Preclinical safety and toxicity assessment of Stathmin 1 bifunctional shRNA formulation following a single intravenous injection in rats

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Stathmin 1 (STMN1) is a critical protein required for microtubule depolymerization and mitotic spindle regulation. STMN1 has been reported to be overexpressed in a variety of human cancers such as Wilm's tumor, acute leukemia, lymphoma, ovarian carcinoma, prostate cancer, breast cancer, head and neck cancer, hepatocellular carcinoma, osteosarcoma, lung cancer and mesothelioma. We have selected STMN1 as a candidate target for knockdown based on its overexpression at the protein level in 86% of 35 paired tumor vs. normal tissues analyzed. We have developed a proprietary bifunctional shRNA with enhanced post-transcriptional gene silencing activity against STMN1 (bi-shRNA-STMN). bi-shRNA-STMN concurrently induces translational repression (mRNA cleavage independent) and post-transcriptional mRNA degradation (cleavage-dependent and -independent). *In vitro* studies using the colon cancer cell line CCL-247, demonstrated effective knockdown of STMN1 expression with G_2/M cell cycle arrest within 24 hours of treatment with consequent growth arrest and apoptosis. A single low dose (10 μg) intratumoral injection of bi-shRNA-STMN in cationic liposomes significantly reduced CCL-247 tumor xenograft growth at day 7 ($p = 0.05$) and day 8 ($p = 0.01$) as compared with the control group treated with DW5 only. Scrambled bi-shRNA control did not significantly alter tumor growth. Increasing the intratumoral injecting dose to 6 consecutive daily injections of 50 μg or 100 μg, bi-shRNA-STMN-lipoplex essentially abrogated osteosarcoma tumor growth as compared with D5W control treated animals ($p = 0.017$ and 0.035 for 50 ug and 100 ug-treated cohorts, respectively). Pre-clinical safety and toxicity of the bi-shRNA-STMN lipoplex were assessed in Sprague-Dawley rats (~150 g). There were 5 groups of rats, each consisting of 60 rats (30 male and 30 female). Three groups were given bi-shRNA-STMN-lipoplex at 1.5, 15, or 150 μg by single IV injection of 300 μl. Control groups were injected with either empty liposomes or the diluent (D5W). Cohorts of 10 rats (5 male and 5 female) from each treatment group were sacrificed at days 2, 7, 14, 30, 60, or 90 post-injection. Assessments included in-life observations for overall health, mortality and measurement of body weight once every week. Assessments at necropsy included gross pathology observation, collection of blood for complete blood counts, serum chemistry analysis and coagulation tests. Tissues were also collected for histopathology analysis. In conclusion, administration of bi-shRNA-STMN-lipoplex was safe with mild lethargy noted for animals that received 150 μg lipoplex, only on the day of injection. There were no treatment related deaths observed. Preliminary results suggest that the maximum tolerated dose of bi-shRNA-STMN lipoplex is ≥150 μg via systemic administration (equivalent to ≥10 mg per 80 kg human based on body surface area conversion).