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MONOGRAPH

SB-509

Zinc Finger DNA-Binding Protein Transcription Factor Activator
VEGF-A Upregulator
Treatment of Amyotrophic Lateral Sclerosis
Treatment of Diabetic Neuropathy

32Ep65
VZ+426
VZ+434
VZ+434b

pVAX-1 DNA plasmid encoding the cytomegalovirus (CMV) promoter, three-finger ZFP (zinc finger protein) DNA-binding domain, the nuclear translocation signal from simian virus 40 large T antigen and the transactivation domain from the p65 subunit of the human nuclear factor NF-κB, targeting the VEGF-A gene sequence: GGGGGTGA

EN: 378544

SUMMARY

SB-509 is a zinc finger DNA-binding protein transcription factor (ZFP-TF) activator that upregulates the expression of vascular endothelial growth factor A (VEGF-A). VEGF-A plays an important role in both angiogenesis and neurological development. Independent research studies show that VEGF-A has direct neurotrophic activity on axonal outgrowth in in vitro culture conditions. The dual angiogenic and regenerative effects of VEGF-A on nerves suggest a potential therapeutic effect in neuropathic disorders. SB-509 stimulates endogenous VEGF-A expression, of all natural isoforms in their proper ratios. In the streptozotocin-induced diabetes model of neuropathy, SB-509 prevented the typical reduction in motor and sensory nerve conduction velocities. This favorable result led to human trials in diabetic neuropathy. A phase I trial showing trends for meaningful clinical improvement in SB-509-treated subjects led to a phase II trial. In the phase II trial, SB-509-treated subjects showed an increase of 55% in intraepidermal nerve fiber density (IENFD) compared to a decrease of 16% in placebo-treated subjects. Although there was no overall difference in clinical outcome measures between SB-509- and placebo-treated subjects, subjects who started out with low IENFD showed clinical improvements correlating with increases in nerve fiber density. Additional clinical studies are in progress in diabetic neuropathy and amyotrophic lateral sclerosis (ALS). These studies have shown that plasmid-mediated delivery of the engineered zinc finger transcription factor is safe and may be a therapeutic option in a variety of diseases.

PREPARATION*

The *ZPF* gene coding for the three-finger zinc finger protein DNA-binding domain, the nuclear translocation signal from simian virus 40 large T antigen, the transactivation domain (amino acids 288-548) from the p65 subunit of human nuclear factor NF-κB and the human cytomegalovirus (CMV) promoter are cloned into the DNA plasmid pVAX-1 (Invitrogen). The *ZPF* gene is constructed by polymerase chain reaction (PCR), assembling oligonucleotides encoding for the α-helix and the β-sheet regions of each three-finger protein. SB-502 targets 9 base pairs of the *VEGFA* gene (GGGGGTGACc) located 438 base pairs downstream of the principal site of transcription initiation (1).

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BACKGROUND

Zinc finger DNA-binding protein transcription factors (ZFP-TFs) can be engineered to interact with the genome at selected sites to regulate gene expression (2, 3). VEGF-A stimulates enhanced vascular permeability and promotes the proliferation, migration and survival of endothelial cells, resulting in angiogenesis (4, 5). VEGF-A mRNA is alternatively spliced to express three major isoforms, VEGF-A_{189}, VEGF-A_{165} and VEGF-A_{121}. These isoforms have high affinity for the protein-tyrosine kinase receptors VEGFR-2 (FLK-1, *KDR*) and VEGFR-1 (FLT-1), and the non-tyrosine kinase receptors NRP1 and NRP2 (6). Although each of the isoforms supports blood vessel development, optimal angiogenesis resulting in normal vascular architecture requires the cooperative effect of all isoforms (7).

VEGF-A plays an important role in neurological development (8). Nervous system angiogenesis is dependent on VEGF-A expression (9). VEGF deficiency leads to developmental retardation and progressive destruction of neural tissue in the brain (10). In addition to its role in vasculogenesis in promoting nervous system development, there is strong evidence that VEGF-A has a primary effect on neurons (11). VEGF has neurotrophic activity and stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in the peripheral nervous system (12). VEGF-A has a direct neuroprotective effect in ischemia models (13), and induces neurite outgrowth and enhances neuronal survival in cultured dorsal root ganglia (14).

