Biomarker Qualification Letter of Intent (LOI)

ADMINISTRATIVE INFORMATION

1. **Submission Title**

   "Second Harmonic Generation (SHG) and Machine-Learning based Model for a Stain-free and Fully-Quantitative Measurement of Fibrosis (qFibrosis) in Non-Alcoholic Steatohepatitis (NASH) Clinical Trials"

2. **Requesting Organization**

   Histoindex Pte Ltd
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3. **Submission Date**

   May 20, 2021

DRUG DEVELOPMENT NEED STATEMENT

Nonalcoholic steatohepatitis (NASH) with liver fibrosis is a serious and life-threatening condition, which was confirmed in the recent publication by the FDA and its new Division of Hepatology and Nutrition.[1] Currently, there are no approved therapies for nonalcoholic steatohepatitis (NASH). Approximately 30–40% of patients who develop NASH will develop fibrosis to a varying degree, and 15–20% of those with fibrosis will go on to develop cirrhosis, which is in turn associated with an elevated risk for decompensated cirrhosis, hepatocellular carcinoma (HCC), requirement for liver transplantation and liver-related mortality.[2] Liver fibrosis predicts liver-related clinical outcomes[3] and has been accepted as a reliable end-point in the assessment of NASH. As NASH is a progressive disease, its increasing prevalence has notable implications in terms of the clinical and economic burden of disease on national and global levels. There is a major and unmet clinical need to accelerate the development of effective therapeutics that result in significant clinical benefit.

Histopathological fibrosis assessment plays a critical role in NASH clinical trials with histology being the only reference standard which correlates with long-term clinical outcomes. However, there remains specific challenges related to how fibrosis is assessed by pathologists and one such challenge is that the histological system for liver fibrosis assessment is not designed to evaluate intervention efficacy. Inter-observer variations between expert hepatologists are considerable and over the years the level of interobserver agreement in scoring liver biopsies has remained modest.[4,5] Furthermore, there is the issue of substantial intra-observer variability which was illustrated in the recent analysis of the EMMINENCE trial.[6] Using a system that provides sensitive and reproducible quantitation of liver fibrosis will help addressing these challenges. The need for improvement and standardization of the interpretation of liver biopsies in NASH trials was highlighted by a panel of 14 pathology experts.[7] Their analyses concluded that the current staging systems do not capture the full spectrum of fibrosis in NASH; the severity of perisinusoidal fibrosis should be captured at all stages and a method to evaluate features of fibrosis regression should be developed.[7]

To illustrate this, it is well understood that with drug intervention, fibrosis activities can be

observed concurrently in all zones including fibrosis changes around portal tracts, central veins, and thinning of bridging fibrosis. The conventional CRN system however, represents static measures of fibrosis and do not capture changes with enough granularity especially in short term interventional studies. Furthermore, the amount of collagen deposition in the liver can be reduced as a result of an intervention, however because of the limited range of the CRN scoring system, a liver biopsy may still be categorized as F3 liver fibrosis as long as there is one bridging fibrosis remaining. The semi-quantitative system defines F1 and F2 as fibrosis changes around the sinusoidal and/or portal regions, while F3 records bridging fibrosis (Refer to Table 1). As a result, crucial information relating to potential intervention efficacy cannot be adequately assessed.

Fibrosis Stage	Histologic Findings
0	None
1a	Mild, zone 3 perisinusoidal
1b	Moderate, zone 3 perisinusoidal
1c	Periportal sinusoidal fibrosis without accompanying zone 3 fibrosis
2	Zone 3 perisinusoidal and portal/periportal
3	Bridging fibrosis
4	Cirrhosis

Table 1. NASH-CRN developed Fibrosis Scoring System. Adapted from Kleiner D.E. *et al*. *Hepatology. 41(6):1313-21, 2005* [4]

Furthermore, the conventional process of a panel of pathologists visually identifying pathological features in stained biopsies under a microscope requires professional but subjective judgement from the pathologists. Subtle pathological changes may also be unrecorded due to the rigid score-based assessment guidelines, inconsistent staining outcome, and/or human errors. Thus, there is a critical need for stain-free, scalable, reproducible, and validated tools in fully-quantitative pathology for the assessment of treatment efficacy in NASH for clinical research. Identification and validation of such biomarker tools could significantly accelerate drug development, especially breakthrough therapy.[8]

BIOMARKER INFORMATION AND INTERPRETATION

1. Biomarker

Biomarker Name: qFibrosis

Type of Biomarker: Histology based, Stain-free imaging modality, measurement of fibrosis along a continuous scale.

