Variability in Liver Biopsy Assessment: Data from the Pegozafermin Phase 1b/2a Study in Subjects with Non-Alcoholic Steatohepatitis (NASH)

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INTRODUCTION

- Fibroblast growth factor 21 (FGF21) is an endogenous hormone that regulates lipid and glucose metabolism and energy expenditure.
- Pegozafermin (PGZ) is a glycoPEGylated FGF21 analog with a prolonged half-life compared to native FGF21 that is currently being developed for treatment of non-alcoholic steatohepatitis (NASH) and severe hypertriglyceridemia (SHTG).
- In a randomized, placebo-controlled Phase 1b/2a POC study in subjects with NASH, PGZ had a significant liver-related and metabolic benefits.
- In an open-label cohort of this POC study that included subjects with biopsy-confirmed NASH (NAS ≥4, fibrosis stage F2 or F3; N=20), PGZ 27mg for 20 weeks led to clinically meaningful histological improvement, as well as significant liver-related (MRI-PDFF, ALT, multiple fibrosis-related non-invasive tests) and cardiometabolic benefits, with favorable safety and tolerability.
- Intra-reader and inter-reader variability in liver biopsy reads is increasingly recognized as a major challenge to drug development in NASH.
- Assessment of NASH trial biopsy slides by different pathologists (or by the same pathologist at different timepoints) may affect subject eligibility and evaluation of the proportion of subjects who have met histological endpoints.

OBJECTIVE

- The objective of the study was to assess the impact on histological endpoints in the Phase 1b/2a POC study of assessment by 4 individual expert NASH pathologists.

METHODS

Study Design

19/20 (95%) patients completed treatment and had end-of-treatment biopsies; 1 patient discontinued treatment due to withdrawal of consent

KEY INCLUSION CRITERIA

- Stage 2 or 3 fibrosis; NAS ≥4 (with a ≥1 score in each of steatosis, ballooning, and lobular inflammation)
- MRI-PDFF ≥8%

KEY EXCLUSION CRITERIA

- History or evidence of cirrhosis
- Evidence of liver disease other than NASH
- Recently diagnosed diabetes or HbA1C ≥9.5%

KEY ENDPOINTS

- ≥2-point improvement in NAS
- NASH resolution
- Fibrosis improvement
- Safety and tolerability

PGZ was administered subcutaneously

Biopsy Reading

- For the primary analysis, biopsies were read centrally at baseline (BL) and end of treatment (EOT) by a single expert liver pathologist with significant experience reading NASH studies.
- In a post-hoc exploratory analysis, a panel of 3 additional expert NASH pathologists — Pathologist A, Pathologist B and Pathologist C — assessed the same BL and EOT slides that had been evaluated by the central pathologist. Slides from BL and EOT were mixed, and the pathologists were blinded to the timepoint.
- Biopsies were scored for lobular inflammation, ballooning, steatosis, and fibrosis.
- The proportion of subjects achieving ≥2-point reduction in NAS, NASH resolution without worsening of fibrosis, and fibrosis improvement ≥1 stage without worsening of NASH were compared.

RESULTS

Baseline Characteristics - All Subjects

Parameter Mean or %	PGZ 27mg QW (n=20)
Age (years)	58.4
Female	75.0%
Weight (kg)	104.6
BMI (kg/m²)	37.0
Type 2 diabetes	85.0%
MRI-PDFF (%)	21.1
ALT (U/L)	47.1
AST (U/L)	36.1
Fibroscan VCTE (kPa)	14.3
Triglycerides (mg/dL)	170.0

