	8 (19.5%)	27 (71.1%)	28 (82.4%)
Improvement in NAS by ≥4 without worsening of fibrosis [nt]	0	6 (15.8%)	16 (47.1%)

*p<0.05 ; **p<0.01; ***p<0.001 vs placebo [Cochran-Mantel-Haenszel (CMH) test]

[a] Consistent with FDA published guidance, no worsening of NASH is defined as no increase in any one or more of steatosis, inflammation or ballooning score; [b] Consistent with FDA published guidance, NASH resolution is defined as a ballooning score of 0 and a lobular inflammation score of 0 or 1, with any score (0 to 3) for steatosis; [c] In contrast to FDA published guidance that permits any steatosis score (0 to 3) for a patient to achieve NASH resolution, this endpoint requires that a patient must achieve a ≥2-point reduction in overall NAS score, in addition to a ballooning score of 0 and lobular inflammation score of 0 or 1, to be deemed a NASH resolution responder; [d] Defined as ballooning score ≥1 at baseline and 0 at Week 24; [nt] post hoc analysis not tested for significance

EFX-treated Subjects Improved SAF-A and Total SAF Scores, in a Dose-dependent Response

SAF-Activity (SAF-A) = Ballooning (0-2) + Lobular Inflammation (0-2)
Total SAF = SAF-A (0-4) + Steatosis (0-3) + Fibrosis (0-4)

Table 3. Baseline SAF-A and SAF scores by treatment group

Baseline	Placebo (N=43)	EFX 28 (N=42)	EFX 50 (N=43)	All EFX (N=85)
Mean (SD) SAF-A score	3.28 (0.8)	2.95 (0.8)	3.21 (0.8)	3.08 (0.8)
Mean (SD) total SAF score	8.00 (1.2)	7.71 (1.1)	8.07 (1.0)	7.89 (1.1)

Table 4. Responder analysis in SAF-A and SAF at week 24

Week 24	Placebo (N=41)	EFX 28 (N=38)	EFX 50 (N=34)	All EFX (N=72)
Proportion of subjects with at least 2-pt improvement in SAF-A, n (%)[nt]	4 (9.8%)	17 (44.7%)	24 (70.6%)	41 (56.9%)
Proportion of subjects with at least 2-pt improvement in total SAF, n (%) [nt]	9 (22.0%)	30 (78.9%)	30 (88.2%)	60 (83.3%)
Proportion of subjects with at least 4-pt improvement in total SAF, n (%) [nt]	0	13 (34.2%)	21 (61.8%)	34 (47.2%)

nt = post-hoc analysis not tested for significance

EFX led to Histologic Improvement and was Associated With Changes in Key Features of Regression

Scoring: Two pathologists independently analyzed liver biopsy images and scored for any of 6 pre-determined features of regression. Biopsies were not paired, and pathologists were blinded to subject treatment and visit sequence.

Table 5. Definitions of features of regression

Feature of Regression [5,6]	Definition
Regenerative change	Hepatocytes of normal appearance separating thin strands of collagen, often occurring as a row of hepatocytes between two strands of collagen
Isolated thick collagen fibers	Thick but short, often square or rectangular, fibers of collagen in the hepatic parenchyma, not associated with a vascular or portal structure
Large lipogranulomas	Large collections of intraportal or pericentral macrophages containing >3 fat droplets
Interrupted septa	Linear strands of collagen with an obvious break in the strand, can be as small as a single hepatocyte width
Isolated arteries	Small arteries located in the hepatic parenchyma and not associated with other vascular structures of portal areas.
Thick and thin collagen	Broad bands of collagen with either an abrupt termination or abrupt transition into a much thinner collagen strand.

