**LETTER OF INTENT**
**DETERMINATION LETTER**
**DDTBMQ000113**

May 14, 2021

Innovative Medicines Initiative (IMI) TransBioLine
Attention: Lidia D. Mostovy
One Health Plaza
Novartis Pharmaceuticals Corporation
East Hanover, New Jersey 07936

Dear Dr. Mostovy:

We are issuing this letter to Innovative Medicines Initiative (IMI) TransBioLine to notify you of our determination on the project submitted to the Center for Drug Evaluation and Research (CDER) Biomarker Qualification Program (BQP). We have completed our review of the Letter of Intent (LOI) deemed reviewable on February 16, 2021 and have determined to accept the LOI into the CDER[1] Biomarker Qualification Program

Your LOI submission proposes qualification for: a single biomarker or a composite panel of safety biomarkers, proposed for identifying patients with potential acute liver injury caused by drugs in whom dose reduction or dose interruption is warranted.

Your next submission, a Qualification Plan (QP), contains details of the analytical validation plan for the biomarker measurement method, detailed summaries of existing data that will support the biomarker and its context of use (COU), and includes 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.

Below, we provide you with specific considerations and recommendations to help improve your preparation for, and submission of the QP. As this biomarker development effort is refined, the submitted data, the specifics of your context of use (including the target patient population), and the design of study(ies) used in the clinical validation of the biomarker will ultimately determine which of these considerations and recommendations are most

[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.

applicable. For more information about your next submission and a QP Content Element outline, please see the BQP Resources for Biomarker Requestors web page.[2]

## CONSIDERATIONS & RECOMMENDATIONS

### 1. Drug Development Need

*Requestor's Drug Development Need Statements:*

There is a strong need for biomarkers that could be used in drug development to identify and stratify patients who progress to develop acute liver failure or develop chronicity in the longer term.

Biomarkers that distinguish adaptation, and therefore recovery, from progression and therefore serious liver injury, in DILI will transform monitoring in clinical trials and strengthen regulatory approval of novel molecular entities.

Concordance between standard toxicological studies performed today and idiosyncratic DILI in humans is poor. Tools to distinguish adaptation from potential DILI are lacking. The standard panel of liver laboratory tests performed today lack sensitivity and specificity and do not predict the clinical course of a patient in whom DILI is suspected.

FDA agrees that additional novel biomarkers to aid in assessment of DILI would be beneficial.

### 2. Biomarker Name & Description

A single biomarker or a composite panel of safety biomarkers

| Marker | Origin |
|---|---|
| High mobility group box 1 (HMGB1) | Detectable in almost all tissues |
| Cytokeratin 18 full-length (K18) | Epithelial cells |
| Cytokeratin 18 Caspase- cleaved fragment (cc-K18) | Epithelial cells |
| Glutamate dehydrogenase (GLDH) | Mitochondrial matrix; primarily in the centrilobular region of the liver; lower levels in the kidney and brain |
| Osteopontin (OPN) | Multiple tissue and cell types including liver |

[2] https://www.fda.gov/drugs/cder-biomarker-qualification-program/resources-biomarker-requestors

| | |
|---|---|
| Macrophage colony Stimulating factor receptor 1 (MCSF1R) | Cytokine receptor on macrophages/monocytes |
| Bile acids | Synthesized by the liver |
| Sphingolipids | Abundant in the liver |

Note that you did not indicate methods for identification or quantification of the specific miRNAs of interest in this submission. If miRNA will be part of the final biomarker or composite panel, additional detail will be needed in subsequent qualification stages to validate the analytic approach and the clinical utility of the miRNA biomarker(s).

## 3.COU Considerations

*Requestor's COU Statement:*

A single biomarker or a composite panel of biomarkers will aid in identifying patients with potential acute liver injury caused by drugs, in whom dose reduction or dose interruption is warranted. Acute liver injury is suspected based on elevations of alanine transaminase (ALT), aspartate transaminase (AST) or alkaline phosphatase (ALP).

*FDA COU Recommendation: A single safety biomarker or a composite panel of biomarkers that aids in identifying clinical trial subjects with potential acute liver injury caused by drugs, in whom dose reduction or dose interruption is warranted. Identification of potential acute liver injury is based on elevations of alanine transaminase (ALT), aspartate transaminase (AST), or alkaline phosphatase (ALP).*

The FDA recommends this change as it more explicitly states the scope of use (subjects in a clinical trial) and the action to be taken (dose alteration). It may be prudent to change the second sentence of the COU to include specific levels at which elevations of liver enzymes would trigger use of the biomarker, if the data supporting such a change becomes available during the qualification process (e.g., 3-5x ULN or > 3x baseline for transaminases and >2x ULN for ALP).