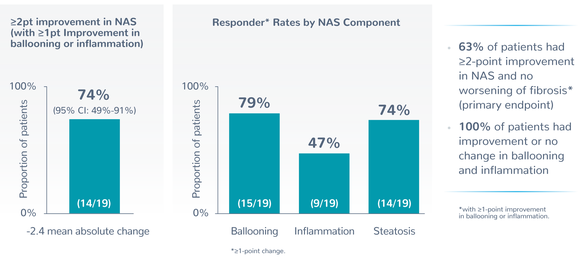
Baseline Biopsy Reading

- The proportion of subjects in NAS grade/stage categories across the study population were generally consistent.
- All subjects were deemed eligible by the central reader (primary analysis).
- The 3 Panel pathologists assessed fibrosis stage as more advanced than the stage determined by the central reader:

Parameter Mean or %	Central Reader	Pathologist A	Pathologist B	Pathologist C
Fibrosis Stage F1/F2/F3/F4 (%)	0/35/65/0	0/16/47/37	5/11/58/26	0/17/50/33

- 6/19 (32%) subjects were assessed with F4 fibrosis by 2 or more 3 Panel pathologists (putative F4). 4/19 (21%) subjects were assessed with F4 fibrosis by all 3 Panel pathologists.

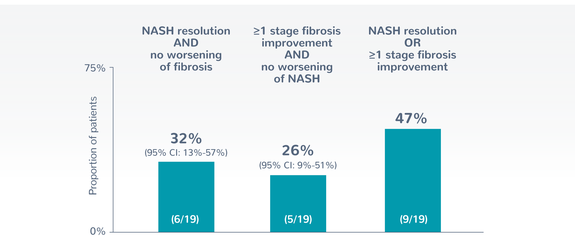
Primary Analysis (Central Reader): PGZ Robustly Improved NAFLD Activity Score (NAS) and All Components of NAS



- **63%** of patients had ≥2-point improvement in NAS and no worsening of fibrosis* (primary endpoint)
- **100%** of patients had improvement or no change in ballooning and inflammation

*with ≥1-point improvement in ballooning or inflammation.

Primary Analysis (Central Reader): PGZ Demonstrated Clinically Meaningful Changes on Key Histological Efficacy Endpoints



Week 20 Biopsy Reading

- Mean EOT scores and mean change from baseline were similar across most parameters per assessment of the 4 pathologists.
- There was more variability at EOT compared to baseline in the proportion of subjects assigned to specific histological grade/stage categories by the 4 pathologists across the study population.

Week 20 Biopsy Endpoints By Individual Pathologist

Parameter	Central Reader	Pathologist A	Pathologist B	Pathologist C
≥2-point reduction in NAS (%)	74	79	79	68
NASH resolution without worsening of fibrosis (%)	32	26	42	47
Fibrosis improvement ≥1 stage without worsening of NASH (%)	26	42	32	12

Week 20 Central Reader Biopsy Endpoints Excluding Putative F4 at Baseline

Parameter	All subjects (n=19)	Excluding putative F4 fibrosis (n=13)*
≥2-point reduction in NAS (%)	74	77
NASH resolution without worsening of fibrosis (%)	32	46
Fibrosis improvement ≥1 stage without worsening of NASH (%)	26	38

*Sensitivity analysis excluding subjects assessed with F4 fibrosis by 2+ Panel pathologists (n=6); cirrhosis was an exclusion criterion in this study.

Baseline Characteristics – Putative F4 Fibrosis*

Parameter Mean or %	Subjects with putative F4 fibrosis (n=6)
Age (years)	60.9
Female	100%
Weight (kg)	92.0
BMI (kg/m²)	33.9
Type 2 diabetes	83
MRI-PDFF (%)	18.25
ALT (U/L)	40.8
AST (U/L)	34.5
Fibroscan VCTE (kPa)	18.42
HbA1c (%)	6.6
Triglycerides (mg/dL)	161.1
Albumin (g/dL)	4.33
Platelets (×10³/µL)	188

*Subjects assessed with F4 fibrosis by 2+ Panel pathologists.