BEST Classification: Pharmacodynamic/response to be used when evaluating patients with NASH to assess fibrosis response to therapeutic intervention and disease monitoring in NASH clinical trials.

2. Analytical Methods

Given that histologic data is the anchor for treatment efficacy assessment, qFibrosis methodology can provide a fully-quantitative histological fibrillar collagen information, along with zonal fibrosis and bridging fibrosis details that would otherwise be a challenge to obtain via a semi-quantitative, and ordinal static system. These features will be quantified both objectively and consistently, enabling an evaluation that is based on machine learning and part of artificial intelligence (AI) pathology.

HistoIndex (HI) employs the state-of-the-art technology to carry out pathological assessment by coupling two-photon emission fluorescence (TPE) imaging, second harmonic generation (SHG) imaging, and digital image analysis algorithms. Genesis®200, an automated SHG/TPE microscope, reads collagen in the SHG channel and the surrounding cellular structure in the TPE channel directly from unstained formalin-fixed, paraffin-embedded (FFPE) biopsies. AI-based algorithm recognizes and segregates the whole biopsy area into three histologic zones, namely, central vein (CV), portal tract (PT), and peri-sinusoid (PS) zones. The definition of zones, with an example shown in a needle biopsy sample are illustrated in Figure 1.

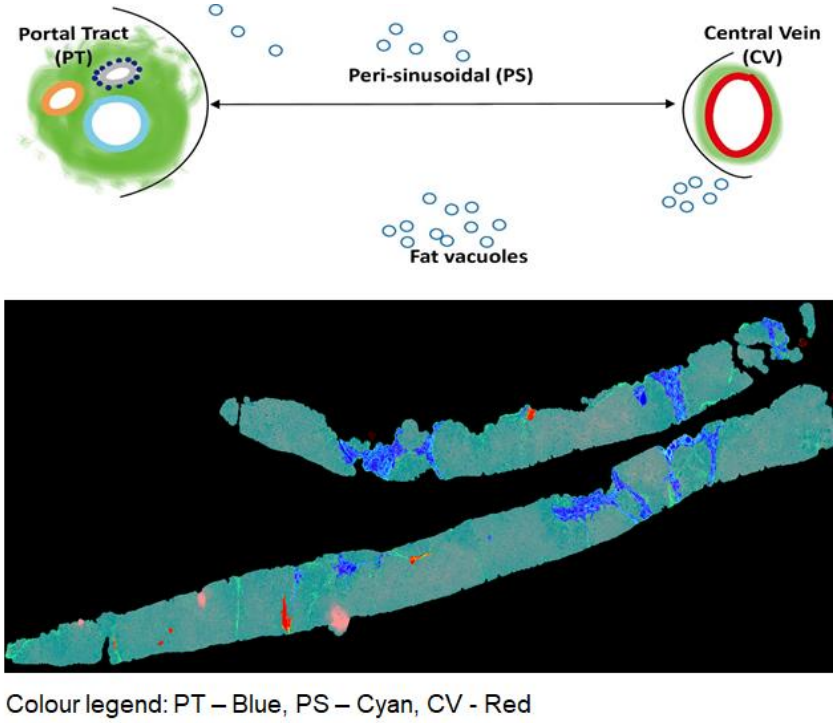

Figure 1. Illustration of zonal regions (top) and a biopsy sample with color-coded zones (bottom).

To evaluate the severity of liver fibrosis, collagen is detected from the SHG channel at the tissue region and quantification of defined architectural parameters that are characteristic of these collagen features, which are combined in an algorithm and generates a qFibrosis continuous value that can be used in fibrosis evaluation.[9]

qFibrosis is a morphology-based AI for digital pathology, which automatically detects and identifies different zonal regions in the liver, and associated morphological features such as collagen length, width, area, and intersections. qFibrosis measurement indicates the severity of fibrotic liver due to NASH, and the higher the qFibrosis value, the more severe the hepatic fibrosis is. This creates an unbiased and repeatable assessment on the biopsies. qFibrosis provides not only the capability to record meaningful morphological features identified by pathology systems such as CRN (Table 1), but also provides a fully-quantitative measurement to all these features.

3. Measurements Units and Limits(s) of Detection:

Fibrosis features used in CRN system will be measured and quantified by the model to determine the final qFibrosis value. The qFibrosis value (arbitrary unit) ranges from 0.00 – no upper limit.

4. Biomarker Interpretation and Utility:

The qFibrosis value is derived from 128 fibrosis parameters by identifying collagen fibers in different zones of the liver architecture.

