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Quantitative Digital Pathology and Imaging methods demonstrate the consistent reduction of liver fat burden in patient treated with LPCN 1144

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BACKGROUND and AIMS

LPCN 1144 is an oral prodrug of testosterone developed as a treatment for pre-cirrhotic non-alcoholic steatohepatitis (NASH) and recently completed the 36 weeks, blinded, placebo controlled, paired-biopsy LiFT Phase 2 clinical study in (NCT04134091). Some subjects continued treatment (Open Label Extension, OLE, NCT04685993) for additional 36 weeks. This analysis evaluates the anti-steatotic effect of the treatments using histological (pathologist), Digital Pathology and artificial intelligence (AI) and Imaging methods to optimize the design of a registration study.

METHOD

STUDY DESIGN AND TREATMENT ARMS

Group	Enrollment	36 w Intervention	N=44
Placebo	NASH diagnosed by histologic assessment of liver biopsy and NASH-CRN stage 1-3 fibrosis. 50 years in average 36.0 kg/m² Mean BMI	Placebo Twice Daily for 36 weeks	15
Treatment A		142 mg eq. T (testosterone) Twice Daily for 36 weeks	15
Treatment B		142 mg eq. T + 238 mg d-alpha tocopherol Twice Daily for 36 weeks	14

LIVER TISSUE HISTOLOGY

FFPE sections (~4 microns) of adequate liver biopsies were stained with Masson Trichrome for Collagen and H&E for assessment of the NAS histological grades (steatosis, ballooning, inflammation)

THREE ASSESMENTS OF THE ANTISTEATOTIC EFFECT WERE STUDIED ON THE SAME SLIDES:

HISTOLOGICAL GRADING

- Biopsy slides are read by an independent pathologists trained on the NASH-CRN staging system
- Steatosis grades are 0 (<5% hepatocytes count with steatosis involvement), 1 (5% to 33%), 2 (33% to 66%) and 3 (> 66%)
- Responders are identified with at least a full categorical stage change

MRI BASED PROTON DENSITY FAT FRACTION (MRI-PDFF)

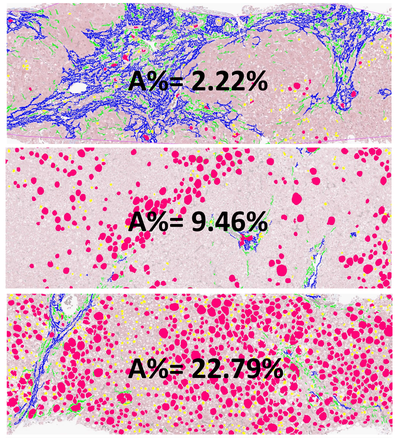
- Most patients underwent an MRI exam resulting in the calculation of their PDFF
- Responders are identified for a >30% relative reduction of PDFF between baseline and end of treatment

DIGITAL PATHOLOGY AND ARTIFICIAL INTELLIGENCE

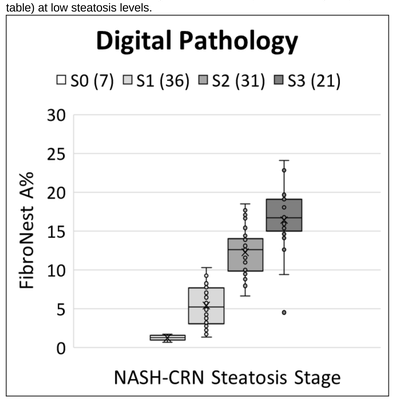
- The same slides prepared for and reviewed by Pathologists were digitized at 20X (0.50 micron/pixel) on a Aperio AT WSI system
- The Masson trichrome digital Images were read using FibroNest®, a single-fiber, high-content quantitative Digital Pathology image analysis and AI automated, full-tissue method
- The method quantifies large and medium macro-steatosis fat vacuoles as well as the area of scar fibrotic tissue (complex and dense fibrosis) to calculate the non-fibrotic parenchymal tissue fat area ratio (A%)
- Responders are identified for a >30% relative reduction of A% between baseline and end of treatment

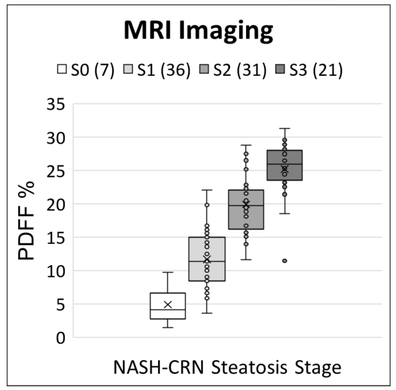
RESULTS

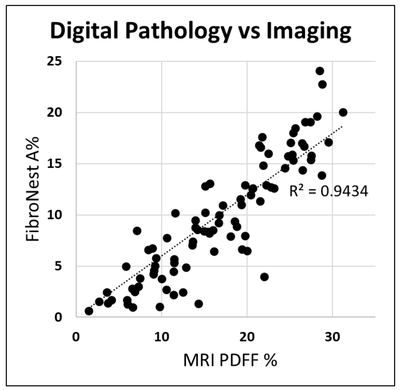
IMAGING VS DIGITAL PATHOLOGY PERFORMANCE