Week 20 Liver Non-Invasive Tests (NITs) – Putative F4 Fibrosis at Baseline*

Parameter Mean or %	Putative F4 fibrosis (n=6)
Relative liver fat reduction by MRI-PDFF (%)	-71.3
MRI-PDFF 30% responders/MRI-PDFF 50% responders	100%/100%
Change in ALT (U/L)	-23
Percent change in ALT (%)	-50.7
Percent change in AST (%)	-48.7
Change in Fibroscan VCTE score (kPa)	-3.8**
Change in FAST score	-0.51**
Percent change in FAST score (%)	-78.5**
Fibroscan VCTE/FAST response*** (%)	60**/100**
Percent change in adiponectin (%)	98.7

- In putative F4 subjects (n=6), fibrosis improvement ≥1 stage without worsening of NASH ranged from 17% to 57%; NASH resolution without worsening of fibrosis ranged from 20% to 50%.

*Subjects assessed with F4 fibrosis by 2+ Panel pathologists.
**N=5; one outlier with poor quality measurement was excluded.
***VCTE >20% reduction; FAST score ≤0.35.

DISCUSSION

Impact on Eligibility

- 32% (6/19) of the subjects would have screen failed as F4 at baseline on post-hoc analysis by the 3-panel pathologists rather than F3 as determined by the study central reader.
- A consensus approach with 2 or more readers may reduce variability.

Impact on Study Endpoints

- The proportion of subjects meeting the guideline-recommended endpoints for NASH clinical trials varied between the 4 pathologists, ranging between 26-47% for NASH resolution without worsening of fibrosis and 12-42% for fibrosis improvement ≥1 stage without NASH worsening.
- Excluding subjects who were assessed as having putative F4 fibrosis at baseline (by 2+ Panel pathologists), in this post-hoc analysis, study histological endpoints would have been met by a higher proportion of subjects in the primary analysis:
  - NAS ≥2 points 75% → 77%;
  - NASH resolution without worsening of fibrosis 32% → 46%;
  - Fibrosis improvement ≥1 stage without worsening of NASH 26% → 38%.

Subjects With Putative F4 Fibrosis* at Baseline

- All subjects with putative F4 fibrosis had well-compensated cirrhosis (Child Pugh A). There were no clinical or laboratory findings suggestive of clinically significant portal hypertension or other complications of cirrhosis.
- At week 20, there was marked improvement in most liver-related and metabolic NITs in subjects with putative F4 fibrosis.
- Safety and tolerability were favorable.
- These findings are reassuring in regards to the safety and potential benefit of PGZ in an F4 population.

*Subjects assessed with F4 fibrosis by 2+ Panel pathologists.

CONCLUSIONS

- This analysis demonstrates the inter-reader variability in biopsy scoring.
- In this post-hoc analysis, a higher proportion of subjects would have met histological endpoints in the primary analysis had the subjects determined to have putative F4 fibrosis at baseline (by 2+ panel pathologists) been excluded.
- A marked beneficial effect on liver NITs and metabolic markers with good safety and tolerability was observed in subjects with putative F4 fibrosis at baseline. Putative F4 subjects were well compensated; no safety issues suggestive of cirrhosis-related complications were reported.
- Study limitations include post-hoc analysis and a small sample size.
- PGZ is currently being evaluated in NASH in the ongoing Phase 2b ENLIVEN study (NCT04929483). Learnings from this study have been incorporated including a 3 panel read with a consensus charter.

DISCLOSURES

Rohit Loomba **– Consultant:** Aardvark Therapeutics, Altimmune, Anylam/Regeneron, Amgen, Arrowhead Pharmaceuticals, AstraZeneca, Bristol-Myer Squibb, CohBar, Eli Lilly, Galmed, Gilead, Glympse bio, Hightide, Inipharma, Intercept, Inventiva, Ionis, Janssen Inc., Madrigal, Metacrine, Inc., NGM Biopharmaceuticals, Novartis, Novo Nordisk, Merck, Pfizer, Sagimet, Theratechnologies, 89 bio, Terns Pharmaceuticals and Viking Therapeutics.

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