



SUBCONTRACT EXECUTIVE SUMMARY

A Human Embryonic Stem Cell-Derived Motor Neuron System for SMA Drug Development¹

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In SMA patients, reduced levels of SMN protein result in death of spinal motor neurons. The basis for the particular sensitivity of motor neurons to reduction in SMN levels is not known. SMN is an essential protein—in most model systems loss-of-function mutations are lethal. The survival of human SMA patients depends on SMN protein produced from a second copy of the SMN gene that is found in humans, but not typical model systems. Due to the unique sensitivity of motor neurons and configuration of SMN genes in humans, it is essential that potential SMN therapeutics be tested in a human motor neuron system. We propose the development of a human embryonic stem (hES) cell-derived motor system specially designed to test the ability of candidate therapeutics to increase SMN levels. As a reference cell type, the system uses cultures containing human motor neurons derived from NIH-approved hES cell lines BG01 and BG02. Our neural cultures (mixed motor neurons or MMN) contain readily identifiable motor neurons (islet-1 and choline acetyltransferase positive cells) that remain viable for over three weeks in culture. We will introduce SMN reporter genes to allow screening for drugs that increase SMN expression (e.g., via specific splicing or transcriptional mechanisms) in human MMN. This system is naturally suited to high-throughput adaptation and can be established quickly (within one year).

The objective and three milestones of this project are:

To establish, verify and transfer a human neural cell system for identification of compounds that increase SMN expression.

- 1) Establish a human mixed motor neuron (MMN) system that expresses SMN reporter minigenes.
- 2) Demonstrate ability of compounds known to increase SMN2 expression in non-neuronal cells to increase SMN2 reporter gene expression in this system.
- 3) Demonstrate transferability of the assay to an outside lab.

The timeline for development is short because the key components are in place. Our MMN cultures are readily available and characterized. The adenoviral vectors to be used to introduce reporter genes are available and effective in neural cells. The adeno-associated vector that we have offers stable integration into a specific non-interfering genomic location, eliminating positional expression effects and saving time. The minigene reporter construct has been previously established in non-neuronal cells by members of this team. Once transferability is demonstrated the assay will be transferred to a central testing facility. The transfer of the systems presents some unique challenges since the neural stem cell system requires a new operator to become proficient in several new cell culture methodologies, however, in directing NIH-sponsored hES stem cell workshops twice a year we have developed the necessary technology transfer expertise.

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