LETTER OF INTENT
DETERMINATION LETTER

DDTBMQ000106
December 3, 2020

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Dear Dr. Erhardtsen:

We are issuing this letter to LITMUS to notify you of our determination on your proposed qualification project submitted to the Center for Drug Evaluation and Research (CDER) Biomarker Qualification Program (BQP). We have completed our review of your Letter of Intent (LOI) submission deemed reviewable on September 3, 2020 and have concluded to **Accept** it into the CDER BQP.[1]

Based on our review of the LOI, we agree that the development of this composite biomarker for prognostic enrichment of patients who are more likely to reach long term events in NASH clinical drug development trials could address an unmet need.

As this biomarker development effort is refined in subsequent BQP submissions, the submitted data, the specifics of your context of use (including the target patient population), the specific analytics and the design of study(ies) used in the clinical validation of the biomarker will ultimately determine which of the comments below may be the most applicable to your qualification effort.

Your next stage of submission, a Qualification Plan (QP), should contain details of the analytical validation plan for the biomarker panel measurement method, detailed summaries of existing data that will support the biomarker panel and its context of use (COU), and include descriptions of knowledge gaps with proposed mitigation strategies. If future studies are planned, please include detailed study protocols and the statistical analysis plan for each study as part of your QP submission. Below, we provide you with specific considerations and recommendations to help improve your preparation for, and submission of the QP. For more information about your next submission and a QP Content Element outline, please see the BQP Resources for Biomarker Requestors web page.[2]

[1] In December 2016, the 21st Century Cures Act added section 507 to the Food, Drug, Cosmetic Act (FD&C Act). FDA is now operating its drug development tools (DDT) programs under section 507 of the FD&C Act.
[2] https://www.fda.gov/drugs/cder-biomarker-qualification-program/resources-biomarker-requestors

Biomarker Considerations

Requestor's Biomarker Description: A composite biomarker which will include a measure of 3 serum molecular biomarkers (hyaluronic acid, amino-terminal propeptide of type III, and tissue inhibitor of metalloproteinase 1), and corrected T1 (cT1) Magnetic Resonance Image (MRI), as components of the Enhanced Liver Fibrosis (ELF) test. The ELF test uses an algorithm to produce a unitless number from measuring the molecular biomarkers hyaluronic acid (HA), amino-terminal propeptide of type III procollagen (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1). The cT1 MRI is used to indicate the free water within the tissue. The final composite marker is not yet determined.

1. For the three serum biomarkers measured by the ELF test, please note that the algorithm that is used to produce the unitless score would need to be provided as part of your QP submission. If there is a differential weighing of the factors, please provide a biologic rationale to support the algorithm's determination and interpretation.

2. It is unclear how the cT1 liver imaging biomarker will be used and with what aspect of liver injury (fibrosis, inflammation, scarring) it is associated. Please provide a description of the impact of this biomarker individually and in the composite biomarker. Please provide a full description of your biomarker, including a clear description of how your device takes input data (such as MR images) and generates the output biomarker value (e.g. cT1 relaxation time).

3. As you state the final composite biomarker is not yet determined. It is unclear from your submission if the final biomarker will be a score of the composite measure, a panel in which each biomarker is independent of each other, or some other type of algorithm. Please provide information for each biomarker and its proposed impact to support the COU before providing analysis of the final composite marker.

4. In your QP submission please provide sufficient detail on the derivation of your composite marker. For example, if each biomarker is weighted, please provide information on the weighted value for each component in your final equation. If an algorithm is used to generate a composite score, please include the algorithm as part of your QP submission.

Context of Use (COU) Considerations

Requestor's COU: The proposed prognostic enrichment biomarkers (liver cT1 and/or the ELF test) should identify patients who are more likely to reach the intermediate and/or the composite long-term events. For this COU the long term events are defined as Death (liver-related or all-cause), liver transplant, complications of cirrhosis (including hepatocellular carcinoma (HCC); variceal bleed; Change in MELD score from less than or equal to 12 to more than 15, and histological progression to cirrhosis. The intermediate endpoint will be defined based on the definition in the reflection paper below.

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FDA Recommended COU: Prognostic enrichment biomarkers in biopsy proven NASH patients with NAS[3] ≥4 and fibrosis stages 2 and 3 (advanced fibrosis) to identify patients who are more likely to experience clinical endpoints such as progression to cirrhosis, hepatic decompensation events (variceal bleeding, ascites, hepatic encephalopathy), death, or liver transplant during the timeframe of a NASH clinical trial.

We have the following comments about COU considerations:

5. Your proposed context of use does not identify patients to be enrolled in clinical trials. Because this biomarker and the context of use is to identify NASH patients who are more likely to develop long-term events, it is recommended the COU be revised to identify this specific patient population.