Collagen fiber parameters are measured as its physical properties such as length, width, intersections. A list of examples for these parameters are demonstrated in Table 2. A full list of parameters can be found in the publication by Liu et al.[9]

No.	Abbreviation	Description
1	CollagenAreaAll	The area of collagen at overall region
2	FiberAreaAll	The area of fibers at overall region
3	FiberAWidthAll	The total average width of fibers at overall region
4	FiberMWidthAll	The total maximal width of fibers at overall region
5	FiberLengthAll	The total length of fibers at overall region
6	FiberPerimeterAll	The total perimeter of fibers at overall region
7	#FiberAll	The number of fibers at overall region
8	#LongFiberAll	The number of long fibers at overall region
9	#ShortFiberAll	The number of short fibers at overall region
10	#ThickFiberAll	The number of thick fibers at overall region
11	#ThinFiberAll	The number of thin fibers at overall region
12	AggAreaAll	The area of aggregated fibers at overall region
13	#IntersectionAll	The number of intersections at overall region

Table 2. List of collagen fiber parameters.

Features used in CRN system are portal sinusoidal fibrosis, periportal sinusoidal fibrosis, zone 3 perisinusoidal fibrosis, bridging fibrosis, and cirrhosis. In the context of qFibrosis, fibrosis are measured by collagen fiber parameters in portal tract zone, central vein, and perisinusoidal fibrosis. While bridging fibrosis and cirrhosis are measured by connecting collagen fibers across all zones.

The AI-based algorithm automatically identifies and quantifies the features used by expert

pathologists when they diagnose using CRN system, so qFibrosis provides a weighted average as a single continuous numerical output.[9] For example, the CRN system records the presence or absence of fibrosis bridging in a dichotomous manner i.e., once bridging fibrosis is observed, the final output will be F3 fibrosis regardless of the extent of fibrosis dynamics in the periportal and pericentral regions. The qFibrosis continuous numerical output, however, is capable of capturing changes within septum even though the static stage has not changed.

The clinical benefits and utility of applying AI digital pathology for assessment of liver fibrosis are multidirectional:

- Digital Quantitation of Fibrosis on a linear scale can be correlated with digital outputs from imaging modalities (MRI-PDFF, MRE, Fibroscan) and serum markers (Non-invasive tests);
- Changes on liver fibrosis overtime or as a result of an intervention will be assessed quantitatively with an objective reproducible methodology;
- Continuous numerical ouput from qFibrosis provides dynamic measure of fibrosis changes across all regions within the biopsy sample even when the changes are too subtle to be recorded as at least 1 stage changes by the static staging system;
- These quantitative data is essential in early phase studies to better estimate the time duration and patient number for later phase studies.

In summary, from a clinical benefit and utility perspective: qFibrosis can identify and capture minute fibrosis changes which is not recorded by the ordinal static CRN system and we propose that this allows for a better baseline & efficacy evaluation with more sensitivity than current conventional approach.

CONTEXT OF USE STATEMENT

A pharmacodynamic/response biomarker that is stain-free and AI-based, to evaluate treatment response based on fibrosis change in liver biopsies from baseline to end-of-

treatment (EOT) in patients enrolled in clinical trials for treatment of non-alcoholic steatohepatitis (NASH).

ANALYTICAL CONSIDERATIONS

In NASH, fibrosis heterogeneity can be substantial and is expected to be greater in post-treatment samples. An example taken from an EOT biopsy sample is shown in Figure 2, in which features from all 4 stages of NASH CRN system can be observed.