Figure 2. Example Histology Images of Features from HARMONY Study

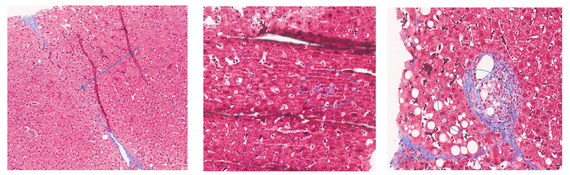


Table 6. Number of Subjects With Definite Features Present at Baseline and Week 24

Feature of Regression	Placebo (N=41)		EFX 28 mg (N=38)		EFX 50 mg (N=34)	
	Baseline	Week 24	Baseline	Week 24	Baseline	Week 24
Regenerative change	7	9	9	13	3	14
Isolated thick collagen fibers	12	16	9	25	6	17
Large lipogranulomas	4	6	4	5	1	12
Interrupted septa	4	14	3	13	9	15
Isolated arteries	4	6	7	6	10	10
Thick and thin collagen	3	2	3	3	6	3

Definite Feature was determined if both pathologists identified a feature of regression independently.

More Biopsies From EFX-Treated Subjects Were Classified as Improved for Fibrosis and Features of NAS

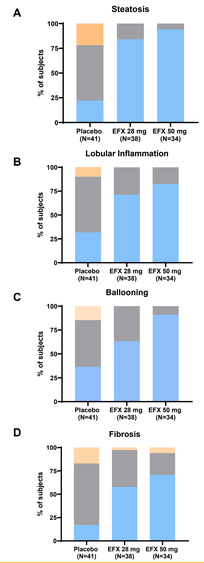
Scoring: Two pathologists analyzed biopsy images (paired by subject, but blinded to timepoint and treatment) and classified, by consensus: biopsy A as same, better, or worse than biopsy B with regard to steatosis, lobular inflammation, ballooning, and fibrosis stage.

Figure 3. Histology images from a paired subject in HARMONY



Better fibrosis and steatosis | Worse fibrosis and steatosis

Figure 4. Proportion of Subjects at Week 24 Classified as Same, Better, or Worse Compared to Baseline With Respect to Steatosis, Lobular Inflammation, Ballooning, and Fibrosis



Week 24 Steatosis	Placebo (N=41)	EFX 28 mg (N=38)	EFX 50 mg (N=34)
Better than baseline	9 (22.0%)	32 (84.2%)	32 (94.1%)
Same as baseline	23 (56.1%)	6 (15.8%)	2 (5.9%)
Worse than baseline	9 (22.0%)	0	0

Week 24 Lobular Inflammation	Placebo (N=41)	EFX 28 mg (N=38)	EFX 50 mg (N=34)
Better than baseline	13 (31.7%)	27 (71.1%)	28 (82.4%)
Same as baseline	24 (58.5%)	11 (28.9%)	6 (17.6%)
Worse than baseline	4 (9.8%)	0	0

Week 24 Ballooning	Placebo (N=41)	EFX 28 mg (N=38)	EFX 50 mg (N=34)
Better than baseline	15 (36.6%)	24 (63.2%)	31 (91.2%)
Same as baseline	20 (48.8%)	14 (36.8%)	3 (8.8%)
Worse than baseline	6 (14.6%)	0	0

Week 24 Fibrosis	Placebo (N=41)	EFX 28 mg (N=38)	EFX 50 mg (N=34)
Better than baseline	7 (17.1%)	22 (57.9%)	24 (70.6%)
Same as baseline	27 (65.8%)	15 (39.5%)	8 (23.5%)
Worse than baseline	7 (17.1%)	1 (2.6%)	2 (5.9%)

Other Significant Findings on Histology: As part of blinded primary efficacy reading, pathologists also noted any significant findings indicative of other liver diseases(s). At Week 24:

- two placebo subjects were noted to have multiple granulomas or dense portal inflammation raising the possibility of a disease other than NASH.
- no findings were noted for any EFX-treated subjects at Week 24.

CONCLUSIONS

- Total SAF and SAF-A scores were improved in more EFX-treated subjects, corroborating the findings of improvement in NASH-CRN fibrosis stage and NAS (steatohepatitis)
- Direct comparison of paired biopsies demonstrates intra-grade (NAS) and intra-stage (fibrosis) improvement, which is not detectable in categorical staging systems
- Consistent with previous reports, histologic features of regression such as interrupted septa or isolated thick collagen fibers were identified on liver biopsies at baseline and post-baseline biopsies
- Orthogonal post-hoc semi-quantitative and qualitative analyses of histopathology reveals progressive dose-dependent response to EFX
- No significant safety findings were noted in EFX-treated biopsies at Week 24

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