6. Your proposed COU to "identify patients who are more likely to reach the intermediate ***and/or*** the composite long-term events" appears to be two different COUs. If you plan to develop the biomarker as a prognostic enrichment biomarker to identify patients more likely to reach long-term events and as a prognostic enrichment biomarker to identify patients more likely to reach intermediate events, you should submit a separate LOI for each COU.

7. You state that this biomarker could be used in all clinical states of NASH drug development including proof- of-concept, dose-ranging, and confirmatory clinical trials. Please provide more information on how the biomarker could be used in each stage of clinical trial development.

8. You statement "Additionally, the exclusion of subjects that reach the intermediate endpoints without treatment from clinical trials, i.e. those that spontaneously regress in disease, will also increase the efficiency of such trials" stated on page 10 of 21 of LOI lacks clarity. Clarify whether you would collect the biomarker and endpoint data in these subjects, or would data from these subjects not be included as part of the dataset. We recommend if a subject meets the enrollment criteria, then collect all the data until the endpoint is reached.

9. In your QP, please consider the types of evidence you will be able to gather and analyze for your COU. Your analytical validation data and proposed clinical information, should be able to support your COU.

[3] NAS: Nonalcoholic fatty liver disease (NAFLD) activity score; with at least one point from steatosis, one point from ballooning and one point from inflammation

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Analytical Considerations

Pre-Analytical Sample Collection, Handling, Stability and Supporting Standard Operating Procedures

10. You have provided literature articles as a reference for stability studies for the biomarkers in serum used for the ELF test. The literature articles provide a summary of the results and do not provide data for the individual samples. Please provide stability data for individual patient samples with the baseline biomarker measurements and the future timepoint biomarker measurements.

11. The LOI states that all MR data undergoes automated quality control checks. Please describe these quality control checks and if these quality control checks make any adjustments to the data which include removing data or modifying the data. Please provide the process if the data fails these automated quality control checks.

Validation: Calibration, Controls, and Verification of Repeat Measures (Variability) and Demonstration of Capability for Full Parameter Range (Performance)

12. The LOI states that analytical validation for the ELF test will be conducted. It is unclear how this validation is going to occur. You should validate the measurement of each biomarker involved in the measurement as well as the final ELF score. Please provide analytical data for each biomarker being measured by this method and the final ELF score. You should also consider that the analytical studies needed to demonstrate that the biomarkers can be used as stated in the COU will depend on how the design of the final biomarker (e.g., whether the ELF test and the CT1 are interpreted independently or whether they are combined into a score), how the result will be interpreted (e.g., looking for changes, using medical decision points), the methods, the patient population, the measuring range, etc.

    We recommend that, in your future Qualification Plan (QP) submission, you provide a description of the final biomarker including a description of the measurement methods (including a description of the traceability of the ELF test, a description of how the final biomarker will be used and any medical decision points), and whether the test is qualitative or quantitative. You should provide detailed protocols used for your analytical validation studies needed to support the COU including a description of the purpose of each study. The protocols should include the following: the method(s) and instrument(s) used, the specimen type (e.g., serum, native, contrived, quality control material), the specific concentrations of each target biomarker (as well as the composite), the number of samples tested, the number of replicates tested for each sample, the number of days, the number of operators, the number of reagent lots used, and any reference materials used. All studies should be conducted using stable samples (i.e., stored and handle using validated conditions). The sample type should reflect the clinical samples that will ultimate be used and

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native patient samples should be used whenever possible (and especially around important medical decision levels).

In general, we recommend that you refer to the following Clinical and Laboratory Standards Institute (CLSI) guidelines when designing your analytical validation studies: EP05-A3 "Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition"; EP06-A "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline"; and EP17-A2 "Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-Second Edition".

13. Please provide a full description of the technical performance of your device/software. Please ensure that this information includes:
    a. measurement reproducibility
    b. measurement performance across MRI system vender, model, and software version
    c. analysis of how liver iron concentration affects the performance characteristics
    d. sensitivity and specificity related to decision points defined in the Context of Use