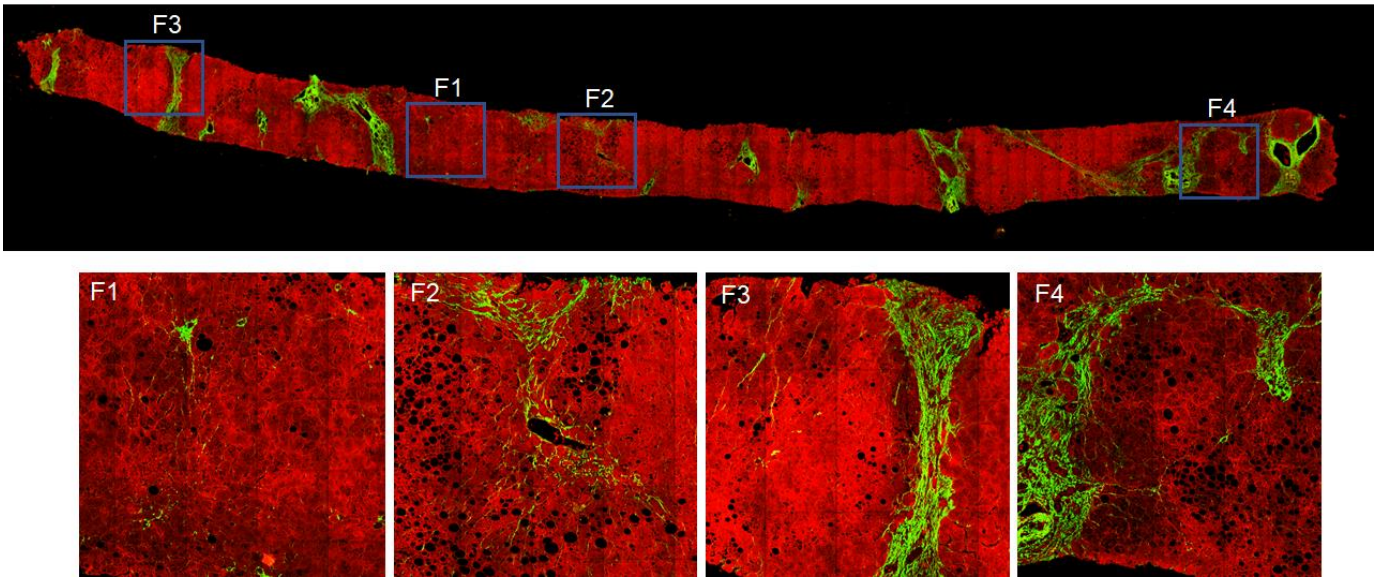

Figure 2: Heterogeneity of fibrosis stages within a biopsy sample taken post-treatment.

There is no clarity on dealing with fibrosis heterogeneity in the CRN system, and it is common to make the conservative calls (i.e. highest stages) during natural history development cases in which no treatment is involved. The implication is such that only significant fibrosis reduction will be recorded by the NASH CRN system.

In recent study by Wang *et. al.*, it has been shown through fibrosis quantification using SHG that specific fibrosis features such as number of fiber strands, length of fiber strands demonstrated good concordance with pathologists scores in a cohort of non-alcoholic fatty liver disease (NAFLD) patients. In addition, the quantification of these fibrosis-related parameters (q-FP) can be used for discriminating fibrosis stages with excellent performance (AUROC of 0.81-0.93).[10]

