LETTER OF INTENT
DETERMINATION LETTER
DDTIST00006

July 1, 2022

Attention: Benjamin Doranz
Integral Molecular, Inc.
www.integralmolecular.com

Dear Dr. Doranz:

We have completed our review of the Letter of Intent (LOI) for Drug Development Tool DDTIST00006 received on April 28, 2021 by the Innovative Science and Technology Approaches for New Drugs (ISTAND) Pilot Program submitted under section 507 of the Federal Food, Drug, and Cosmetic Act.

> **Submission Title:** Specificity Screening of Biotherapeutics for Improved Safety Profiling in IND Applications Using the Membrane Proteome Array (MPA)

FDA has completed its review and has agreed to accept your LOI into the ISTAND Pilot Program.

Your next submission, a Qualification Plan (QP), should contain details of the analytical validation plan for the measurement method, detailed summaries of existing data that will support the proposed tool and its context of use (COU), and include descriptions of knowledge gaps and how you intend to fill those gaps. If future studies are planned, please include detailed study protocols and the statistical analysis plan (SAP) for each study as part of your QP submission. In preparing your next submission, please address the scientific issues and the recommendations outlined below.

1. Context of Use (COU)
    a. In your submission, you state that this tool has been optimized for all major biotherapeutic modalities to date, including IgG, IgM, scFv, VHH nanobodies, bispecifics, peptides, RNA aptamers, and CAR T cells. This is a very broad COU, and we suggest that you consider prioritizing and limiting the modalities in your initial COU to those for which data can be submitted to support their inclusion in your proposed COU. Please see the analytical considerations below.
    b. You state that "The MPA has now been used to test the specificity of hundreds of biotherapeutics from different biotech and pharmaceutical companies as a commercial research service, and about 50% of recently surveyed customers have indicated that they anticipate filing an IND using data from the MPA screen." Provide a summary of these programs by showing the range of biotherapeutic modalities and

the intention to submit under an IND.

c. Specify a COU statement specific to each modality that the QP will address. Include all known and anticipated limitations of use, including but not limited to: investigational therapeutic size, weight, conformation, subcellular or body compartment localization (i.e., volume of distribution); subcellular or body compartment localization of intended target; lack of soluble protein targets included in the MPA.
d. Clarify what types of CAR T cells would be applicable to your MPA technology. Please include in your clarification any limitations regarding the antigen-binding domain (e.g., scFv vs. other types of binding domains), number of unique antigen-binding domains (e.g., dual- targeted CAR T cells), impact of hinge/spacer length, and types of signaling and costimulatory domains.
e. Clarify whether data obtained with the MPA are intended to be used in conjunction with or to replace data obtained using alternative technologies (e.g., Tissue Cross-Reactivity using immunohistochemistry techniques, CAR T cell cytotoxicity assessment on a panel of human tissues, CAR T cell in vivo animal studies, etc.) for all proposed biotherapeutic modalities. If your MPA technology is intended to replace current assays used to assess specificity, please provide data to demonstrate equivalency or superiority.
f. Discuss how off-target binding data would be presented and interpreted in reports generated for regulatory submissions.

2. Technical Description
    a. You state that your MPA library covers 94% of the human membrane proteome. Please specify the membrane proteins that comprise the missing 6% of membrane proteins, if known. Are any functional classes or protein families disproportionately missing?
    b. Describe how your MPA cDNA library captures human genetic diversity and expression. How do the 625 different protein isoforms contribute to human gene diversity and expression within your cDNA library?
    c. Describe any limitations for membrane protein expression using your MPA platform. Are there any inherent limitations to expressing specific functional classes or protein families?
    d. Address whether your MPA library includes proteins specific to all stages of human development such as adult, juvenile, neonatal, placental and/or fetal proteins.
    e. Describe the limitations for the interpretability of your MPA platform data given the lack of soluble protein targets.
    f. Describe the limitations for the interpretability of your MPA platform data given the absence of potential binding sites (or exposure of unintended sites) specific to multi- subunit protein complexes, since individual proteins that comprise multi-subunit complexes will not be co-expressed.
    g. Describe how off-target binding events will be reported. Will any quantitative binding information be provided?
    h. Specify the standards (e.g., positive and negative controls) you intend to include in each experiment when assessing a novel product, following MPA assay qualification (if successful). Are different standards needed for each unique biotherapeutic modality (i.e., IgG mAb, IgM mAb, nanobody, bispecific mAb, peptide, RNA aptamer)

to be evaluated (e.g., use human mAb isotype as a negative control for a novel human mAb product of the same isotype only)?

i. You describe three methods by which CAR T cell specificity can be evaluated on your cell expression array: (i) scFv binding; (ii) CAR T cell binding; and (iii) CAR T cell functional activation. Please clarify if a single approach will be used or a combination of all three approaches will be used to evaluate CAR T cell specificity.

j. Regarding your CAR T cell binding and functional screening assays, please clarify the following:
   a. The number of donors from which a CAR T cell product would be manufactured for evaluation using your MPA technology.
   b. If CAR T cells will be manufactured using the proposed clinical manufacturing process and what limitations on the manufacturing process (e.g., activation method, CD4/CD8 ratios, vector introduction methods, etc.) exist for use with the MPA technology.
   c. Whether there are specific product release criteria (e.g., percent CAR-positive cells, cell viability, CD4/CD8 T cell ratio, T cell phenotype, etc.) that CAR T cells would need to meet for assay interpretability.
   d. If changes to the hinge region, spacer region, intracellular signaling region, or intracellular co-stimulatory region would necessitate re-evaluation of a CAR T cell product in which the antigen-binding domain is unchanged.