14. You provided a summary protocol to evaluate the effect of potential endogenous and exogenous interfering substances. The list of interferants does not appear to include all potential exogenous and endogenous interfering compounds in the intended use population. For example, creatinine, glucose, cholesterol, chenodeoxycholic (bile acid) and lactate are common endogenous interferents that may be relevant to this population and rosuvastatin, verapamil, tetracycline, and pseudoephedrine are common exogenous interferents. In addition, the proposed testing concentrations for the interferents appears to be low and may not provide sufficient information on the performance of the biomarkers in the ELF test in the presence of potential interferents at concentrations that could be reasonably expected in the intended use population. For example, you intend to test conjugated and unconjugated bilirubin at 5 mg/dL and 15 mg/dL, respectively. It is recommended in CLSI Guideline EP37 - Supplemental Tables for Interference Testing in Clinical Chemistry, that both conjugated and unconjugated bilirubin be tested to at least 40 mg/dL to adequately characterize test performance in the presence of total bilirubin. You should carefully assess the risk in the intended study population of the presence of any potentially interfering compound. We encourage you to refer to CLSI EP07 – A3 Interference Testing in Clinical Chemistry and CLSI EP37 - Supplemental Tables for Interference Testing in Clinical Chemistry for recommendations concerning interference testing.

Confirmation of Transparency of Analytics Technical Parameters

15. You state that the algorithm to determine cT1 is propriety property of Perspectum Diagnostic. For the biomarker to be used by other drug developers, enough information of the cT1 measurement will need to be made public. In the QP submission, please indicate the information of the cT1

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measurement that will be made public and how this information should be adequate for others to replicate this measurement.

16. The biomarker will ultimately be qualified independent of the measurement method used to assess the biomarker. Please be aware that qualification of the biomarker requires generalization and public dissemination of the performance specifications necessary to ensure that the biomarker can be measured with any test meeting those performance specifications.

17. Section 507 of the FD&C Act includes transparency provisions that apply to your submission. Analytical information about the assays, device, and software may be publicly posted if the biomarker is successfully qualified by the Agency. To ensure the biomarker can be used as a drug development tool by any interested party, please confirm technical parameters and other pertinent information about the assays, device, and software that may be made public. The biomarker qualification process does not endorse the use of any specific device, assay or software with a qualified biomarker.

Clinical Considerations

Background

18. In your QP, please ensure your clinical protocols and proposed studies will support your proposed COU. If your studies will be collecting information related to other ongoing biomarker development efforts (including other COUs), for clarity, please only include study information related to this specific biomarker/COU project.

19. Information that is provided in the LOI and in the attachments are conflicting. For example, the LOI states that samples have been stored for up to 18 years, but the attachments do not appear to refer to any study that is from this time period.

20. Based on the feedback provided in this letter, please provide a revised decision tree diagram. This diagram should include the updated COU, and clinical trial design input.

Interpretive Criteria (Cut-offs/Boundaries), Application & Validation in population

21. In your Meta-cohort and LITMUS studies please provide, with respect to the context of time, when the liver biopsies were taken with respect to when the blood samples were collected, and when the cT1 MRI taken. Provide justification that the sequence of studies over the pre-specified time interval specified does not affect the outcome of your analysis.

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22. Attachment 2 ELF information proposes some threshold values that may be used to determine if a patient should be included in a clinical trial. Please provide analysis on how variation and values of the individual biomarkers may affect this value. Please explain if an increase or decrease in one biomarker predicts an increase or decrease in the other biomarkers.

Gaps and Proposed Studies

23. There are assumptions that are being used that the ELF score will increase as fibrosis increases. Please address the following comments:

    a. With the continued progression of cirrhosis to advanced (decompensated) cirrhosis, does the ELF score decrease or continue to increase? If the ELF score might be reduced as fibrosis declines in advanced stages of disease, might there be a potential to incorrectly categorize a patient with advanced cirrhosis as moderate fibrosis or vice versa?

    b. Healthy volunteers and patients with moderate fibrosis[4] had similar reference ranges of hyaluronic acid due to high biological variability. Therefore, a healthy volunteer could be misclassified as having moderate fibrosis. Clarify how this misclassification would be handled.

    c. It appears ELF scores are higher[5] in men compared to women; and, afternoon values are higher relative to morning values. Clarify whether these variabilities in ELF scores could significantly impact the stated objective of COU.

    We recommend you characterize the performance of the ELF score in healthy volunteers and advanced cirrhosis to better quantify the accuracy and precision of ELF.

24. We recommend you enroll patients with fibrosis of other organs for example, patients with pulmonary fibrosis but with normal liver (no NASH). This will help assess whether fibrosis in other organs might increase the ELF scores in patients who do not have NASH with fibrosis.

25. In the clinical interpretive criteria section of the LOI, you state that the cT1 cut-off will be identified from a NAFLD cohort based on a combination of data from the Oxford-Reading study and LITMUS Imaging study. It is unclear if these patients went on to develop NASH and have long term outcome events. Please provide the initial assessment of these patients and, also long-term assessments of these patients which will support the context of use.