Most patients underwent MRI and liver biopsy exams at baseline and end of treatment. Small macro vesicular is not quantified by the FibroNest method and might explain slight differences in performance (see p-value table) at low steatosis levels.







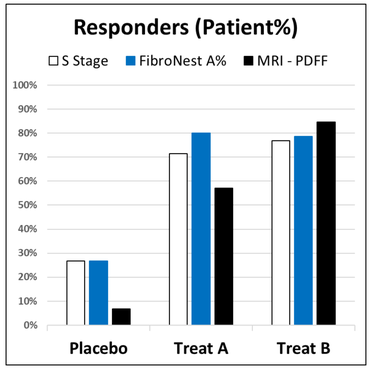
MRI PDFF (p-values)			FibroNest A% (p-Values)		
S0-S1	S1-S2	S2-S3	S0-S1	S1-S2	S2-S3
0.000162	2.4E-10	0.000147	3.95E-12	3.8E-14	0.000463

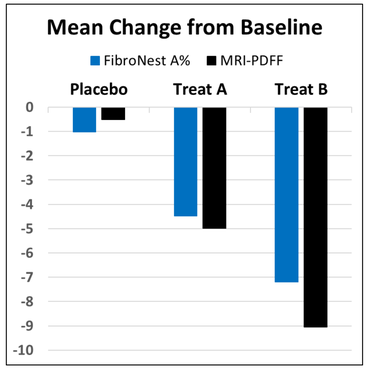
RESPONDER ANALYSIS

Group sizes ranged from 13 to 15 patients per group.
Responders were identified with a relative reduction from baseline of PDFF and A% of >= 30%, and with a 1-unit reduction for the histological S-stage.

While sampling the liver at different scales, the two digital imaging methods (MRI-PDFF and Digital Pathology by FibroNest) agreed to demonstrate a strong antisteatotic effect of the treatments (Mean Change from Baseline).

The three perspectives identified comparable level of responses.





		Placebo	Treat A	*pValue*	Treat B	*pValue*
Pathology - S Grade	**Total N**	**15**	**14**		**13**	
	Mean Change From Baseline	-0.200	-0.867	*0.070*	-1.214	*0.003*
	Mean R% Change from Baseline	0.011	-0.397	*0.058*	-0.590	*0.004*
Absolute Change (>=1)	Responders N	4	10		10	
	Responders %	26.7%	71.4%		76.9%	
Digital Pathology - FibroNest A%	**Total N**	**15**	**15**		**14**	
	Mean Change From Baseline	-1.017	-4.484	*0.043*	-7.194	*0.007*
	Mean R% Change from Baseline	0.060	0.117	*0.926*	-0.532	*0.018*
R% Change (>=30%)	Responders N	4	12		11	
	Responders %	26.7%	80.0%		78.6%	
Imaging - MRI - PDFF	**Total N**	**15**	**14**		**13**	
	Mean Change From Baseline	-0.510	-4.974	*0.074*	-9.055	*0.006*
	Mean R% Change from Baseline	0.040	0.089	*0.909*	-0.388	*0.012*
R% Change (>=30%)	Responders N	1	8		11	
	Responders %	6.7%	57.1%		84.6%	

References

1. Petitjean, L. *et al.* Evaluation of the performance of a novel Digital Pathology method for the continuous quantification of Steatosis, Ballooning and Inflammation in liver biopsies and its correlation with NASH-CRN scores in patients with NASH. *International Liver Congress*, *EASL* (2022).

2. Chen, L. *et al.* Evaluation of a novel histology-based fibrosis phenotypic composite score and its correlation with NASH-CRN Fibrosis scores in patients with NASH. *International Liver Congress*, *EASL* (2020).

3. Chen, L. *et al.* Novel Digital Pathology quantitative image analysis and AI method detects the treatment effect of NASH drug candidates with a performance that benchmarks Imaging based measurements. *International Liver Congress*, *EASL* (2022).

Conclusion

LPCN 1144, both alone and in combination with d-alpha tocopherol, demonstrates a strong antisteatotic effect. Digital Pathology and Imaging methods for the continuous assessment of these effects are highly correlated and provide a robust support of the pathologist-based results.

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