



SUBCONTRACT EXECUTIVE SUMMARY

Disruption of the Mouse *Smn* Locus with A *Lox P* Flanked *tTA* Flanked Expression Cassette to Allow Inducible Expression of SMN¹

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We have created animal models of SMA by introducing the human *SMN2* gene onto a mouse that has a disrupted *Smn* gene or *Smn*^{-/-}. By introducing the SMNA2G transgene and SMNΔ7 transgene we have extended the range of phenotype of these mice. These mice develop SMA due to low SMN levels and replicate human SMA. The SMA mice have recently been approved to be deposited in Jackson Laboratories for more facile distribution. We have made these mice available to a large number of investigators. In addition we have made an inducible SMN:luciferase transgene which will express both SMN and luciferase when induced by expression of tTA. This transgenic line is currently breed onto the SMA mouse background. The SMN luciferase construct is a cDNA based construct and the SMA mouse background contains the *SMN2* gene. We therefore have all the components except one to answer when high levels of SMN are important in order to prevent SMA. The missing ingredient is a tTA transgene that can express tTA such that you get correction of SMA. This is essential before the question as regards timing of SMN expression can be answered. We have chosen a very specific manner in which to obtain the correctly expressed tTA namely an insertion into the *Smn* locus. This will take longer than making a tTA transgene under the *SMN2* promoter however it is considerably more reliable and has the additional advantage of generating a different knockout allele and allowing a separate system of reactivation using *cre lox*. In addition we have already prepared *SMN2* promoter tTA lines and are testing them at present it is clear that using this methodology is not straight forward to obtain the required tTA lines. In addition we are attempting to construct a SMN2 transgene that can be switched to SMN1 at a specific time.

In this proposal we will insert a tTA construct into the SMN gene such that it disrupts the *Smn* gene but allows expression of tTA. The tTA construct will be flanked by *loxP* sites so that removal of the tTA by expression of *cre* will allow re-expression of the *Smn* gene. Generation of this line will achieve three goals:

- 1) a SMN-disrupted allele equivalent to current knockout alleles. This also simplifies breeding in that the tTA and the knockout allele are co-inherited.
- 2) the tTA under the control of the endogenous SMN gene will be used in conjunction with a tTA inducible SMN/luciferase transgene that we have generated to obtain inducible SMN expression.
- 3) the use of specific *cre* drivers will allow removal of the tTA and thus re-expression of SMN from the endogenous locus. The time and place of *cre* expression will determine when and where SMN expression will be restored.

Basically there is one goal to obtain a mouse with insertion of tTA into the SMN locus that can be used to determine when high levels of SMN are important in motor neurons. This can be divided as follows for the project

- 1) Prepare a targeting construct capable of inserting a *loxP* flanked tTA expression cassette into the SMN gene.

This will simultaneously disrupt the mouse SMN gene.

- 2) Prepare ES cells with the targeted mutation
- 3) Obtain mice with the targeted insertion
- 4) Cross to SMA mice and use either the tetracycline or *cre lox* system to determine when high levels of SMN are important. This project is estimated to take a year.

¹ A proposal to support this subcontract was submitted to the SMA Project's RFP JL-32903-001, "An Inducible Mouse Model of Spinal Muscular Atrophy." SAIC provides management support for The SMA Project to the NINDS through contract N01-NS-3-2356.