The dual angiogenic and regenerative effects of VEGF-A on nerves suggest a potential therapeutic effect in neuropathic disorders. The protective effects of VEGF-A on motor neurons have been demonstrated in several studies. Neuronal overexpression of the VEGF-A

receptor VEGFR-2 has shown promise in preventing motor neuron degeneration (15). Overexpression of VEGF-A delays neurodegeneration in SOD1G93A/VEGF$^{+/+}$ mice (16), and VEGF-A also protects motor neuron-like NSC34 cells against cell death (17).

Potential strategies to administer VEGF-A therapeutically include: 1) direct delivery of the protein to neuronal tissue; 2) administration of the gene for VEGF-A to induce overexpression; and 3) stimulation of expression of the endogenous gene. SB-509 employs the latter strategy. The investigational agent SB-509 is a formulated plasmid encoding a ZFP-TF, the structure of which is based on the Cys2-His2 zinc finger class that binds to the endogenous VEGF-A promoter and activates its transcription (1). The transcripts undergo natural splicing, resulting in the production of the three major protein isoforms. The greatest potential advantage of plasmid delivery of the transcription factor is the ability to stimulate VEGF-A production locally in the direct vicinity of the nerve rather than systemically. Indeed, systemic untargeted delivery of VEGF-A is not desirable, as VEGF-A is a growth factor with potent effects on the microvascular system and systemic untargeted angiogenesis could lead to cancer growth and/or promote diabetic retinopathy. Antibodies to VEGF-A have entered standard practice to treat several forms of cancer and to suppress diabetic retinopathy (18-20). Locally administered plasmids persist at the injection sites and have minimal systemic distribution (21).

PRECLINICAL PHARMACOLOGY

SB-509 has been studied in several animal models across multiple species. Significant capillary angiogenesis has been demonstrated in muscle following i.m. injection in rabbits (22) and mice (23). In the streptozotocin-induced diabetic rat model, i.m. administration of SB-509 prevented the typical reduction in motor and sensory nerve conduction velocities that occur in this animal model of diabetic neuropathy (24). Increased levels of VEGF-A were confirmed in sensory ganglia of SB-509-treated streptozotocin-induced diabetic rats (25).

SAFETY

To date, there have been no issues of safety in the phase I and II studies (26). The primary theoretical concerns are that elevated levels of VEGF-A could stimulate cancer cells and promote retinal neovascularization. A total of 164 patients have been treated with SB-509 and increased serum levels of VEGF-A were not detected. In addition, no malignancies were reported in this SB-509-treated population. Fecal occult blood tests, mammograms and prostate-specific antigen level determinations have been performed to detect the most common malignancies, with no evidence of change. Fundus photography and fluorescein angiography have been performed and clinical findings of retinal pathology have not been reported. The most common adverse event in the trials has been transient injection-site reactions.

CLINICAL STUDIES

The favorable effects of SB-509 in animal models of diabetic neuropathy led to initial studies in humans (27). Initial phase I studies were performed in patients with moderate to severe diabetic neuropathy. The efficacy variables included the Neuropathy Impairment Score-Lower Limb (NIS-LL), which is a composite score evaluating muscle strength, sensory findings and reflexes, nerve conduction velocities and vibration perception threshold. SB-509 was administered by direct i.m. injection along the course of the major nerve trunks on the lower extremity. Injections were placed in the tibialis anterior, gastrocnemius and soleus with 0.5-cc injections and the lateral quadriceps with 1-cc injections. At the highest dose level, treatment included 22 injections per leg, with a 1-inch 25-gauge needle for the legs and a 1.5-inch 25-gauge needle for the thighs, both using a 1-cc insulin syringe. Subjects were followed for 180 days after this single injection. There were clinically meaningful improvements in NIS-LL and vibration perception in patients treated with the highest doses, and there was a trend toward higher sensory and motor nerve conduction velocities in the SB-509-treated patients as well.