3. Analytical Considerations
   a. In your submission, you stated that the MPA has undergone analytical validation. Please specify the major parameters that were assessed during the validation of the flow cytometry assay with your QP.
   b. You stated that plasmid library, protein expression, and specific target detection were validated. Please specify the criteria that were used in this validation with your QP.
   c. You indicated that the MPA can successfully be used for specificity profiling of diverse biotherapeutics. Please define the criteria of biotherapeutics sample analysis for each modality.
   d. Describe the purity of the plasmid expression system (cDNA library). What is the relative abundance of endogenous membrane proteins from the eukaryotic cell line expression systems? How might the presence of endogenous membrane protein affect the interpretation of a positive binding event?
   e. We note that there may be differences in post-translational modifications occurring in different cell types proposed for the MPA platform. Describe whether the cell line selected affects the results obtained. Describe the advantages and disadvantages of utilizing these different cell lines for these experiments.
   f. Describe how you confirm that the membrane proteins from your library are expressed as full-length and in their active conformations.
   g. Please comment on the reproducibility of your MPA assay and how variability in protein expression is controlled for.
   h. You stated that for specific binding, monoclonal antibodies should bind only one target, with all other target proteins binding below pre-set statistical thresholds (e.g., 3 standard deviations of mean binding across the library on both replicates). Please specify the rationale used to set up these criteria.

i. Comment on whether the MPA can detect binding to proteins with a high level of amino acid sequence similarity to the intended protein target (i.e., paralogs).
j. You stated that the MPA has a success rate of >90% in identifying the correct target in specificity screens (in the remaining cases, the antibodies are usually too sticky or weak to run on the MPA). Please define the sensitivity of the assay (e.g., binding affinity range for potential targets) and the specificity screening criteria.
k. Please comment on the screening assay false-positive and false-negative criteria and rates. Please include in your discussion the potential for false negatives due to either missing proteins in the library or aberrant expression and the potential for false positives due to overexpression.
l. You have not provided a summary of the analytical validation of your CAR T cell screening assays in your submission. Please provide comprehensive information regarding analytical validation for: (i) your CAR T cell binding screening assay; and (ii) your CAR T cell functional activation screening assay.
m. We have the following additional comments regarding your T cell functional activation screening assay:
    a. CAR T cell activation can be induced by an allogeneic response against mismatched HLA if the CAR T cell expresses its endogenous T cell receptor. Please provide a discussion, with supporting data, regarding how alloreactivity is differentiated from off-target activity and how potential alloreactivity may affect the sensitivity and specificity of your screening assay.
    b. CAR T cell activation can be affected by the density of target antigen on the cell surface. Please provide a comprehensive discussion, with supporting data, regarding your rationale for the antigen density on the expression cells and how this correlates to antigen density on respective normal human tissues.
    c. Please clarify how the threshold for CAR T cell activation is defined. Please discuss how this threshold may vary across different CAR T cell products.

4. Nonclinical Relevance
    a. How well does the MPA technology recapitulate the current standards (e.g., TCR using immunohistochemistry methods)?
    b. What are the specific benefits and limitations of the proposed MPA technology compared with the current standards?

5. Statistical Considerations
    a. Provide a detailed SAP.
    b. In the QP, please label all axes.
    c. In Figure 1, subfigures do not have y-axis labels. Please provide the labels and appropriate units.
    d. In Figure 3, subfigures do not have y-axis labels. Please provide the labels and appropriate units for Target Binding. Please provide data-based summary regarding “current methods for profiling the specificity of biotherapeutics being poorly predictive of cross-reactivity against the native human proteome and having low sensitivity”.

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.

If product sponsors plan to use the tool prior to qualification to support regulatory review for a specific Investigational New Drug (IND), New Drug Application (NDA), Biologics License Application (BLA) or Abbreviated New Drug Application (ANDA) development program, they should prospectively discuss the approach with the appropriate CDER or CBER division.

The ISTAND encourages collaboration and consolidation of resources to aid 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 Integral Molecular, Inc. directly.

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[1].

Please contact the ISTAND Pilot Program at ISTAND@fda.hhs.gov, should you have any questions (refer to DDTIST00006 in the subject line).

Sincerely,

/s/

Mary Thanh Hai, MD
Director (acting), Office of Drug Evaluation Science (ODES)
Deputy Director for Clinical
Office of New Drugs (OND)
Center for Drug Evaluation and Research (CDER)
U.S. Food and Drug Administration (FDA)

/s/
Karen L. Davis-Bruno, PhD
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/s/
Wilson W. Bryan, MD
Director, Office of Tissues and Advanced Therapies (OTAT)
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[1]https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificationProgram/ucm535881.htm

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