LOI DETERMINATION LETTER

DDTBMQ000095

May 7, 2020

Elisabeth Erhardtsen, DVM,
Senior Regulatory Director
Nordic Bioscience A/S
Herlev Hovedgade 205-207, 2730
Herlev, Denmark

Dear Dr. Erhardtsen:

We are issuing this Letter of Intent (LOI) Determination Letter regarding 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 LOI submission deemed reviewable on December 18, 2020 and have concluded to **Accept** it into the CDER BQP.[1] We support and encourage the study of biomarkers for Non-Alcoholic Steatohepatitis (NASH).

You have proposed a composite biomarker consisting of 2 biomarkers, PRO-C3 and the FAST score, as a diagnostic screening biomarker among individuals with non-alcoholic fatty liver disease (NAFLD) to identify those at high risk of having liver fibrosis stages 2 or 3. 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.

Based on our review of the LOI, we agree there is an unmet need, and the development of this composite biomarker for diagnostic screening of NAFLD patients before liver biopsy, may be helpful to reduce the number of patients requiring liver biopsy as part of enrollment in NASH clinical drug development trials.

When you are prepared to make a submission to the next stage in the 507 DDT qualification process, please prepare a Qualification Plan (QP) submission that addresses the scientific issues and the recommendations outlined below. A QP contains details of the analytical and software validation of the biomarker measurement method, detailed summaries of existing data that will support the biomarker and its context of use (COU), and descriptions of knowledge gaps and how you propose they will be mitigated. If future studies are planned, please include detailed study protocols and the statistical analysis plan for each study as part of your QP submission. We have provided initial comments based on your LOI and hope these comments may be useful as you proceed with the preparation of your initial QP submission.

When evaluating biomarkers prospectively in clinical trials, sponsors are encouraged to submit study data

[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.

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.

To better understand the benefits of the identified biomarker as a DDT, and to continue to refine the COU, please address the recommendations below in your future QP submission. We acknowledge that some of the responses to questions and comments below may already be included in your publications or other publicly available resources, (such as the Epitope Registry or at www.epitopes.net). However, for completeness, we recommend that they be adequately summarized in the QP.

Biomarker Considerations

Requestor's Biomarker Description: Composite biomarker which will include PRO-C3, the FAST score (Liver Stiffness Measurement (LSM) and Controlled Attenuation Parameter (CAP) measured by Fibroscan device, and AST). PRO-C3 is a propeptide fragment that measures formation of type III collagen. The FAST score combines a liver stiffness measurement, Controlled Attenuation Parameter, and circulating AST measurement from venous blood. The final composite marker is not yet determined.

1. 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.

2. 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.

Context of Use (COU) Considerations

Requestor's COU: Diagnostic screening biomarker, which, among individuals with non-alcoholic fatty liver disease (NAFLD) identifies those at high risk of having liver fibrosis stages 2 or 3 (based on the non-alcoholic steatohepatitis clinical research network (NASH-CRN) scoring system), and which

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could subsequently be included in NAFLD clinical trials for drug development after further confirmation of their diagnosis with liver biopsy.

FDA Recommended COU: *Diagnostic enrichment biomarker intended for use, in conjunction with clinical factors, to identify patients likely to have liver biopsy histopathologic findings of nonalcoholic steatohepatitis (NASH) and with a nonalcoholic fatty liver disease activity score (NAS) ≥4 and liver fibrosis stages 2 or 3 (by Brunt/Kleiner scale); and thus appropriate for inclusion in liver biopsy-based NASH drug development clinical trials focused on pre-cirrhotic stages of NASH.*

We have the following comments about COU considerations:

3. Your proposed context of use identifies patients to be enrolled in NAFLD clinical trials. Because this biomarker and the context of use is to identify patients who are more likely to develop NASH, FDA's recommended COU more clearly identifies the patient population who can be enrolled into NASH drug trials.