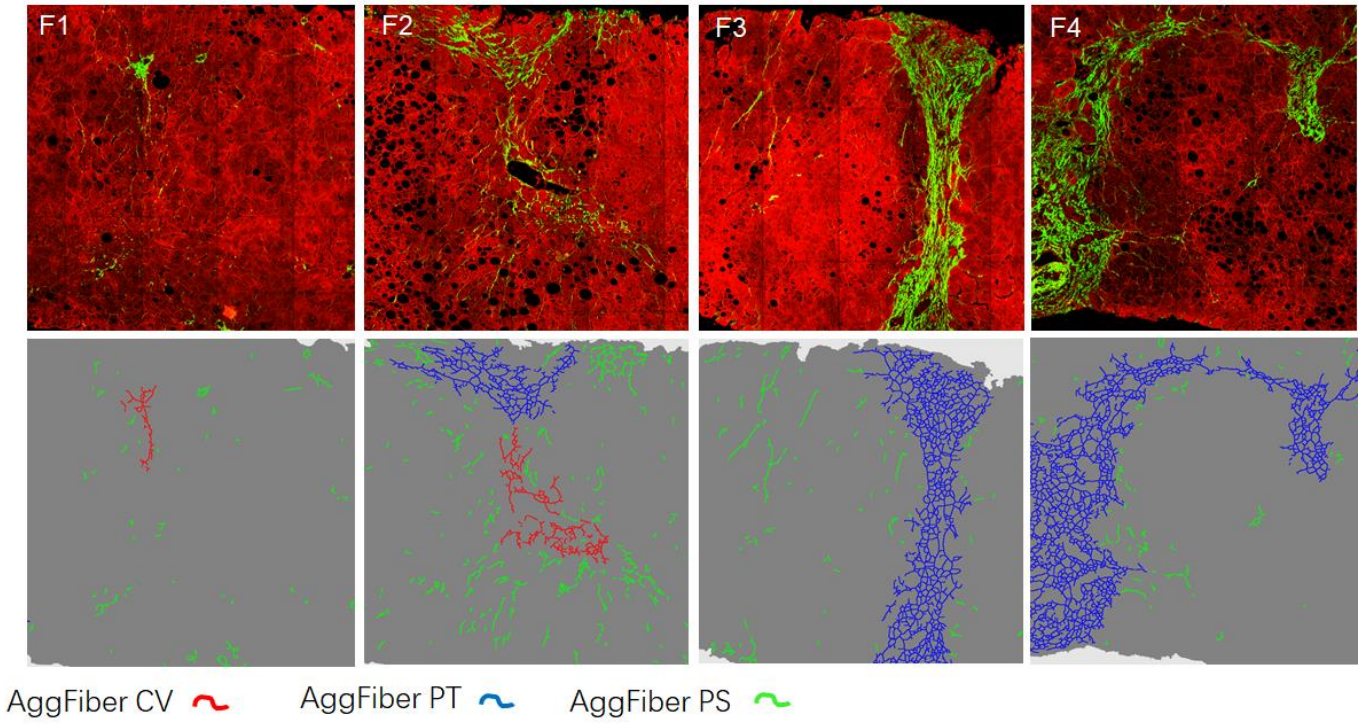

Figure 3: Examples of how AI can identify fibrosis stage specific features: isolate central vein fibrosis for F1; central vein and portal tract fibrosis in proximity for F2; extension of portal tract fibrosis to other portal tract for F3; extension of portal tract fibrosis in circular formation (nodule) with no/little perisinusoidal fibrosis inside the nodule.

As shown previously in Table 1, key fibrosis feature(s) are typically stage-specific according to the definition established by the CRN system, i.e., F1 is characterized by sinusoidal fibrosis, F2 is both sinusoidal and portal, bridging fibrosis in F3 and cirrhotic nodules in F4. Based on this, the qFibrosis algorithm is designed by identifying these morphological features as described by the CRN system in a machine learning-based manner to provide an objective fibrosis evaluation. The heterogeneity within a post-treatment biopsy sample has been previously shown in Figure 2, and its corresponding color-coded images showing specific fibrosis parameters as identified by the qFibrosis algorithm is presented in Figures 3. In these figures, we demonstrate that the qFibrosis algorithm can also identify specific features as was described in the CRN system. F1 shows only zone 3 CV fibrosis parameters, F2 shows both zone 3 central vein and portal tract fibrosis, F3 shows clear bridging fibrosis with aggregated collagen fibers connecting between 2 points (PT to PT or PT to CV), and F4 with clear nodular cirrhosis with negligible perisinusoidal fibrosis inside the nodule. From these images, it is clear that fibrosis

progression is not a linear process and cannot be accurately measured by collagen proportionate area (CPA) or a simple area average method based on image pixels.[11]

qFibrosis is built by combining parameters from all stages of fibrosis, such as perisinusoidal fibrosis and bridging fibrosis, so that these features are added linearly with fixed coefficients. And it has been demonstrated that it can recognize stage-specific fibrosis features according to the NASH CRN system by evaluating the weightage of various fibrosis features with scores from expert histopathologist.[9] In addition, it quantifies all these features across the entire biopsy sample simultaneously in a reproducible manner. [9] This enables the use of qFibrosis readout to go beyond the limitations of the static and ordinal CRN system.

The objective of this LOI is to demonstrate the concept of drug efficacy evaluation in NASH clinical trials following the validation of fibrosis parameter-based quantification, as compared with the CRN system approach. In a pilot analysis with the FLIGHT-FXR study involving paired liver biopsies in patients with NASH (NCT02855164) (Figure 4), we observed no change in fibrosis stages (based on CRN system) in approximately 60% (59% - 64%) of patients for all treatment arms. When applying the qFibrosis assessment, a lower proportion of patients are categorized as no change, i.e., 44% of the placebo patients and approximately 30% in treated patients (140 μg and 200 μg) showed no change according to qFibrosis and its associated qFibrosis stages (categorized with cut-off values). [9] These results provide evidence that qFibrosis is more sensitive than the CRN system in recording heterogenous fibrosis changes which is expected in patients with treatments, and more importantly, it suggests that there may be more “intra-stage” fibrosis changes in treated patients than in placebo patients.