## 4. Analytical Considerations

4.1 The biomarker(s) that you proposed in your letter of intent (LOI) is/are still in the early phase of development, therefore, it is too early to provide more detailed feedback on the design of specific analytical validation studies needed to support the proposed context of use (COU). The design of analytical studies to demonstrate that the proposed biomarker(s) can be used as stated in the COU will depend on multiple factors including the following: the type of biomarker (e.g., composite panel or individual biomarkers); how the result will be interpreted (e.g., looking for a change from baseline, using medical

decision points or cut-offs); the sample types; the pre-analytical methods used; the patient population; the measuring range; whether the test(s) are qualitative or quantitative. Please see some general considerations below as you develop your biomarker assay(s).

4.2 You provided analytical considerations in section 6 of your letter of intent (LOI) and supporting information as follows: 1) validation testing conducted for Keratin 18 and caspase-cleaved Keratin 18 immunoassay kits for use in human plasma; and, 2) the plan for the validation for immuno-LC-MS/MS based assays for the quantification of Macrophage Colony Stimulating Factor 1 Receptor (MCSF1R), High mobility group protein B1 (HMGB1), Osteopontin (OPN), and Glutamate Dehydrogenase 1 (GLDH) concentration in human plasma. In support of the validation of Keratin 18 and caspase-cleaved Keratin 18 immunoassay kits, you provided results from testing limits of quantitation, accuracy and precision, parallelism, reproducibility, and analyte stability. However, the testing and results provided for the Keratin 18 assays and proposed for the LC-MS/MS assays are not adequate, difficult to interpret, and likely not robust enough to support the analytical reliability of these assays for the proposed COU. Please refer to the table below for the additional analytical validation studies that should be performed. We highly recommend that you perform your analytical validation studies following the recommended methods, study designs, and applicable data analyses described in Clinical and Laboratory Standards Institute (CLSI) guidelines such as those listed in the table below. Please also note that parameters such as traceability, drift, and carryover are important and depend on the technology used to measure the biomarker(s) and should also be considered when validating a test. Finally, for a combination of the individual biomarker measurements into an algorithm, additional analytical performance studies are required based on the composite scores of all the biomarkers measured. We suggest that you refer to the “Class II Special Controls Guidance Document: Ovarian Adnexal Mass Assessment Score Test System” (available at https://www.fda.gov/media/80370/download) for a discussion of recommendations applicable to tests that measure separately one or more proteins obtained from patient specimens.

| Performance characteristic | CLSI guideline |
|---|---|
| Method verification specific to mass spectrometry technology | C62 “Liquid Chromatography-Mass Spectrometry Methods”-First Edition |
| Precision (including but not limited to repeatability, within laboratory, between lots, between instruments, between operators, between sites reproducibility) | EP05-A3 “Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline”-Third Edition |
| Linearity of the analytical measuring range | EP06-A “Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach, Approved Guideline” |

| | |
|---|---|
| Detection capability (Limit of Blank, Limit of Detection, Limit of Quantitation) | EP17-A2 “Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline”-Second Edition |
| Interference[3] (endogenous and exogenous substances) | EP07 “Interference Testing in Clinical Chemistry”, and EP37 “Supplemental Tables for Interference Testing in Clinical Chemistry” |
| Reference intervals | EP28-A3c “Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory”- 3rd Edition |

4.3 The supporting information document (Section 3.5, page 20/173 for the Keratin 18 and caspase-cleaved Keratin 18 immunoassay, and 72/173 for immuno-LC-MSMS assays), you state pre-specified acceptance criteria for the accuracy and precision studies of ±20% .These acceptance criteria are not sufficiently stringent, as such high imprecision could have an impact on the usefulness of these assay results for clinical decisions. We typically recommend a total within-laboratory %CV of ≤10% for devices that employ technology similar to these assays. We also recommend that you pre-define acceptance criteria for each analytical validation study in the context of the cumulative effect that different sources of error, including bias or systematic differences as well as imprecision, have on test performance. Moreover, you should define acceptance criteria for each parameter such that your total analytical error does not preclude the determination of clinically meaningful differences in the biomarker(s).

4.4 You did not indicate methods for identification or quantification of the specific miRNAs of interest in this submission. If miRNA will be part of the final biomarker or composite panel, additional details need to be provided in subsequent qualification stages to validate the analytic approach and the clinical utility of the miRNA biomarker(s).

4.5 We suggest that, in your future Qualification Plan (QP) submission, you provide a clear description of the protocols that include the following: the method(s) and instrument(s) used, the specimen (e.g., plasma, native, contrived: diluted, spiked, pooled), quality control material, the specific concentrations of each target biomarker, 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 and also refer to item 4.2 when designing your validation studies.

[3] Because immunoassays may employ biotinylated detection antibodies and biotin intake levels higher than the recommended daily allowance may cause interference with these tests (https://www.fda.gov/MedicalDevices/Safety/AlertsandNotices/ucm586505.htm), we recommend testing biotin interference of up to 3500 ng/ml for streptavidin-biotin-based assays.

All studies should be conducted using stable samples (i.e., stored and handled using validated conditions compared to a fresh sample). In addition, the sample type and matrix type should reflect the clinical samples that will ultimate be used and native patient samples should be used whenever possible (and especially around important medical decision levels or cut-offs).