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

Analytical Considerations

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

5. You state your intention was to collect 130 serum samples for a reference range study. Approximately 50% of the samples were from males and 50% from females. The samples were collected, frozen, and stored at -70°C from May 2016 to January 2017. The samples were then shipped to Nordic Bioscience on dry ice. At Nordic Bioscience, the samples were stored at -80°C until they were thawed, aliquoted, and stored at -80°C for testing. We have the following comments concerning the reference range study:

    a. It is not clear where the samples were collected or how the population(s) from which the samples were collected are representative of the intended study population in the United States. Please provide information demonstrating that the samples that were collected are representative of the US intended study population.
    b. Samples were stored at -70°C from May 2016 to January 2017 and then stored at -80°C for an unspecified length of time before thawing, aliquoting with re-storage at -80°C for an unspecified length of time. Your sample stability testing includes storage at -80°C for up to 12 months, and sample stability up to three freeze/thaw cycles. The sample stability validations studies do not appear to reflect how the samples were handled, storage

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duration, or and stored in the reference range study. Please ensure that the samples used in the studies were stored and handled under appropriate conditions that would not affect the performance of the PRO-C3 test.

6. It is unclear whether two separate patient samples were collected for analysis of PRO-C3 and AST, respectively. Please state if different collection methods were used to analyze these two biomarkers from each patient, and as result two individual samples were collected from the same patient. If only one patient's sample was used to analyze both PRO-C3 and AST, please provide testing to show that the sample as collected and stored was stable for both PRO-C3 and AST.

7. You state that the data for the Meta-cohort group was collected. Please provide the protocols for data collection for the LSM and CAP measures for this group. Please also explain how the data collection for these measures was consistent or the method of collection did not impact the analysis for this group.

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

8. You indicate that the LITMUS project is anticipated to take place over five years. Please provide a plan on how you will ensure that the performance of your assay will be consistent between lots of the PRO-C3 assay you plan to use. You should develop strategies to understand the differences in lot-to-lot performance. The composite biomarker may be difficult to develop if there are performance differences between PRO-C3 assay lots.

9. You plan to perform a high dose hook effect study by spiking synthetic PRO-C3 peptide into a native serum sample up to a concentration of approximately 10000 ng/mL. You should ensure that samples spiked with synthetic PRO-C3 mimic native samples (e.g., that the performance of the PRO-C3 test with samples spiked with synthetic PRO-C3, is not significantly different from the performance with native samples). Alternatively, you could perform your studies with native samples.

10. 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 PRO-C3 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

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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.

11. AST will be measured and be included in the FAST Score. You have not provided how AST will be measured. Please provide the assays that will be used to measure AST and the performance characteristics of these assays. If the assays in the study are FDA cleared devices, please provide the 510K clearance number. Please also consider that performance of the AST assays may have an effect on the overall performance of the composite biomarker.

12. There are significant differences between your proposed use of the Fibroscan® device and what it has received in the 510K clearances. In your QP, please provide your analytical validation data for the device based on your proposed COU. The analytical validation previously provided for the 510k submissions may be applicable, but please provide an explanation on how these data support your context of use. Additional analytical validation testing may be needed based on your proposed context of use and needed performance to support your context of use.

Confirmation of Transparency of Analytics Technical Parameters

13. 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

14. In your QP, please ensure your clinical protocols and proposed studies will support your proposed context of use. 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.

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15. Please clarify how you intend to develop the composite biomarker: for example, within your proposed COU, do you intend to develop them for sequential use (with FAST only being obtained with a "positive" PRO-C3) or concurrent use? If the final application is to use sequential testing (PRO-C3 as a "gatekeeper" to determine need for FAST) then labeling for FAST would be limited for use as a biomarker only contingent upon a "positive" PRO-C3. Ultimately the labeling for your proposed biomarker(s) would depend on the studies that were conducted

16. Please clarify if the liver biopsy interpretation used as the reference will be the report obtained in the setting of clinical care or whether the biopsies will be reviewed by expert hepatopathologist(s).

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

17. You state that there should be less than a 6 month-time interval between the LSM and CAP assessment and when the AST is assessed. It is unclear when the PRO-C3 will be assessed over this time interval. Please state when samples will be collected and analyzed for PRO-C3. Also provide a mechanistic rationale or justification as to why the 6-month interval is optimal for a timing interval for assessment of the PRO-C3, the serum AST assessment, and the LSM and CAP(Fibroscan®) assessments.