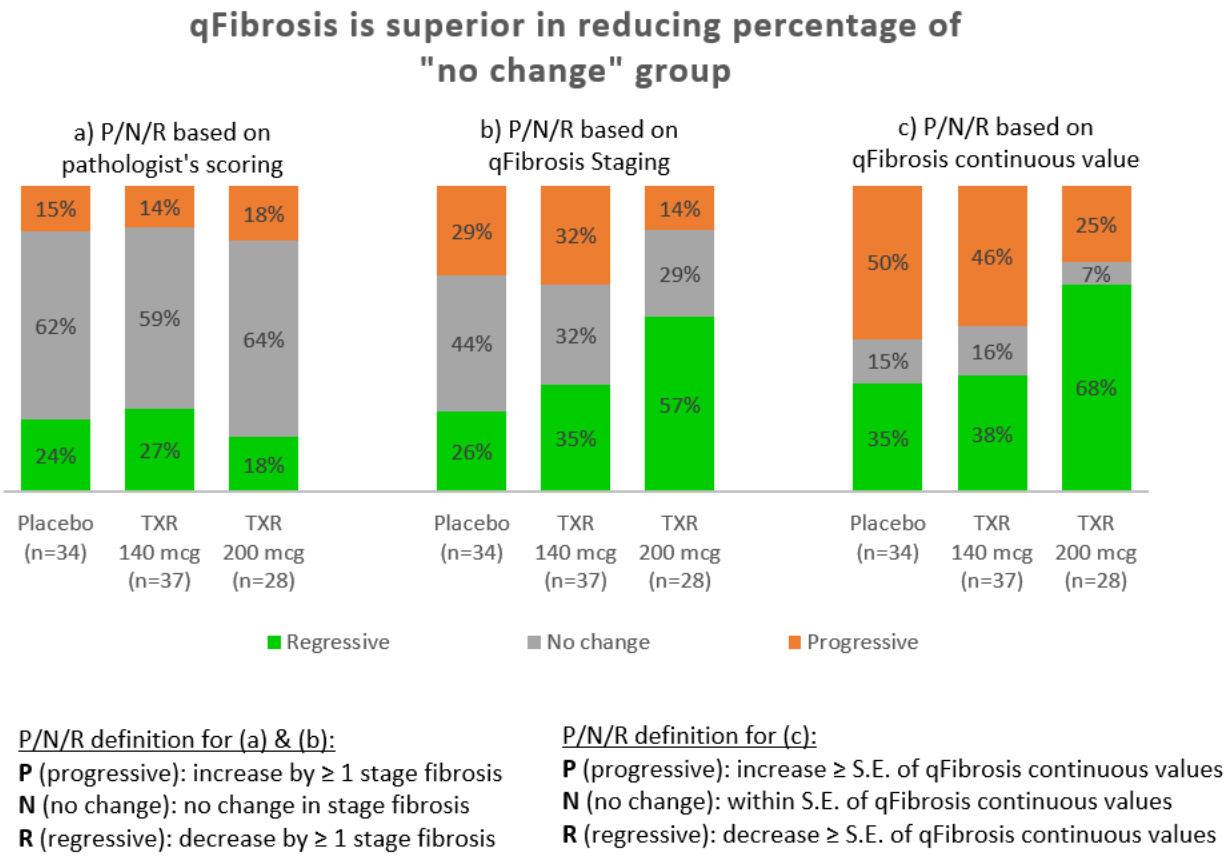

Figure 4: Changes in liver fibrosis between baseline and end of treatment taken from the FLIGHT-FXR study

To further demonstrated the limitation of ordinal static staging system, we evaluated the fibrosis response by qFibrosis continuous value. In this approach, the no change group is defined by the standard error of the qFibrosis continuous value measurement.[12] This further reduced percentage of patients in the no-change to 7-16%. Therefore, by employing qFibrosis as a continuous measure, we can avoid the error introduced by static system such as the CRN system, and measure fibrosis as a continuum. Furthermore, qFibrosis provides greater sensitivity to measure changes in fibrosis for short term trials.

It was also reported in another NASH study, phase 2 study of Resmetirom, there is 47% of the treated patients with F3 fibrosis stages at baseline showed a more than 1 point reduction in fibrosis by qFibrosis, while there is 0% in the placebo group with significant statistical differences (see Figure 5). This finding also suggests the qFibrosis can capture subtle fibrosis changes in intervention studies which might not be properly recorded by

the ordinal and static NASH-CRN system.

