A Novel, Locally Delivered TrkA Inhibitor for the Treatment of Joint Pain: Efficacy in Preclinical Models of Arthritis

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Introduction: Arthritic joint pain and associated functional impairment is a major cause of disability, and there is a high unmet need for effective and safe analgesic treatments. Recent clinical trials for intravenous (IV) infusions of antibodies which block nerve growth factor (NGF) have demonstrated outstanding pain relief in osteoarthritis (OA) patients (1, 2), however an increased drug-related risk for accelerated OA progression was observed in some participants. As part of an alternate strategy for targeting this pathway, we have identified a novel small molecule compound, GZ389988, which potently inhibits TrkA, the high-affinity receptor for NGF. GZ389988 was formulated for intraarticular (IA) delivery, and its effects on local knee pain were tested in rats using both a monosodium iodoacetate (MIA) induced model of OA, as well as an arthritis flare model mediated by streptococcal peptidoglycan polysaccharide (PGPS).

Methods: Identification and characterization of TrkA inhibitor GZ389988: Small molecule drug candidate screening was conducted to select inhibitors of Trk family tyrosine kinases. Compound ‘hits’ were refined based on selectivity against non-related kinase representatives, and low aqueous solubility. Selected compounds were further evaluated using a CellSensor cell-based TrkA assay (Invitrogen), and additional screening was performed using the DiscoveRx KINOMEScan 442 kinase panel and Millipore safety and liability panel. Acute cytotoxicity was assessed for a variety of cell types, including primary human chondrocytes and synoviocytes (Articular Engineering), by exposing cultured cells to increasing concentrations of GZ389988 for 24h and quantification using the Neutral Red dye uptake assay (3). In vivo biocompatibility testing was conducted by IA injection of GZ389988 or placebo/vehicle into rat knee joints. Animals were monitored for clinical parameters for up to 7 days post-injection, whereupon knee joints were processed for histopathology scoring of cartilage and subchondral bone, and synovial inflammation, proliferation, fibrosis and neovascularization.

Preclinical rat OA model: Unilateral knee OA was initiated by IA injection of MIA (1mg; Sigma). One week later, diseased (ipsilateral) joints were treated with a single IA injection of GZ389988 or placebo/vehicle (n=8/treatment group), and differences in hind-limb weight distribution were measured weekly for 4 weeks using a commercial Incapacitance Tester (4). In some cases, GZ389988 was administered to the
contralateral knee instead, to test for the occurrence of systemic compound distribution affecting the 
ipsilateral joint.

Preclinical rat arthritis flare model: Unilateral primary knee arthritis was induced by IA injection of PGPS 
(0.125mg; Lee Laboratories). Two weeks later, animals received a single IA injection of GZ389988 or 
placebo/vehicle into the diseased (ipsilateral) joint (n=12/treatment group). Disease reactivation flares 
were triggered by IV administration of PGPS (5) at weeks 1 and 3 following drug (or placebo) treatment. 
Gait analysis was conducted by obtaining inked pawprint impressions from animals which were allowed 
to ambulate freely along a papered walkway. Gait scoring and quantification was performed using 4 
distinct pawprint pairs per animal, collected daily for 4 days following each reactivation.

**Results:** GZ389988 was identified as a potent inhibitor of TrkA. Similar IC50 values were observed for 
TrkA, TrkB and TrkC, and the growth factor receptor cFMS (CSF1R), however GZ389988 was highly 
selective against a wide panel of other kinases. In addition, GZ389988 has very low aqueous solubility, 
thus facilitating its formulation for local administration. No cytotoxicity was observed in cell-based 
assays using criteria of LD50/IC50 ratio >100. In vivo biocompatibility studies with GZ389988 showed 
neither a treatment effect on body weight or clinical appearance, nor any significant histopathologic 
changes in joint tissues.

In the rat MIA model of OA, a single IA injection dose of GZ389988 into the ipsilateral joint provided a 
significant reduction in local joint pain (weight-bearing imbalance) for 4 weeks. Treatment of the 
contralateral joint with GZ389988 had no effect on ipsilateral joint pain (Fig. 1), illustrating the absence 
of a substantial systemic effect following IA administration. GZ389988 was also efficacious in the PGPS 
arthritis model, demonstrating a significant improvement of gait analysis scores following painful 
disease reactivation flares (Fig. 2).

**Discussion:** Elevated levels of NGF are associated with tissue injury, and have been observed in a 
number of painful conditions including inflammatory arthritis and OA (6). In addition, TrkA, which is the 
high-affinity receptor for NGF, is expressed by OA synovial fibroblasts and is upregulated in response to 
NGF (7). A number of clinical trials have been conducted in OA patients receiving IV treatment with NGF-
blocking antibodies, and have demonstrated significant analgesic efficacy with excellent responder 
orates. However, unexpected adverse events (AEs) affecting either the index or non-index joints of some 
patients led to a clinical hold being imposed by the FDA and a comprehensive data review at an Arthritis 
Advisory Panel meeting held in March 2012. Although the exact cause of this observed rapidly 
progressive OA is unclear, the rate of AEs was highest for patients receiving co-administered NSAIDs, 
such that exclusion of such co-treatment will be a likely requirement for resuming clinical studies. Our 
approach toward targeting the NGF signaling pathway to treat joint pain, including strategies for risk 
mitigation, has been to develop a locally-delivered inhibitor of the NGF receptor, TrkA. In addition to the 
efficacy data presented here, pharmacokinetic studies and safety pharmacology and preclinical 
toxicology data support the development of GZ389988 as a novel therapeutic for this application. 

**Significance:** A novel TrkA inhibitor, GZ389988, has been identified and formulated for intraarticular 
administration. A single injection of GZ389988 demonstrated significant local efficacy in two preclinical 
arthritis pain models, providing proof of principle for developing this therapeutic modality to treat joint 
pain.
Figure 1. Efficacy of GZ389988 in rat MIA model of joint pain. Disease was initiated by a single IA injection of MIA into the knee joint (ipsilateral). One week later, a single dose of GZ389988 was administered by IA injection into the ipsilateral or contralateral knee. Differences in hind limb weight-bearing distribution were measured immediately prior to injection of MIA (baseline; BL) and GZ389988 (Time 0), and weekly thereafter for 4 weeks. Data are presented as mean ± SEM.

Figure 2. Efficacy of GZ389988 in rat PGPS model of joint pain. Disease was initiated by a single IA injection of PGPS into the knee joint (ipsilateral) three weeks prior to the first reactivation. A single dose of GZ389988 was administered by IA injection into the knee one week prior to the first reactivation. Disease reactivation (painful flare) was induced on study days 0 and 14 by IV administration of PGPS. Gait analysis was performed based on pawprint areas, and scored as follows: 1=mild limping/pain; 2=moderate limping/pain; 3=marked limping/pain; 4=severe hopping/pain. Data are presented as mean ± SEM.