SCIENTIFIC ABSTRACT

SB-509-0601: A Phase 2 Repeat Dosing Clinical Trial of SB-509 in Subjects with Diabetic Neuropathy

Vascular Endothelial Growth Factor-A (VEGF-A) represents a novel approach in the treatment of diabetic neuropathy. VEGF-A is well known as a potent angiogenic factor, but it has also been shown to be a potent neurotrophic and neuroprotective factor. Studies have shown that the hVEGF-A_{165} cDNA, delivered as naked DNA, can reverse neuropathy in diabetic animals (streptozotocin-treated rats, alloxan-treated rabbits) (Schratzberger *et al.*, 2001). A Phase 1 clinical trial testing hVEGF-A_{165} cDNA has suggested that this approach may be beneficial (Simovic *et al.*, 2001). A larger, placebo-controlled clinical trial is currently underway.

The VEGF-A mRNA is alternatively spliced to express three major protein isoforms, VEGF-A_{189}, VEGF-A_{165}, and VEGF-A_{121}. Recent studies suggest that the combination of all three isoforms provides a more potent angiogenic response than any of the isoforms alone (Whitlock *et al.*, 2004). The same may be true for the neurotrophic and neuroprotective effects of VEGF-A. Treatments that rely on cDNA clones for the delivery of a single isoform of VEGF-A to damaged neurons may miss the beneficial effects of the other isoforms.

Sangamo BioSciences, Inc. has developed a plasmid DNA (SB-509) that expresses an engineered transcription factor (TF) that binds the endogenous VEGF-A promoter and activates transcription of the endogenous VEGF-A gene. This TF is based on a Cys_2-His_2 Zinc Finger DNA binding domain. The VEGF-A transcript produced is subjected to the aforementioned alternative splicing, naturally producing the three major protein isoforms. It has been shown, in side-by-side comparisons, that activation of the endogenous VEGF-A gene by using engineered transcription factors produces non-leaky vasculature with normal vessel architecture (Rebar *et al.*, 2002), in contrast to vessels produced in response to a single isoform, which leak and have defective architecture (Elson *et al.*, 2001).

Based on the technical advantage of using engineered transcription factors for endogenous gene regulation, we believe this approach will extend to subjects with Diabetic Neuropathy. This plasmid DNA has been tested in a Phase 1 trial in subjects with Diabetic Neuropathy. In the Phase 1 study 24 subjects were treated at the dose levels of 1, 3, 5, 10, 15, 30 and 60 mg. Treatment was well-tolerated and there were no dose-limiting toxicities or drug-related serious adverse events. There were ten treatment-related adverse events, nine were in subjects treated with SB-509 and one in a subject treated with placebo. All AE's were reversible.

Treatment with SB-509 has been well tolerated up to the dose of 60 mg/subject. The proposed Phase 2 trial will be performed in subjects with diabetic neuropathy. Naked DNA encoding the VEGF-A engineered transcription factor is formulated in a non-ionic polymer known to enhance DNA delivery to skeletal muscle cells. The formulated DNA will be injected into the muscles of the upper and lower limbs of subjects with diabetic neuropathy. The primary objective is to compare in subjects with diabetic neuropathy the effect of SB-509 versus placebo on a pre-defined multi-endpoint analysis that includes visual analog scale for pain intensity (VASPI), total neuropathy score (TNS), nerve conduction velocity (NCV), quantitative sensory testing (QST), and epidermal nerve fiber density (ENFD) and regeneration (ENFDR). The secondary objective is to evaluate the safety of SB-509 as compared to placebo in subjects with diabetic neuropathy.