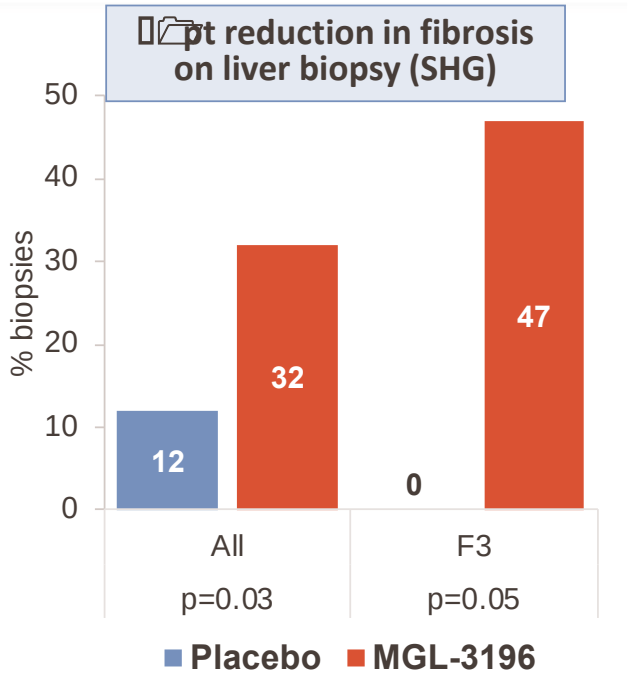

Figure 5: Percentage of patients showed more than 1 point reduction in fibrosis by qFibrosis in Phase 2 Resmetirom study.[13]

Therefore, qFibrosis is a more sensitive biomarker for treatment efficacy evaluation for use in NASH clinical trials, and such quantification data would be essential for the regulators' consideration in both i) NASH development programs which to date have had a high failure rate (in both phase II & phase III), and to support ii) NASH drug approval processes in the future.

The statistical analysis plan (SAP), including the statistical method and analysis as well as sample size calculations, for these analytical and clinical validation studies will be developed and finalized prior to the start of these studies and will be described in the Qualification Plan.

CLINICAL CONSIDERATIONS

1. **Use Statement**

For **diagnostic** purposes, the review of liver histology by an expert hepatopathologist using a conventional microscopy currently is and will always be the essential first step. For **quantitation of changes** in NASH features, especially progression or regression of liver fibrosis, in longitudinal cohorts evaluating the disease course over time, or as a result of treatment intervention (e.g., pharmacological treatment; lifestyle changes, bariatric surgery, etc.) the use of AI digital pathology will markedly improve the scope and accuracy of the overall information. Thus, AI digital pathology and the information it provides will extend the outputs of hepatopathologists and the two approached will work hand-in-hand.

In NASH clinical trials, our fully-quantitative qFibrosis methodology allows for rich architectural interrogation which can reveal finer measurements of disease progression and regression that is eluded by the ordinal CRN system. qFibrosis readout can provide quantitative zonal fibrosis as well as bridging fibrosis changes which are both significant for the evaluation of treatment efficacy for drugs in NASH clinical trials.

In early phase trials, duration of the trial may not be adequate to observe fibrosis changes which is significant enough to move 1 stage up or down based on the CRN system. In addition, the changes in fibrosis in these short term studies are often masked by noises from inter or intra pathologist discrepancies, which resulted a large number of patients showed no significant fibrosis changes (1 stage progression or 1 stage regression) in many studies. qFibrosis provides quantitative, highly dynamic measures for fibrosis changes in continuous scale which can be used to assist decision about whether to move the drug forward, or the length of study needed for larger studies.

It is also beneficial to record these quantitative data by utilizing AI-based digital pathology methodology, such as qFibrosis, for future studies. These multi-dimensional data provides additional insights, which are currently not available from conventional ordinal and static staging system, to better understand and planning NASH intervention studies.

2. Proposed Conditions of Qualified Use

Proposed population for use	▪ Adults, 18 years and older.

	▪ Patients enrolled in a NASH clinical trial, with either a NASH confirmed biopsy or biochemical criteria and/or imaging evidence of steatosis/steatohepatitis/fibrosis in addition to known risk factors for NASH.
Whole slide image considerations for clinical trial use	▪ Formalin-fixed paraffin-embedded (FFPE) ▪ Genesis®200 imaging device

3. General Clinical Validation Plan

The objective of the clinical validation for the use of qFibrosis in clinical trials will be to verify the patients whom were initially identified as “no change” by pathologists, and later identified as “regression” by qFibrosis, shows signs of fibrosis regression either by 3rd biopsy or other clinical data.

qFibrosis has been published in numerous peer-reviewed journals and presented at several conferences.[9,12,14,15] We are also currently involved in the evaluation of drug efficacy in more than 15 NASH clinical trials and many NASH clinical studies. In terms of the maturity of qFibrosis, it has been tested for repeatability/reproducibility. Briefly, we used sample slide across different machines to evaluate the qFibrosis assessment, and the qFibrosis stage is consistent across all machines, with >95% agreement for our inter and intra-system repeatability.