18. Given the potential for variability in inflammatory activity over time in NASH, a longer median time interval between liver biopsy, Fibroscan® and AST assessments in the meta-cohort may affect the interpretation of the data.
    a. Please clarify the median time interval and IQR between liver biopsy and biomarker assessments.
    b. There is a potential concern that up to a 6-month time interval between Fibroscan® (LSM/CAP) and phlebotomy from which AST is assessed may lead to variability in results (see above) especially if a significant proportion of these results will have a time window that approaches 6 months. Of note, the derivation and validation cohorts of the FAST score from the publication by Newsome et al. tended to have a very narrow median window, except for the Turkish (and to a lesser extent the US) validation cohort which did allow for a wider time interval between Fibroscan® and AST. The AUROC for the FAST score in the Turkish cohort at 0.74 was qualitatively less than for the other cohorts. However, whether this was due to such a large time window or is related to other factors remains unclear.

19. 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 LSM and CAP were measured. 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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20. Please clarify the use of the FIB4 index in your hypothesis testing section of the SAP. FIB4 was developed to produce a 3-category result (rule in cirrhosis, rule out cirrhosis and indeterminate result). The traditional cutoff of >3.25 for a "rule in" result for FIB4 was developed to identify more advanced fibrosis (primarily F4, advanced F3) in hepatitis C patients. The NASH CRN evaluated FIB4 in NAFLD to identify patients with advanced fibrosis (F3/F4) and in this cohort determined that a FIB4 index of ≥ 2.67 had an 80% positive predictive value while a FIB4 index ≤ 1.30 had a 90% negative predictive value for advanced fibrosis. Although this was an application of a biomarker for NAFLD/NASH, the COU was still able to identify F3/F4 (advanced fibrosis and cirrhosis); and not for identifying individuals with moderate hepatic fibrosis (F2/F3).[2] Explain, therefore, how the FIB4 performance characteristics will be compared with the FAST and PRO-C3 results in your hypothesis testing.

Gaps and Proposed Studies

21. 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

22. You state that not all patients in the Meta-cohort group have been evaluated by the Fibroscan device. Please provide the number of patients in this cohort who have both PRO-C3 and Fibroscan data. Please explain how the final composite biomarker will be computed with more PRO-C3 data than Fibroscan data.

23. Please clarify if 300 or 100 patients will be used to determine cut-offs – per your briefing package (SAP section, page 96 of LOI) it appears that there will be 300 patients to determine the cutoffs for PRO-C3 and 300 for FAST in the Meta-cohort study. Given the uncertainties in response immediately above we encourage greater number of patients to be included in these considerations. In addition, please clarify the number of patients that will be used to perform validation testing from the LITMUS study (100 enrolled in the Prospective Biopsy study or all 600 patients).

24. For the statistical testing in the LITMUS study, you plan to compare relative sensitivity and

[2] 2. Shah AG, Lydecker A, Murray K et al. Comparison of Noninvasive Markers of Fibrosis in Patients with Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol* 2009;10: 1104-12.

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specificity of PRO-C3 and FAST versus FIB-4. To show that proposed markers outperform FIB-4, it is not sufficient to show superiority in only one of the two accuracy characteristics. You should either demonstrate superiority on both, sensitivity and specificity, or you could show superiority on one (e.g. sensitivity) and non-inferiority on the other (e.g. specificity).

25. To ensure diagnostic accuracy of PRO-C3 and FAST, you should propose adequate success criteria by specifying targeted accuracy levels for each of the summary measures (i.e. sensitivity, specificity, false positive rate etc.). The success could be evaluated, for example, by utilizing respective confidence bounds for each measure in the LITMUS study.

Other:

Please note that section 507 of the FD&C Act includes transparency provisions that apply to your submissions. Certain information contained within your submissions may be made publicly available on the Internet, as required by section 507. For examples of transparency and prior submissions see the Biomarker Qualification Submissions webpage.[3]

If you have questions, please contact the CDER Biomarker Qualification Program (CDER-BiomarkerQualificationProgram@fda.hhs.gov) via email. We look forward to working with you on this beneficial project.

Sincerely,

Christopher L. Leptak -S

Digitally signed by Christopher L. Leptak -S
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[3] https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificationProgram/ucm535881.htm

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