Mahzi Therapeutics Inc.
470 Noor Ave, STE B, #1036
South San Francisco, CA 94080

June 24, 2024

Re: Mahzi's CIRM Application CLIN1-14825#3

Dear Independent Citizens Oversight Committee and Application Review Subcommittee of the California Institute for Regenerative Medicine:

In response to reviewers' comments, and prior to the June 27th meeting, we'd like to provide additional information regarding our comparability plan. Specific details were not provided previously as we focused on responding to reviewers' comments from the previous round of review.

1. **Product characterization for comparability**: The extent of analytical evaluation needed to assess manufacturing changes in comparability studies generally increases with the stage of product development. Mahzi's current stage of development is at pre-IND, thus the amount of data needed is far less than if MZ-9138 were in the clinic or commercially approved. Furthermore, Mahzi plans to seek FDA's feedback on the analytical comparability assessments prior to initiating the work, with the comparability data included in the original IND application. Demonstrating analytical comparability will allow Mahzi to utilize the GLP toxicology studies completed using material from the prior vendor.

   Keep in mind that though Mahzi is changing CDMOs, key elements of MZ-9138 are unchanged, including no changes to the construct, the transgene sequence, or regulatory elements. Mahzi is in possession of retains from multiple development lots manufactured by the former CDMO, including the lot utilized for the IND-enabling toxicology studies. This toxicology lot was extensively characterized by the former CDMO and will be similarly characterized by the new CDMO side-by-side with a new representative lot. This side-by-side comparison will include evaluation of >20 attributes spanning safety, identity, strength, potency, and quality (see appendix). The final three CDMOs under consideration all have prior experience successfully supporting clients with analytical comparability assessments at this stage of development, demonstrating their requisite internal capabilities and experience to successfully support MZ-9138 through a similar exercise. Additionally, preliminary comparisons of "former" and "new" material has already been done utilizing a smaller subset of key assays (e.g., vg titer by ddPCR, full/empty ratio by AUC, infectious titer by TCID50, and purity by SDS-PAGE/Silver staining); this comparison was done as part of a feasibility assessment performed to support the selection of our new CDMO and showed high congruency between the two lots.

   If analytical comparability is not accepted by regulators, Mahzi will assess a small number of animals (mouse and NHP) at the highest doses tested, which are translatable to human dose levels, to show comparative biodistribution and

tolerability. If biodistribution of vDNA and mRNA are comparable between material from the two vendors, which is what leads to histopathological findings, Mahzi believes that there will not be a need to repeat the histopathological assessments but plans to store all tissues for full assessment if there are any differences assessed that would require further investigation.

2. **Number of CDMOs Used**: One CDMO was used to make research-grade vector for non-GLP studies while a process was being developed at what we hoped would be our GLP and GMP manufacturer. An unfortunate sequence of events led us to seek a new CDMO. We understand the complexity of switching at this stage and we will ensure the most robust comparability testing will be conducted, in conjunction with regulatory feedback. Making a manufacturing switch at this earlier, preclinical phase was weighed against the scenario of continuing with the former CDMO through IND and making a CDMO switch after MZ-9138 was in the clinic. However, this latter scenario would involve a much more involved and time-consuming comparability package. The decision to bridge now, at the preclinical stage prior to IND submission, de-risks the chances of a comparability plan that is either not accepted by the FDA or requires a protracted clinical assessment of comparability. External SMEs were also engaged in this decision-making process, and there was unanimity in their assessments that bridging to new material now would be preferred over doing it after the program was in the clinic. Mahzi was also advised that it is not uncommon for bridging at this stage of development to include only an analytical comparability assessment and require no additional animal studies. While Mahzi believes this analytical comparability-only approach will be successful for MZ-9138, we are nevertheless planning for a contingency scenario where the FDA requests in vivo animal data to support the comparability assessment.

3. **Potency Assay**: Mahzi has selected a contract lab to move forward with once CMC activities restart. The lab selected has a demonstrated track record of developing potency methods for AAV therapies including commercially approved programs, and just supported the successful development and qualification of a transgene expression assay for a program with the same serotype (AAV9) and promoter (synapsin) which was recently cleared by the FDA. Feasibility studies performed by their lab on MZ-9138 material demonstrated the applicability of their assay's cell line and associated method for MZ-9138 given the number of similarities shared between the two therapies.

Sincerely,

DocuSigned by:
Yael Weiss
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Appendix 1: Proposed Testing Panel for MZ-9138*

Attribute	Analytical Method(s)

Vg Titer <USP 1127>	ddPCR
% Full Capsids / Full-to-Empty Ratio	AUC, cryoTEM
Infectious Titer	TCID50 / qPCR
Potency by Transgene Expression	In Vitro Cell Culture / qPCR
Capsid ID	ELISA
Transgene ID	Sanger Sequencing
Protein ID <USP 1104>	SDS-PAGE / Western Blot
Purity <USP 1056>	SDS-PAGE / Silver Stain
Pluronic Concentration	TBD
Osmolality <USP 785>	Freezing Point Depression
pH <USP 791>	Electrochemical, Potentiometry
Submicron Aggregation	SEC-MALS, DLS
Subvisible Aggregation <USP 787/788/789>	AMM
Particle Size Distribution	DLS
Appearance <USP 790>	Visual Inspection
Endotoxin <USP 85>	LAL, Kinetic Chromogenic
Bioburden <USP 61>	Membrane Filtration
Mycoplasma <USP 63>	PCR
Sterility <USP 71>	BAC-T, Membrane Filtration
Container Closure Integrity <USP 381>	Vacuum Decay, Dye Ingress
Residual Host Cell Protein <USP 1132>	ELISA
Residual Host Cell DNA <USP 1130>	qPCR, dPCR
Residual Plasmid DNA <USP 1127>	qPCR, ddPCR
Residual E1A <USP 1130>	qPCR
Residual Affinity Ligand	ELISA
Residual Detergent	TBD
Residual Endonuclease	ELISA
Adventitious Viruses <USP 1050>	In Vitro Cell Culture
Replication Competent AAV	PCR

* Attributes list and their associated test methods in some instances vary slightly between CDMOs; the final MZ-9138 test panel will be decided once CDMO is selected.