LETTER OF INTENT
DETERMINATION LETTER
DDTBMQ000109

Stemina Biomarker Discovery, Inc.
Attention: Elizabeth Donley
Stemina Biomarker Discovery, Inc.
504 S Rosa Rd., Suite 150
Madison, WI 53791

Dear Elizabeth Donley:

We are issuing this letter to Stemina Biomarker Discovery, Inc., 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), submitted under section 507 of the Federal Food, Drug, and Cosmetic Act. We have completed our review of your Letter of Intent (LOI) deemed reviewable on May 4, 2021 and have concluded to **Accept** it into the CDER BQP[1].

Based on our review of the LOI, we agree there is an unmet need and support the development of this safety biomarker, as a component of a weight-of-evidence assessment, for detecting the potential for human developmental toxicity *in vitro* using human pluripotent stem cells at the nonclinical stage of drug development for small molecule drugs.

In your next submission, a Qualification Plan (QP), you will describe the detailed approaches involved in calculation of the biomarker and threshold, describe the analytical validation plan for the biomarker measurement method, provide detailed summaries of existing data that will support the validation of the biomarker threshold and its context of use (COU), and include descriptions of knowledge gaps and how you propose they will be mitigated. Data sufficient for our reviewers to independently determine that your assay has utility for the proposed context of use should also be provided. Please include detailed study protocols and the statistical analysis plan for each future planned study as part of your QP submission. The QP should outline the data to be submitted in the Full Qualification Package.

Below, we provide you with specific considerations and recommendations to help your preparation and submission of the QP. As this biomarker development effort is

[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.

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 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. Biomarker description:
Measurement of the metabolite ratio of ornithine to cystine.

FDA agrees with your biomarker description.

2. COU Considerations

Requestor's COU Statement:
The ratio of ornithine to cystine may be used as a safety biomarker for detecting human developmental toxicity potential *in vitro* using human pluripotent stem cells at the nonclinical stage of drug development for small molecule drugs candidates expected to be embryo-fetal toxicants as described in Annex 2 of the ICH S5(R3) guideline.

FDA COU Recommendation: The ratio of ornithine to cystine may be used as a safety biomarker for detecting human developmental toxicity potential *in vitro* using human pluripotent stem cells at the nonclinical stage of drug development for small molecule drug ~~candidates expected to be embryo-fetal toxicants~~ as part of a weight-of-evidence assessment as described in ~~Annex 2 of~~ the ICH S5(R3) guideline.

2.1 Remove the use of the word "candidate" from the COU (FDA recommended COU is outlined above) as it is not necessary and could give the impression that this biomarker is for the screening stage of small molecule drug development. FDA does not regulate drug screening.

2.2 Acceptance of the LOI is contingent on the removal of the words "expected to be embryo fetal toxicants" and "Annex 2". Any use case for an "alternative assay" for embryofetal developmental toxicity, as described in ICH S5(R3) includes:

- An evaluation of the biological plausibility of the model including a description of embryo-fetal development (e.g. cell migration, differentiation, vasculogenesis, neurulation, gastrulation) and subsequent development adverse effects studied with the model. In addition, any limitations of each of

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

the individual assays should be discussed. The description should include a discussion and supporting data to show that the duration and timing of exposure supports the prediction of MEFL *in vivo.*

We have concluded that there is no plausible means by which your assay, and the resultant biomarker that it generates, can meet this criterion for a qualified alternative assay. It may have potential utility, however, as an additional datapoint in a weight-of-evidence assessment of embryofetal developmental toxicity.

The biomarker should be used as part of a weight of evidence since at this stage the biological plausibility is not expected to be sufficient to qualify the biomarker as a stand-alone drug development tool to predict MEFL *in vivo*.

2.3 Explain in detail the methods that will be used to determine the applicability domain of your proposed biomarker with respect to what drug and drug classes it would apply to and those which it would not. In addition, please explain in detail the outcomes of these methods, outlining the appropriate applicability domain of your assay.

3. Analytical Considerations

3.1 The QP should include a description of what measures are taken to characterize the test articles used in the qualification studies. For example, how compound identity and purity was confirmed. Explain how impurities and other factors such as binding affinity are taken into consideration.

