

single or repeated i.t. pIL10 encapsulated in micro-particles prepared from FDA-approved biodegradable copolymers of polylactide and polyglycolide (PLGA) (PLGA- pIL10). Lastly, we examined whether i.t. rhodamine-labeled PLGA-micro-particles could be visualized in spinal cord using confocal microscopy in naive rats. Behavioral measures were assessed prior to & at 3 & 10 days post CCI. I.t. injections were given on day 10 post-CCI, consisting of pIL10 (100 ug/injection), control plasmid encoding jellyfish green fluorescent protein (pGFP; 100 ug/injection), vehicle (3% sucrose in phosphate buffered saline), PEI- pIL10 (10 ug/injection) or PLGA- pIL10 (350 ug PGLA/injection). For repeated injections, a 2nd injection was given on day 13. Allodynia was reassessed every 1 to 4 days. Allodynia was stable in control treated rats but was reversed (prolonged reversal lasting 40+ days), in rats given 2 i.t. injections of: 1) pIL10 2) PEI- pIL10 & 3) PLGA-IL10. Single i.t. injections of pIL10, PEI- pIL10 or PLGA- pIL10 produced a brief 3-6 day reversal of allodynia. Importantly, rhodamine-labeled PLGA-micro-particles were visible immediately & at 3 days after i.t. injection. Ongoing studies using i.t. rhodamine-labeled PLGA-micro-particles (either without DNA or with pGFP DNA) are aimed at examining the spread, the distribution in superficial spinal cord or deeper parenchymal layers & gene expression. This approach to pain control represents a dramatic departure from all other available therapies. Support: Avigen & NIH HL56510, DA015656, DA018156 & DA015642.

645. ITRs Are Necessary for IL-10 Encoding Naked Plasmid Induced Reversal of Chronic Pain State in Rat

Travis Hughes,¹ Stephen J. Langer,¹ Evan M. Sloane,² Erin D. Milligan,² John Mahoney,² Brian Jekich,² Kirk W. Johnson,³ Ray E. Chavez,³ Steven F. Maier,² Linda R. Watkins,² Leslie A. Leinwand.¹

¹MCD Biology, University of Colorado, Boulder, CO;

²Psychology, University of Colorado, Boulder, CO; ³Avigen, Inc., Alameda, CA.

Controlling chronic pain in humans is a major unresolved problem. Spinal cord astrocytes and microglia are critically involved in the creation and maintenance of diverse enhanced pain states via the release of proinflammatory cytokines. Interleukin-10 (IL-10), a potent anti-inflammatory cytokine, suppresses proinflammatory cytokine production and activity. Control of chronic neuropathic pain such as sensitivity to light touch (allodynia) requires chronic spinal delivery of IL-10. Chronic pain can be surgically induced in rats by loose ligation of chronic cat gut around the sciatic nerve in four places. This creates a chronic constriction injury (CCI) as the nerve bundle swells against the irritating ligation. The CCI model produces an enhanced pain phenotype for 3 months. We have demonstrated that this pain is reversed transiently (~4hrs.) through intrathecal injection of recombinant IL-10 protein and that two temporally spaced injections of an IL-10 expression vector containing inverted terminal repeats (ITRs) reverses this pain long term (3+ months, Milligan et al., these proceedings). The role of ITRs in gene expression is not well known. However, there is evidence that they possess transcription promoter and enhancer activity (Flotte et al., 1992 *Am J Respir Cell Mol Biol.* 7:p349-56 and 1993 *J Biol Chem.* 268: p3781-90). Based on this and other evidence in the literature we sought to determine if the ITRs in our expression vector, which are located 5' of the CMV enhancer and Chicken β -actin promoter (5' ITR) and 3' of the SV 40 poly A tail sequence (3' ITR), influence reversal of pain in the CCI model. To this end, we deleted both ITRs

from the expression vector and found that it was incapable of producing pain reversal. We next asked if one or both ITRs are necessary for pain reversal. We found that plasmids containing just the 3' or 5' ITR were both effective in reversing the chronic pain state. Ongoing studies seek to determine the effect of multimerization of the injected plasmid on pain reversal and gene expression and to further characterize the role of ITRs on gene expression and pain reversal in the CCI model.

646. Zinc Finger Protein Transcription Factors as Potential Therapeutic Agents for the Treatment of Neuropathic Pain

Siyuan Tan,¹ Andrew McNamara,¹ Yann Jouvenot,¹ Dave Krisky,² Darren Wolfe,² Bradley Compos,¹ Xiaohong Zhong,¹ Joe Glorioso,² H. Steven Zhang,¹ Philip D. Gregory.¹

¹Gene Regulations, Sangamo BioSciences, Richmond, CA;

²Department of Molecular Genetics and Biochemistry, University of Pittsburgh, School of Medicine, Pittsburgh, PA.

Neuropathic pain is a complex disorder resulting from injury to the nerve, spinal cord or brain. Current therapies for neuropathic pain using small-molecule drugs are limited by a range of drug-related side effects, primarily due to their lack of specificity in targeting the receptor/channel of choice. Gene therapy approaches to the treatment of neuropathic pain address this problem in two ways. Firstly, the difficulty in selectively targeting key biologically validated pain receptors at the protein level is circumvented by treatments that can derive specificity at the DNA level and drive the regulation of the target gene. Secondly, the effector molecule can be coupled to a delivery vector that specifically targets the organ of interest – in this case the dorsal root ganglia (DRG) – thus creating a more “local” treatment.

We have developed zinc-finger protein transcription factors (ZFP TFs) for the repression of target gene expression. Such ZFP TFs hold therapeutic promise as they have been shown to be highly efficacious and yet function with singular specificity within the mammalian genome. We have chosen to focus our efforts on the development of ZFP TF repressors against two well-validated targets: Tyrosine kinase A receptor or high-affinity NGF receptor (TrkA), and the sodium channel Nav1.8 (or PN3, SCN10A). Here we report that we have successfully obtained TrkA-specific ZFP TFs capable of potent target gene repression in cell line models *in vitro*. Consequently, we have initiated experiments aimed at evaluating the efficiency and efficacy of viral delivery into the DRG neurons using non-replicating herpes simplex virus (HSV) vectors. HSV vectors are naturally neurotropic and have been demonstrated to transduce the DRG *in vivo* with high efficiency following a single subcutaneous injection. We are currently evaluating our ZFP TF reagents when delivered by HSV vectors in both primary cell DRG cultures and subsequently in animal models of neuropathic pain. Our preliminary data has indicated that i) DRG neurons could be transduced to high efficiency by viral HSV vectors; ii) HSV vectors drive good expression of the gene specific ZFP TFs and; (iii) HSV driven ZFP TF expression results in target gene repression in DRG neurons. These data support the pre-clinical evaluation of ZFP TF in additional animal models of neuropathic pain.