Target ALS *In Vivo* Target Validation Core

Dear Investigator,

I wish to draw to your attention to the launch of an exciting new mechanism through which you can enhance the translational impact of your ALS research. The Target ALS *In Vivo* Validation Core will allow investigators to evaluate the effect of modulating candidate therapeutic targets on the ALS phenotype in the standard mouse model of the disease.

Target ALS is a consortium formed in 2013 that aims to bridge strong scientific rationale with translational research to stimulate investment by industry in novel ALS drug development programs. One of the goals of Target ALS is to encourage proof-of-concept validation of promising targets in *in vivo* models of ALS so as to create strong data packages to support drug discovery strategies. This may be done using AAV vectors expressing shRNA/cDNA for a given gene, or using tool compounds.

Building on the expertise of multiple Target ALS laboratories, we have created new facilities based entirely within contract research organizations (CROs) that are capable of evaluating targets for their disease-modifying capacity in ALS. Investigators – whether or not already funded by Target ALS - will need only to submit proposed targets for evaluation with a brief (2-3 pages) justification and proof of biology for the shRNA/cRNA/small molecule proposed to perturb expression or activity of the candidate.

Following prioritization by the Target ALS Independent Review Committee (IRC), the facility will perform the central experiments for selected targets and return the data to the investigator. The costs of the experiments will be covered in part by Target ALS for selected projects from academic institutions. For for-profit bodies, or academic investigators wishing to pursue targets not selected by the IRC, this service will be provided on a real-cost basis consistent with throughput.

Confidentiality and intellectual property (IP) rights of the investigator:

- Data from the studies will be confidential – Target ALS has established confidentiality as part of the contractual agreement with fee-for-service organizations
- Result of the projects will be sent directly to the investigator – Target ALS central administration will see the data to ensure successful completion of the study
- The IP rights from the study will reside entirely with the investigator/respective organization – Target ALS does not have an interest in access to the IP or financial gains that may come from results of the study. The contractual agreement with fee-for-service organizations ensure that the IP resides entirely with the investigator
- Notwithstanding the above engagement concerning confidentiality, Target ALS believes that the precompetitive space in ALS therapeutic research will be enriched by communication of both positive and negative data from such studies. We therefore encourage both academic and for-profit investigators to allow the top-line results from their studies supported through this mechanism to be reported in the Target ALS database to be made public.
- Target ALS will only fund studies that originate from not-for-profit organizations. It is the responsibility of applicant investigator to give a written assurance that the study is not being carried out in partnership with a for-profit organization. If the study is in partnership with a for-profit organization,

the applicant has to give written assurance that the for-profit organization has obtained a license from Northwestern University for use of SOD1G93A mice.

Endpoints and study design

The primary biological readout to assess the effect of target modulation will be preservation of neuromuscular junctions (NMJs) in SOD1 G93A (high copy) mice (on C57BL/6J background). This endpoint was selected for several reasons: (a) the strong conservation of specificity for FF motor neurons between SOD1 mice and sporadic ALS patients; (b) its position as the first morphological change observed in patients and mice; (c) its high experimental reproducibility as reflected by power calculations. A 15% or greater preservation of NMJ counts at P65 will be used as the criterion to determine a meaningful effect of target modulation.

Examples of study designs

AAV-based study

Experimental groups

- SOD1 mice (P1 or P21) – Control, n=10 (mixed gender)
- SOD1 mice (P1 or P21) – AAV, n=10 (mixed gender)

Route of administration

AAV administered ICV

Experimental readouts

- Body weights twice a week
- Neuromuscular junction counts in tibialis anterior muscle at P65
- Brain, spinal cord, soleus and gastrocnemius muscles will be sent to investigator

Small molecule-based study

Experimental groups

- SOD1 mice – Control, n=10 (males)
- SOD1 mice – Test compound dose 1, n=10 (males)
- SOD1 mice – Test compound dose 2, n=10 (males)

  Or
- SOD1 mice – Control, n=15 (males)
- SOD1 mice – Test compound, n=15 (males)

Route of administration

IP or PO (P35)

Experimental readouts

- Body weights twice a week
- Neuromuscular junction counts in tibialis anterior muscle at P65
- Brain, spinal cord, soleus and gastrocnemius muscles will be sent to investigator

It is likely that follow-up or extension (e.g. lifespan) studies will be necessary for final publication of the data or establishment of the POC data package. Since the principal goal of the Target Validation Core is to achieve rapid head-to-head comparisons of as many targets as possible, these further experiments are not covered by the initial Target ALS funding. Nevertheless, for novel targets having shown clear positive effects, the CRO facilities will be made available at cost to pursue relevant studies.

Project costs

For projects from investigators at not-for-profit institutions selected by the IRC, Target ALS will cover the whole cost of the study based on standardized study designs above. Additional endpoints (e.g. grid test) will be at the investigator's own expense.

For projects from investigators at not-for-profit institutions not selected by IRC and for-profit organizations, the core facility will be available at cost. Please contact Manish for further details.

Project application and review process

The investigator should submit a project proposal (2-3 pages) on or before Friday, May 15, 2015 by 5.00 PM EST to the executive director (Dr. Manish Raisinghani, mr3166@columbia.edu).
Project proposals should give a clear exposition of the biological rationale for *in vivo* validation of the proposed target. The total length includes the following sections: Biological Rationale (with data justifying *in vivo* validation); Target validation approach (AAV or small molecule/biologic); Data showing efficacy of shRNA/cDNA for AAV approach or pharmacokinetics, target engagement and adequate amounts for small molecule/biologic. Following sections will not count against the three page maximum: Literature cited; Assurance by the investigator that the study is not in partnership with a for-profit partner or in the case such a partnership exists that the for-profit partner has a license to use SOD1G93A mice from Northwestern University; Two-page biosketch of the investigator.

The Target ALS Independent Review Committee will complete project proposal review by **Tuesday, June 30, 2015**. Proposals will be ranked on the basis of the following criteria: Biological rationale, Novelty and Feasibility for ALS therapy development. Specifically, the criteria will not include perceived druggability of the named target, since one of the goals is to identify new pathways that contribute to the ALS disease phenotype and only subsequently to identify ways in which they can be modulated in the clinic. Nevertheless, targets whose inhibition is predicted to confer benefit will generally be prioritized over targets requiring activation or over-expression. Since the principal goal of this core is target validation, Target ALS will give lower priority for financial support to projects that plan to use new tool compounds for targets already shown to play a role in SOD1-G93A mice. Projects that involve use of AAV and are ranked below the cut off for Target validation core will be given support to produce AAV that can be tested in the investigator's own lab.

Project initiation

Projects will commence as soon as possible after the results are announced depending on the time needed to organize logistics of individual projects.

Please send any questions or comments to Manish Raisinghani (mr3166@columbia.edu). We look forward to hearing from you.

Regards,
Chris Henderson
Director, Target ALS