Dear Board Members, 11-28-2022

I take this opportunity to address several issues raised by the reviewers evaluating our recently submitted TRAN3 (TRAN3-14001) grant. The primary goal of this proposal is to complete the development and FDA-required regulatory filing of a novel spinal subpial injection needle to be used in a prospective clinical trial(s) for the treatment of neuropathic pain and amyotrophic lateral sclerosis (ALS). While several aspects of our proposal were praised by the reviewers, they also identified several shortcomings which were not sufficiently addressed. I take the liberty of addressing, what I believe are the major issues, and provide you with additional information which directly addresses the key concerns identified by reviewers. I will be brief.

A: Does the project have the necessary significance and potential for impact?

Reviewers comments:

1. Yes, if the combination product regulatory path can be navigated.
2. They have developed a subpial injection device, but whether this is superior to other devices that have been developed for delivery of cell-based or gene-based therapies to the spinal cord is not clear nor is it discussed.
3. While I am not an IP lawyer, I am aware of a very similar patented device that was used in ALS trials at another institution. It is remarkably similar to the planned device in this application. So much similarity exists that this should be addressed since this may impact the ultimate use of this planned device.

Applicant's response: **1)** We have already completed the pre-IND meeting for a combination product targeting neuropathic pain by subpial delivery of GAD65 and VGAT genes using the AAV9 vector. This information was provided in our grant application (p.33). FDA review identified no major regulatory issues. The path for regulatory approval requires the completion of the set of standard activities (CMC, and large animal safety/toxicology) used for a combination product in the gene therapy field. **2)** We have provided data and references from our already published work which demonstrate the superior potency of subpial AAV9 vector delivery (compared to intrathecal or direct spinal parenchymal injection) and which is characterized by wide-spread or segment-targeted transgene expression using subpial injection approach (p.17, Fig.3, p.18, Fig.4, p.19, Fig). In those studies, we have also demonstrated a potent treatment effect in animal models of ALS and chronic neuropathic pain and which can not be achieved by intrathecal or direct parenchymal vector delivery by using already existing devices (see next paragraph). **3)** As requested by one of the reviewers we have provided a copy of the expert opinion on the patentability of our subpial injection device. This document provides a detailed evaluation of the differences between our current subpial injection device vs the device cited by the reviewer. This opinion document describes substantial differences between both devices. In line with this expert opinion, the patent for the subpial injection system was already granted in two jurisdictions (patents: 2,975,447; 10,980,566). It appears that this additional, reviewer's requested information, was not provided to the review panel.

B: Is the rationale sound?

Reviewers comments:

1. The rationale for a new system is not discussed, and limitations in existing approaches are not discussed.
2. This is not clear due to the lack of discussion regarding other devices that have been used in clinical trials for cell delivery. The need for a new system is not clear.
3. There is limited data supported by other publications.
4. No quantitative data is provided.

Applicant's response: **1)** We have provided the rationale for the need to develop a new spinal cord delivery device for the delivery of gene and cell-based therapies in our grant application as well as in our previously published peer review work (p.7, parag.1; p.11, parag.3; p.15, parag.1; Ref. No.1, 2, 3). **2)** All currently used experimental clinical cell spinal cell-delivery devices use a concentric needle for spinal parenchymal cell injections. Our clinical team has an extensive experience with these devices from our previous cell-replacement trial in spinal trauma patients (Cell Stem Cells 22, 941-95, 2018), (https://www.ncbi.nlm.nih.gov/pubmed/29859175). We can attest that none of these devices can be used for subpial therapeutic(s) delivery. That was the primary reason for the development of a new "L"-shaped subpial needle injection device that does not require parenchymal penetration while delivering viral vectors (or cells) broadly into multiple spinal segments. **3)** We have referenced four of our peer review published papers (Ref.No.1, 2, 3, 11) which describe the subpial delivery system and the treatment potency achieved in rodent ALS model and neuropathic pain model after subpial delivery of treatment AAV9-based vectors. As this technology is relatively new there are no other publications available from other groups which would employ this approach. **4)** We have provided detailed qualitative and quantitative data on transgene expression in adult pigs and NHP in the application (p.17, Fig.3, p.18, Fig.4) and in more detail in referenced published papers (Ref.1,2,3,11). The data on broad human stem cell migration after single-level subpial delivery in immunodeficient rats were previously published (Marsala et al., Stem Cells Translational Medicine, 2019, 1-12), (https://www.ncbi.nlm.nih.gov/pubmed/31800978).

C: Is the project well-planned and designed?

Reviewers comments:

- ….the FDA says there is no approved injectable cell/gene therapy, so they can't approve a device to inject a treatment that isn't approved. This adds risk to the proposal of potentially funding a device for which the indication never gets to the IND stage. The regulatory path is complex and therefore cannot be separated from the therapeutic.

Applicant's response: **1)** As previously described in our application the first disease target we plan to treat using subpial injection is chronic neuropathic pain by delivery of two treatment genes (GAD65 and VGAT). **2)** The pre-IND meeting was already completed and all IND-enabling studies including subpial needle development are planned to be executed according to FDA feedback. No major issues were identified by FDA regarding the proposed gene therapy or subpial injection device planned to be used in the clinical trial.

D: Is the project feasible?

Reviewers comments:

- While the proposed work could be done, the ultimate chance of success and eventual regulatory approval is low.
- They need an indication for the intervention alongside the device.
- Major regulatory hurdles with a combination product.

Applicant's response: **1)** As described above the pre-IND meeting on the proposed treatment of neuropathic pain by subpial delivery of two therapeutic genes (ie combination product) was already completed (p.33, paragr. 3) with no major issues identified by FDA. **2)** The development of the two key components of the subpial injection device (spinal platform and XYZ manipulator) was already completed and master files were submitted to FDA. This information was provided as additional updated data during the review period. **3)** We are seeking funding for subpial needle development only. Because the use of a subpial needle is much less invasive (compared to an already approved concentric needle) and doesn't require spinal parenchymal penetration we feel confident that we have a clear path for the subpial device approval (as a combination product for the treatment of neuropathic pain in this stage). The preparation of the CLIN1 application to develop gene-based therapy for neuropathic pain using subpial treatment vector delivery is in progress. Securing the funding for the development of a subpial needle is critical for the successful completion of all required IND-enabling studies.

Summary and rationale for requested funding

- ✓ **We have developed and extensively tested in a large animal model(s) a novel subpial vector and cell delivery technique by using the subpial approach**
- ✓ **This delivery method was found to be highly effective in delivering gene delivery vectors and cells into the entire length of the spinal cord or targeted spinal segment in small and large animal models in adult animals**
- ✓ **We have demonstrated a potent treatment effect in rodent models of ALS and neuropathic pain after subpial delivery of treatment vectors**
- ✓ **The pre-IND meeting for the proposed treatment of neuropathic pain was completed and the path and structure of IND-enabling studies is well defined**
- ✓ **Funding is urgently needed to complete the development of a clinical grade subpial needle to be used in proposed IND-enabling studies and expected the clinical trial to treat chronic neuropathic pain**

Sincerely

Martin Marsala, MD