Assessment of pre-clinical safety of GMP grade GNE-lipoplexes after repeat intravenous injections in Balb/c mice

Anagha P. Phadke[1], Chris M. Jay[1], Phillip B. Maples[1], Neil Senzer[1,2], John Nemunaitis[1,2] and Alex W. Tong[1]

[1]Gradalis, Inc., Dallas, TX; [2]Mary Crowley Cancer Research Centers, Dallas, TX;

Hereditary Inclusion Body Myopathy (HIBM) is an adult-onset, muscular disease caused by mutations in the GNE gene which results in reduced GNE enzyme activity and hyposialylation of cellular proteins. In the current study we assessed the pre-clinical safety of 4 monthly intravenous injections of the GNE plasmid vector encapsulated in the cationic liposome (DOTAP:cholesterol) into Balb/c mice. Four groups of 25 female mice received either the GMP GNE-lipoplex (at 10 ug, 20 ug or 40 ug in 80 ul total volume) or empty liposomes. Animals were injected on days 0, 30, 60, and 90. Five mice from each treatment cohort were sacrificed at days 2, 31, 63, 91 and 122. Body weights were measured every week and mice were monitored for signs of toxicity. Complete blood counts were performed on mice sacrificed on days 31, 91 and 122. Serum chemistries were performed on mice sacrificed on days 2, 63 and 122. Our data did not demonstrate toxicity in mice treated up to 4 times with empty liposomes. Administration of the 10 or 20 ug dose of GNE lipoplex did not generate fatality, but induced transient behavioral alterations (lethargy, ruffled fur and hunched posture) that resolved by 48 hrs. Leucopenia (at 10 or 20 ug dose) and liver enzyme alterations (at 20 ug dose) were observed after the 2nd IV injection. All mice tolerated additional injections and exhibited normal CBC and liver enzyme values at sacrifice (on day 91). Treatment with 40 ug dose of GNE-lipoplex was lethal to 9 of 25 injected animals within 24- 48 hr. Three (3) mice died after the 1st injection and 4 and 2 of the surviving mice, died after the 2nd and the 3rd injections, respectively. Surviving mice that were injected with the 40 ug dose demonstrated behavioral alterations (lethargy, ruffled fur and hunched posture) that resolved after 48 hr of treatment, with marked leucopenia and alterations in liver enzymes (ALT and AST). No treatment related deaths were observed after the 4th injection of 40 ug administered to the 5 surviving (out of 10) animals that received 3 injections. These mice tolerated the 4th injection while exhibiting a partial recovery in CBC and liver enzyme values on day 91. Tissues collected at necropsy are currently being assessed for histopathology and expression of the recombinant human GNE mRNA transgene. Based on these findings the maximum tolerated dose of GNE-lipoplex is < 40 ug when administered systemically at multiple times to Balb/c mice.