The favorable results from the phase I study led to the design of a phase II study in patients with diabetic neuropathy. In this study, SB-509-601, intraepidermal fiber density was added as an additional secondary endpoint. Epidermal nerve fibers are type C and A-δ small-caliber unmyelinated nerve fibers. They have a primary sensory role and are not well assessed by conventional measures, such as nerve conduction velocity, amplitude or most forms of quantitative sensory testing. Intraepidermal fiber density measurement requires a 3-mm skin biopsy on the distal lateral thigh, with rapid fixation and freezing. Skin sections are prepared and stained for axons by immunohistochemistry. The numbers of fibers or axons per millimeter are then counted. The time course of decline in fiber density parallels the course of diabetic neuropathy (26).

In the SB-601 study, subjects with clinical diabetic neuropathy confirmed by abnormal nerve conduction measures were treated with SB-509 or placebo three times on day 1, day 60 and day 120 of the study. A total of 110 subjects were enrolled, with 75 subjects receiving SB-509 and 35 subjects receiving placebo. The efficacy measures included clinical, nerve conduction and quantitative vibration thresholds, as well as intraepidermal fiber density. At 180 days, no overall difference was seen between placebo- and SB-509-treated subjects in the NIS-LL, nerve conduction velocity and vibration testing results (26). However, when analyzed in conjunction with measurement of nerve fiber density, a subgroup of subjects with low baseline nerve fiber density had clinically significant improvements in NIS-LL and nerve conduction velocities. There was an increase in nerve fiber density in SB-509-treated patients overall, compared to a decrease in placebo-treated patients. Sural nerve conduction velocities increased by 2.6 m/s compared to 1.2 m/s in placebo-treated patients. Furthermore, patients with more severe measures of vibration threshold and nerve conduction showed a greater improvement in NIS-LL and nerve conduction velocity. In addition, SB-509-treated subjects showed an increase of 55% in nerve fiber density compared to a decrease of 16% in placebo-treated subjects. Taken together, these findings suggest that patients with more advanced diabetic neuropathy may have a greater response to SB-509. There have been no long-term trials as yet to determine how long the response may last post-injection.

The potential regenerative effects of VEGF-A on neurons raise hope that the agent could be beneficial following injury to the spinal cord. The combined dynamics of angiogenic and neuronal stimulation

could also be therapeutic after cerebrovascular incidents. Another area of current clinical study includes amyotrophic lateral sclerosis.

CONCLUSION

Gene therapy is evolving rapidly, with novel treatment approaches for a number and variety of diseases. Trials are under way in multiple hereditary disorders, such as cystic fibrosis and hemophilia (28). Typically, vectors are used to introduce functional genes into the host to correct deficient or abnormal gene products. Plasmid DNA-mediated delivery is one method of gene transfer that does not use viral vectors for administration and is considered to be safe (29). Many recombinant DNA vaccines are under study in clinical trials, and several veterinary vaccines are currently in use (30). Trials of plasmid-mediated replacement of defective endogenous genes in diseases such as cystic fibrosis are under way (31).

SB-509 is a unique agent in the conceptualization of genetic reengineering. The SB-509 plasmid encodes for a specific ZFP-TF that upregulates endogenous production of VEGF-A and its isoforms in the proper ratios. Consequently, the gene product sequences are identical to those of the host genome and are subject to natural autoregulation. SB-509 has undergone extensive testing in cell cultures and animal models. VEGF-A has both angiogenic and direct neurotropic effects. Consequently, it is an appealing candidate for the therapy of several neurological conditions, including diabetic neuropathy, amyotrophic lateral sclerosis, spinal cord injury and stroke. Sangamo BioSciences moved rapidly to a development program to explore the use of SB-509 in diabetic neuropathy. A phase I and a phase II trial have been completed and a phase IIb trial is ongoing. The results encourage further confirmatory studies in larger diabetic neuropathy trials.

The excitement about the use of SB-509 in diabetic neuropathy is in the proof of concept that plasmid delivery of zinc finger transcription factors is feasible and effective. This technique of modulation of the delivery of endogenous gene products is applicable to a wide variety of disease states.

SOURCE

Sangamo BioSciences, Inc. (US).

DISCLOSURES

The author has been an investigator on a Sangamo-sponsored trial of diabetic neuropathy.

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