In this letter of intent, we plan to use data from Novartis’s NASH Phase 2B FLIGHT-FXR study (NCT02855164) as well as TANDEM study (NCT03517540) on combination NASH therapy to validate the utility of qFibrosis. We are also exploring the possible inclusion of other clinical trial data with investigational drug. In these studies, the change in fibrosis stages from baseline to end of treatment biopsies will be computed using conventional pathologist reads with the CRN system and compared with the corresponding change in qFibrosis values (stages and continuous). For patients whom was initially identified as “no change” by pathologist, and later identified as “regression” by qFibrosis continuous value, we will evaluate their fibrosis data by assessing their histopathological data like fibrosis stage by pathologists from 3rd liver biopsy, specific fibrosis features like thinning of septa, elastography or other wet biomarkers (like $ProC_3$) after their EOT time point. With data

past EOT time point, it can verify the fibrosis trend identified by qFibrosis continuous value, which may be over looked by ordinal static CRN system. This information will provide critical information for designing later stage (phase 3) trials to ensure sufficient duration for observing significant fibrosis changes, and for power analysis to estimate the number of patients required.

Overview of Risks and Benefits

From a clinical study standpoint, the potential benefits of qFibrosis compared to the conventional fibrosis staging lies in its objective, consistent and fully quantitative assessment of liver fibrosis, owing to the use of an automated and stain-free system to analysis of treatment response as a result of a therapeutic intervention.

The potential risks of our system are associated with failure of the system to perform as expected. It is possible that faults in the product, such as a malfunction or a latent design flaw, could lead to an erroneous information, causing incorrect test results. HistoIndex will mitigate potential risks by conducting validation tests, which would be defined in the Qualification Plan (QP).

SUPPORTING INFORMATION

1. White Paper:

Attachment: FDA BQP Application White Paper

This White Paper attached is a supplementary paper which provides a brief introduction to HistoHepa-F.

2. Previous Oral / Poster Presentation:

1. Digital presentation at the 2020 *The Liver Meeting Digital Experience (TLMx).* **Safety and Efficacy of Tropifexor in Patients with Fibrotic Nonalcoholic Steatohepatitis: 48-week Results from Part C of the Phase 2 FLIGHT-FXR Study** by Arun Sanyal.
2. Poster presentation at the 2020 *Digital Paris NASH Meeting.* **Dynamic Fibrosis Features in Post-treatment Biopsies and its Interpretation** by Elaine Lay Khim Chng, Dean Tai, Yayun Ren and Pierre Bedossa.
3. Poster presentation at the 2020 *NASH-Tag*. **Concomitant Zonal Quantification of qSteatosis and qFibrosis in a Sub-study from EMMINENCE, a 12-month Phase 2b NASH study of MSDC-0602K** by Stephen Harrison, Dean Tai, Yayun Ren, Elaine Chng, and Howard Dittrich.
4. Poster presentation at the 2019 *The Liver Meeting (AASLD).* **qFIBS for the Automated Quantitative Evaluation of Histological Evolution in Pediatric Nonalcoholic Steatohepatitis** by Feng Liu, Jingmin Zhao, Leow Wei-Qiang, Ya-Yun Ren, Xiaoxiao Wang, Xiaohe Li, Huiying Rao, Wei Zhang, Aileen Wee and Lai Wei.
5. Poster presentation at the 2019 *The Liver Meeting (AASLD).* **Steatosis and Fibrosis Measured as Continuous Variables on Paired, Serial Liver Biopsies in the Resmetirom (MGL-3196) 36-week Phase 2 NASH Study** by Stephen A Harrison, Dean Tai, Ya-Yun Ren, Rebecca A. Taub, Mustafa Bashir.
6. Oral presentation at the 2018 *The Liver Meeting (AASLD).* **New Automated Evaluation Tool qFIBS - Quantitative Assessment for Fibrosis, Inflammation, Ballooning, and Steatosis in Patients with Non-Alcoholic Steatohepatitis** by Feng Liu, Boon Bee George Goh, Dina Tiniakos, Aileen Wee, Leow Wei-Qiang, Jingmin Zhao, Huiying Rao, Xiao-Xiao Wang, Qin Wang, Wei-Keat Wan, Kiat Hon Lim, Manuel Romero-Gomez, Salvatore Petta, Elisabetta Bugianesi, Chee-Kiat Tan, Stephen A. Harrison, Quentin M. Anstee, Jason Chang and Lai Wei.

PREVIOUS QUALIFICATION INTERACTIONS AND OTHER APPROVALS

This is the first regulatory interaction for this proposed biomarker.

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