[4] Jabor A, Kubíček Z, Fraňková S, Šenkeříková R, Franeková J. Enhanced liver fibrosis (ELF) score: Reference ranges, biological variation in healthy subjects, and analytical considerations. Clin Chim Acta. 2018 Aug;483:291-295.
[5] Lichtinghagen R, Pietsch D, Bantel H, Manns MP, Brand K, Bahr MJ. The Enhanced Liver Fibrosis (ELF) score: normal values, influence factors and proposed cut-off values. J Hepatol. 2013 Aug;59(2):236-42.

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26. You state the final composite biomarker has not been determined at this time. Explain whether the final composite biomarker will be determined after analyzing the meta-cohort group, or after analyzing both the meta-cohort and LITMUS group. If the final composite biomarker will be established after analyzing both groups, it is unclear how the final composite biomarker will be verified.

Statistical Considerations

Below are statistical comments for your consideration in preparing a statistical analysis plan (SAP) to be included in your qualification plan.

27. In the LOI, the SAP focused on the question "whether liver cT1 mapping by magnetic resonance imaging and/or the ELF blood test can act as a prognostic enrichment tool for use in clinical trials in patients with NASH." The LOI also indicated the planning of determining optimal cut-off points in identifying NAFLD/NASH patients more likely to progress in disease in the Metacohort Study for ELF score and the LITMUS imaging study for cT1. However, the SAP mentioned that you do not plan to select definitive cut-off points but intended to evaluate the prognostic performance of both markers. Please note that if the context of use of qualifying the composite biomarker is for prognostic enrichment, clinical utility on degree of enrichment by multiple cutoffs should be reported.

28. Please provide the number of patients who have both ELF test and liver cT1 MRI data by study. You should clearly specify the study or studies to be used along with specific covariates that will be included to validate the final composite biomarker while explaining how the final composite biomarker will be computed.

29. For each study, present the number of NASH patients with both liver biopsy and biomarker measurement (i.e., ELF or cT1, depending on the study) at baseline. Present the numbers by disease severity. You should have sufficient number of patients with observed data (both at baseline and after a sufficient duration of a follow-up) within each disease severity category.

    In addition, include analysis of the population which the validation will be based on (e.g., all recruited patients, patients with available biopsy and the biomarker measurements only).

30. You plan to use observational studies as validation studies. We are aware that you plan to build multivariable Cox models with a first goal of identifying potential confounding effects of other baseline variables, including sex, age, BMI, NAS score and fibrosis stage. Please clarify if all observational studies including registries involve treatment/medication in some subjects. If the answer is yes, you should include plans to handle potential confounding effects of,

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treatment/medication.

31. You proposed ELF/cT1 as enrichment biomarkers for NAFLD/NASH. To demonstrate the utility of the biomarker under this broad spectrum of disease, ensure that you have sufficient number of patients representing specific disease indication (i.e., NASH), and provide prognostic clinical utility on NASH patients separately.

    Furthermore, ensure that you have sufficient number of patients in all four categories of fibrosis stage (i.e., <F2, F2, F3, and F4) to enable the composite biomarker's ability to show evidence of prognostic clinical utility.

32. To express the prognostic performance of the respective biomarkers, you plan to construct time-dependent ROC curves. Given the presence of censoring, you plan to provide estimates of the cumulative sensitivity and dynamic specificity, at five, ten and fifteen years. Provide the algorithm for the cumulative sensitivity and for the dynamic specificity at designated years. You should also report sensitivity and specificity at multiple cutoffs.

33. In addition to imaging acquisition charter, liver cT1 related data to be submitted should include date of biopsy and date of imaging taken.

Please address each of the specific considerations and recommendations and any data requests cross-referencing the numbered list above in a separate addendum to your QP submission.

When evaluating biomarkers prospectively in clinical trials, sponsors are encouraged to submit study data using Clinical Data Interchange Consortium (CDISC) standards to facilitate review and utilization of data. Data sharing and the capability to integrate data across trials can enhance biomarker development and utilization. If sponsors intend to include analyses of these biomarkers to support regulatory decision making for a specific Investigational New Drug (IND) development program, they should prospectively discuss the approach with the appropriate CDER division. Any groups (academia, industry, government) that would like to join in this effort or have information or data that may be useful can contact Dr. Elisabeth Erhardtsen (eer@nordicbio.com), the primary point of contact for this project.

Should you have any questions or if you would like a teleconference to clarify the content of this letter, please contact the CDER Biomarker Qualification Program via email at CDER-BiomarkerQualificationProgram@fda.hhs.gov with reference to DDTBMQ000106 in the subject line. For additional information and guidance on the BQP please see the program's web pages at the link below.[6]

[6] https://www.fda.gov/drugs/drug-development-tool-ddt-qualification-programs/cder-biomarker-qualification-program

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Sincerely,

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