## 5. Clinical Considerations

We have the following comments about your algorithm shown in Figure 7-1 for consideration during qualification plan development.

5.1 We do not think that current biomarker data is robust enough to safely allow AST or ALT to rise to >20 xULN before stopping drug as long as your biomarker panel "does not exceed DILI threshold." Upper limits of normal for ALT in the US vary widely. For men, it can range from 35 to 79 U/L which means 20 xULN would be from 700 to 1580 U/L (Neuschwander-Tetri BA, et al., Arch Int Med 2013). That is too high to continue drug based on a novel biomarker.

5.2 You do not specify an upper limit for ALT or AST to enter your algorithm. Occasionally DILI presents with abrupt ALT or AST rise. It is not clear whether a patient with an ALT much higher than 5 xULN (e.g. 10-20 xULN) should be allowed to enter the algorithm. You should consider a ceiling of ALT or AST, depending on data you provide to support your proposal.

5.3 We believe most of the biomarker literature pertains to hepatocellular injury. Please justify your plan to use this panel in patients who have predominantly cholestatic liver injury as suggested by the ALP > 2x ULN criterion.

5.4 You will need to specify the turnaround time on your biomarker panel. ALT and AST are typically done within 24 hours, so investigators will be ready to make decisions on drug stop or dose reduction quickly.

5.5 We suggest you exclude patients with cirrhosis from this algorithm.

5.6 Please specify if biomarker levels will be determined at baseline or monitored periodically especially in trials where patients have abnormal baseline liver enzymes and/or bilirubin due to chronic liver disease (e.g. non-alcoholic fatty liver disease). Such data may inform how your biomarkers could help manage potential DILI in these patients for whom Hy's Law may not apply.

5.7 We note that you plan to recommend dose reduction or dose interruption in accordance with protocol safety risk mitigation plan if biomarker panel exceeds DILI threshold. Please clarify your specific dose reduction strategy (e.g., fold reduction) and the rationale that supports the planned dose reduction strategy (e.g., dose-response relationship from the DILI safety analysis).

## 6. Statistical Considerations

6.1 As part of your Qualification Plan, please include a Statistical Analysis Plan (SAP) that describes the statistical methods you intend to use to support qualification of candidate biomarkers. Our preliminary statistical comments can be found below. We may have additional comments on your planned approach at the time of submission of the SAP.

6.2 You plan to conduct (i) TransBioLine Pro-Euro-DILI Registry of prospective drug-induced liver injury cases and (ii) case-control study evaluating biomarkers and genetic factors associated with the development of non-alcoholic steatohepatitis (NASH) and alcoholic steatohepatitis (ASH) for clinical validation of the biomarker. In the supporting document, you noted that confirmatory data analysis will evaluate evidence using traditional statistical tools such as significance, inference, and confidence. Provide the specifics on the hypotheses including the parameters to be tested, the test level, what inferences are to be made based on the hypotheses along with the success criteria in the SAP for regulatory feedback.

6.3 You proposed a receiver operator characteristic (ROC) curve analysis to determine each of the six candidate biomarkers as well as their combinations for detection of DILI patients. However, in obtaining sensitivity and specificity from ROC curve analysis, specific DILI threshold criteria for those biomarkers need pre-specification to distinguish between DILI and non-DILI status for each patient. We remind that only after promising biomarkers are selected and thresholds are proposed, then should a clinical validation study be performed on an independent data set.

6.4 You indicated that biomarkers will be considered predictive of DILI if both the ROC area under the curve (AUC) and the lower end of the 95% confidence interval (CI) are >0.5. It is not clear if this criterion is sufficient (e.g. level of 0.5 essentially corresponds to making decision by random selection). See our comment 6.2.

6.5 You also planned to compare the reference ranges with the dynamic ranges in Table 4-2 using appropriate parametric or non-parametric tests depending on the biomarker distributions. Specify how the comparison procedure works based on those ranges and the purpose of it.

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, requesters 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 plan to use

the biomarker prior to qualification to support regulatory review for a specific Investigational New Drug (IND), New Drug Application (NDA) or Abbreviated New Drug Application (ANDA) development program, they should prospectively discuss the approach with the appropriate CDER or CBER division.

The BQP encourages collaboration and consolidation of resources to aid biomarker qualification efforts. Any individuals or groups (academia, industry, government) that would like to join in this effort, have information or data that may be useful can contact Dr. Mostovy.

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 DDT BMQ#000113 in the subject line. For additional information and guidance on the BQP please see the program's web pages at the link below.[4]

Sincerely,

Christopher L. Leptak -S

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Christopher Leptak, MD, PhD
Director, CDER Biomarker Qualification Program
Office of New Drugs
Center for Drug Evaluation and Research

Joseph G. Toerner -S

Digitally signed by Joseph G. Toerner -S
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Date: 2021.05.14 09:28:08 -04'00'

Joseph Toerner, M.D.
Acting Division Director
Division of Hepatology and Nutrition
Office of Inflammation and Immunity/Office of New Drugs
Center for Drug Evaluation and Research

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