3.2 Demonstrate that the stem cell models you have chosen are unique or are superior to other cell model types.

3.3 Demonstrate that the assay is reliable with respect to the different iPS cell lines that you are using. Describe the metrics used to demonstrate that the iPS cells can yield consistent results regardless of source and what standards will be in place to ensure quality and reproducibility of the model.

3.4 Provide detailed information on the stability of the compounds throughout the analysis process.

3.5 Provide additional assay and validation information by expanding the types of compounds to be used. Refer to the ICH S5(R3) guidance annex 2 for the specific compounds.

4. Pharm/Tox Considerations

4.1 Explain how the use of your proposed biomarker compares to the current standard of the combined predictability of two animal studies. Currently the standard is to provide

animal testing in two species, one rodent and one nonrodent. The results of the two animal models are analyzed together to assess the effects on embryo-fetal development (EFD). Explain how your proposed biomarker would compare and how it would provide an unequivocal and/or more robust model for detecting developmental toxicity.

4.2 Explain the rationale for the use of 10x Cmax vs 25x Cmax. Refer to the FDA embryofetal developmental integration guidance to help put signals into context. Provide detailed information on how the in vitro concentrations and in vivo Cmax are determined. How are factors that can impact these determinations addressed (e.g., non-specific binding in vitro, protein binding in vivo)?

4.3 Explain how the o/c ratio is relevant to developmental toxicity and how it relates to molecular or cellular events that result in adverse apical outcomes.

4.4 A robust IVIVE analysis plan needs to be developed for the proposed biomarker. This should be broken down to the level of pharmacological class and how each will be evaluated. A rationale and plan for how the results of this assay will be utilized should be included.

4.5 Risk mitigation strategies should be applied including additional analyses to address the limitations included by the submitter in the original LOI. Explain what limitations are present and how this would define the scope of the use of your proposed biomarker.

5. Statistical Considerations

In your statistical analysis plan (SAP), to be submitted at the QP stage, address the concerns raised below. We may have additional comments/requests pending the SAP submission.

5.1 The proposed biomarker was not quantitatively validated, the results in Tables 1 and 2 were not statistically analyzed and cannot be used for validation.

5.2 The determination of threshold shown in Figure 1 also lacks detailed quantitative evaluation and validation.

5.3 The proposed analytical method did not consider any variabilities associated with the procedure, including the impact of different types of stem cells, the measurement errors, variabilities from different labs and inter-lab variabilities, etc. Normalized data with reference is generally used for comparison to test. It may reduce but cannot eliminate variabilities. These variabilities may substantially affect the accuracy and precision of the method.

5.4 The current proposal of the validation study lacks details in the experimental design, such as the optimal numbers of repeated measures, the choice of both positive and negative control compounds and their concentrations, etc.

5.5 The planned validation study should include an SOP that details how you will ensure that the blinding of the compounds will be maintained for valid prediction.

5.6 The SAP should include the derivation of threshold for o/c biomarker with validation, in vivo outcomes and devTOX^{qp} prediction.

6. General Considerations

6.1 Clinical Considerations

In the section *Clinical Considerations under subsection 2) Benefits and risks of applying the biomarker in drug development*, you have included uses that are not consistent with your current context of use. In the QP, you should discuss uses for your biomarker that are consistent with your proposed context of use. The information in your proposed decision tree should also be revised to reflect the amended COU.

6.2 Supporting Information

In the section *Supporting Information in subsection 4) Description of alternative comparator, current standards(s), or approaches*, the information listed does not appropriately reflect the fact that the current method of comparison compares the predictive nature of the two animal models combined not each animal model individually. Please revise the language noted here as well as the additional locations it is discussed throughout the LOI.

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 Elizabeth Donley (email: BDonley@stemina.com). 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 DDTBMQ000109 in the subject line. For additional information and guidance on the BQP please see the website https://www.fda.gov/drugs/drug-development-tool-ddt-qualification-programs/biomarker-qualification-program.

Sincerely,

Jeffrey N. Siegel -S

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Jeffrey Siegel, M.D.,
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Karen Davis-Bruno, Ph.D.
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