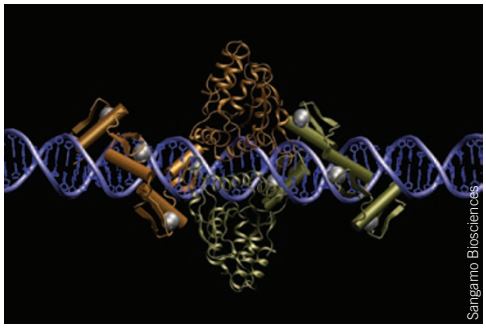
Sangamo's lead zinc-finger therapy flops in diabetic neuropathy

On October 3, 2011 Sangamo Biosciences announced it was halting its lead program SB-509 program in diabetic neuropathy after the zinc-finger protein (ZFP) therapy failed to meet its end points. Although the failure represents a considerable setback for the diabetic neuropathy program, Sangamo remains buoyant about ZFPs as a therapeutic platform, arguing that challenges associated with SB-509's ambitious target indication conspired to thwart the drug.

Sangamo has built its name as the pioneer of engineered ZFPs as therapeutics. ZFPs are DNA-binding proteins that attach to unique sequence motifs and participate in gene regulation. Each 'finger' recognizes a particular three-base set (GNN and ANN triplets) and by choosing the proper number and arrangement of motifs, one can construct specialized ZFPs that promote targeted regulation. What's more, considerable interest focuses around the potential of ZFPs, when joined through a linker to an endonuclease like *Fok1,* in editing or correcting endogenous genes containing disease-causing mutations.



A zinc-finger protein (Zn is silver) recognizes and binds exact DNA sequence. Sangamo engineers zinc-fingers to edit and correct genes carrying disease-causing mutations.

The ZFP gene activator employed in SB-509 was first described in 2001 (*J. Biol. Chem.* **276**, 11323–11334, 2001). SB-509 is a plasmid encoding three ZFPs that target a GGGGGTGAC site in the vascular endothelial growth factor A (*VEGFA*) gene. The ZFPs are linked to a p65 transcriptional activator that is constitutively expressed by a cytomegalovirus early promoter/enhancer and switches on *VEGFA* after binding. The ZFPs are injected intramuscularly in a formulation containing Poloxamer 188, sodium chloride and tris-HCl.

According to Alastair Mackay, an analyst at GARP Research & Securities, in Baltimore, many investors were already tentative about the ZFP technology. "The outside community was split," he says, "with bears saying for some time that this latest trial would not succeed, while bulls were saying that the trial had good prospects for success." To be sure, peripheral diabetic neuropathy is an ambitious target. This common complication of diabetes is actually a heterogeneous syndrome of pathologies, in which patients gradually accumulate damage to the microvasculature in their extremities. This leads to reduced neuronal input and poorer signal conduction in affected tissues, causing pain and/or increasing loss of sensation and leaving diabetic neuropathy sufferers vulnerable to serious injury and infection. The only available treatments are palliative, a combination of analgesics and antidepressants, and attempts to develop efficacious therapeutics have consistently failed.

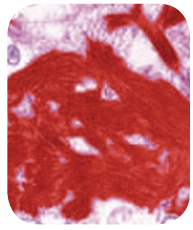
Although VEGFA modulation could theoretically address a host of disease conditions, Sangamo CEO Edward Lanphier cites several reasons why his company saw diabetic neuropathy as the right indication. "VEGFA is first and foremost a potent neurotrophic factor," he says. "Peripheral diabetic neuropathy involves both nerve loss as well as loss of microvascular structure, and the vascular and neurotropic functions made VEGFA ideally suited for treating this disease." According to Lanphier, rodent studies indicated that SB-509 could promote revascularization and repair, potentially accelerating the time required to see symptom improvement. Importantly, SB-509 seemed to promote production of all of the various VEGFA isoforms, increasing the chances for the ZFP to elicit a full spectrum of VEGFA's effects.

In 2006, the Juvenile Diabetes Research Foundation (JDRF) partnered with Sangamo, providing financial support for their '601' phase 2 trial, which enrolled 110 patients with mild to moderate peripheral diabetic neuropathy. The therapy proved safe, with only minimal adverse effects, although efficacy results were a mixed bag and patients in the treatment arm as a whole failed to achieve significant benefit relative to the placebo group.

It didn't help that in the vast majority of people, diabetic neuropathy can be forestalled with glucose control, explains Helen Nickerson, a member of the JDRF scientific staff for complications therapies. "The placebo-group patients

IN brief

Amyloid disease drug approved



Steve Gschmeissner/Science Photo Library

Transthyretin amyloidosis deposits.

The first medication for a rare and often fatal protein misfolding disorder has been approved in Europe. On November 16, the EU gave a green light to Pfizer's Vyndaqel (tafamidis) for treating transthyretin amyloidosis in adult patients with stage 1 polyneuropathy symptoms. Chemist Jeffery Kelly, now at the Scripps Research Institute in La Jolla, California, began working on transthyretin amyloidosis, including familial amyloid polyneuropathy (TTR-FAP), in 1989. He later founded FoldRx in Cambridge, Massachusetts, to develop a small-molecule kinetic stabilizer for transthyretin in its correctly folded form *(Nat. Biotechnol.* **27**, 874, 2009), and in 2010, New York–based Pfizer acquired FoldRx. The approval was based on a study of 128 TTR-FAP patients. Although 45% of patients randomized to Vyndaqel showed improved or stabilized nerve function, compared with 30% of patients on placebo, the results fell short of statistical significance, and Vyndaqel also missed its quality-of-life end point. However, a secondary analysis adjusting for patients who left the study early for liver transplant (the only current therapy) met both end points. Nevertheless, Philip Hawkins, clinical director of the National Amyloidosis Centre in London, calls the EU approval "puzzling." "The clinical results seem disappointing," he says. Kelly disagrees. "It's unequivocal that the drug works," he says.

The autosomal dominant disease is most commonly caused by a V30M transthyretin gene mutation. It affects only 5,000–10,000 people worldwide. But Vyndaqel has potential for much wider use, as unstable transthyretin proteins, which can accumulate as amyloid fibrils and deposit in a variety of organs including the heart and kidneys, probably affect millions of people. Pfizer's potential competitors include Alnylam Pharmaceuticals in Cambridge, Massachusetts, with a lipid nanoparticle short interfering RNA for transthyretin, and Isis Pharmaceuticals in Carlsbad, California, with an antisense compound. Both are in phase 1 trials, as is GlaxoSmithKline's ligand to serum amyloid protein. Dolobid (diflunisal), an old nonsteroidal anti-inflammatory drug, is in phase 3. Kelly especially likes Alnylam's approach, which has effectively silenced transthyretin in clinical studies. "The tafamidis drug will be used in combination with RNAi [RNA interference] suppression in the pretty near future," he predicts. In the US, Pfizer filed a new drug application (NDA) with the Food & Drug Administration in February 2011, but received a Refusal to File letter on the grounds that the application was incomplete. Pfizer spokeswoman Victoria Davis, in an email, says Pfizer "is on track with the NDA refiling." **Ken Garber**