B. Stride frequency also trended towards normalization post IA BoNT B, approaching statistical significance (p= 0.0624). Evoked pain behaviors improved following IA BoNT B, but did not reach statistical significance. No weakness of the hindlimb was noted following IA BoNT B (grasp p= 0.7872 cling p=0.5433)

**Conclusions:** Chronic degenerative arthritis pain can be quantitated in a murine model using visual and computerized gait analysis, and joint tenderness scores. Visual and computerized gait analysis both showed a significant impairment in gait in arthritis mice, improving after IA BoNT B suggesting a substantial analgesic effect. Evoked pain responses increased overall with arthritis, decreasing with IA BoNT B, but not reaching statistical significance. Further studies are underway to expand on these results.

These findings support the hypothesis that chronic arthritis pain may be amplified by neuropeptide release in the periphery due to efferent neurogenic signals. Interruption of neuropeptide release by IA BoNT B appeared to decrease pain responses in the joint and improve gait abnormalities. IA BoNT B was safe: no increased variability in NOx responses was observed across human subjects (Figure 1 left). In the presence of IFNγ costimulant, IL17 induced average 2-fold increase in NOx production above that of IL17 alone (IL17: average NOx = 22 μM, IL17 + IFNγ: NOx = 45 μM, Figure 1 left). In contrast, stimulation with IL17 alone led to a significant elevation in PGE2 levels in all 4 samples (average 14-fold increase, Figure 1 right). As for the NOx result, co-stimulation with IL17 and IFNγ led to a large increase in PGE2 release, more than 24-fold over control values (Figure 1 right, IL17 + IFNγ PGE2 release = 1.36 ng/ml; Control PGE2 release = 0.06 ng/ml). In general, the induction of NOx and PGE2 release by IL17 and IFNγ co-stimulation was significantly larger than that due to TNFα stimulation, for which few changes in NOx and a 10-fold increase in PGE2 was observed.

**Conclusions:** The results of this study demonstrate that degenerative human IVD cells may respond to IL17 stimulation with increased production of the inflammatory mediators, PGE2 and NOx, with a more robust effect observed in the presence of IFNγ. Degenerative and herniated IVD explants are known to contain IFNγ, and IFNγ is known to act synergistically with IL17 to promote release of inflammatory mediators in other cell types. This work demonstrates that the responsiveness of IVD cells to IL17 and IFNγ is consistent with other cell types, and suggests a potential role for these cytokines in contributing to IVD pathology.

**References:**

from viral mediated proof of concept to development of a protein therapeutic, we determined in rats and rabbits the intraarticular pharmacokinetics of sFlt01, a VEGF neutralizing Fc fusion protein, and subsequently the feasibility of sustained release formulation for prolonging residence time in the joint at or above therapeutic levels for 30–90 days. N-methyl pyrrolidone/poly(lactic acid) (NMP/PLA) depot is considered here as the formulation strategy. Methods: Iodinated sFlt01 PK in rat: 65ug 125I-sFlt01 was intraarticularly administered to rat knees 4 weeks after meniscectomy surgery. Radioactivity of the knee joints was measured at sacrifice 1, 6, 24, 72, and 144hrs post injection. sFlt01 PK in rabbit: 150ug sFlt01 was intraarticularly administered to rabbit knee 4 weeks after ACL surgery. Rabbits were sacrificed at 6hr, 8hr, 24hr, 18hr, and 48hr post injection. sFlt01 level in serum, urine, synovial fluid, articular cartilage and synovium was quantified by ELISA. sFlt01 sustained release: NMP/PLA depots were formed by dissolving PLA in NMP in a 40:60 w:w ratio. Lyophilized sFlt01 was added to a concentration of 0.8-1.7 w% and dispersed by sonication. Depots were formed by pipetting ~10ug of material into PBS, allowing the NMP to dissipate and the depot to solidify. In vitro release was conducted under physiologic conditions (PBS + Tween 20, 37°C). Drug release was quantified by BCA. VEGF binding activity was assessed by Biacore and ELISA. Results: Iodinated sFlt01 PK in rats: The knee joint terminal half life of sFlt01 is estimated to be 11 hours in OA rats. There is a very small shift in terminal elimination half life of sFlt01 in joint between OA rats and non-OA rats. sFlt01 PK in rabbits: In general, terminal elimination half life of sFlt01 in joint synovial fluid, synovium and cartilage is very similar in OA and non-OA groups. Exposure in joint synovial fluid, synovium, and cartilage is higher in non-OA group than that in OA group. Systemic exposure in the serum is comparable between OA and non-OA animals. Absorption of sFlt01 into systemic circulation appears to be very slow. sFlt01 is not detected in urine, kidney, heart, liver and lung. sFlt01 sustained release: The NMP/PLA depot showed a nonlinear release pattern with ~15% released in 26 days. sFlt01 released from NMP/PLA depots demonstrated the ability to neutralize VEGF, as determined by an ELISA assay. Biacore studies showed that sFlt01 released from NMP-PLA depots lost some capacity to bind to protein A, indicating modification to the Fc portion, but that captured sFlt01 still strongly bound VEGF.

\[
\begin{array}{cccc}
\text{t_{1/2} (hrs)} & \text{Synovium} & \text{Cartilage} & \text{Synovial Fluid} & \text{Serum} \\
\text{OA} & 9.84 & 7.02 & 7.77 & 66.5 \\
\text{Non-OA} & 9.42 & 6.19 & 10.3 & 71.5 \\
\end{array}
\]

Conclusions: Joint half-life of sFlt01 is as short as hours, suggesting that sustained release formulation is necessary to prolong sFlt01 residence time in the joint. NMP/PLA depots were shown to deliver sFlt01 for ~30 days. New formulations will be in need to further prolong release, characterize late stage burst and sFlt01 alterations, and to test pharmacokinetics in vivo.

500

SYMPTOMATIC OUTCOMES IN A PHASE 1 SAFETY AND TOLERABILITY STUDY OF OP-1

D.J. Hunter, M.C. Pike, B.L. Jonas, E. Kisin, J. Krop, T. McAlindon


Purpose: There are no proven therapies that modify the structural changes in osteoarthritis (OA). Preclinical data suggests that intra-articular recombinant human Osteogenic Protein-1 (OP-1) has reparative effects on cartilage, as well as on symptoms of joint pain. The objective of this study was to determine the safety and tolerability as well as dose-limiting toxicity and maximal tolerated dose of intra-articular OP-1. The secondary objectives were to determine the symptomatic responses through 24 weeks.

Methods: This was a Phase 1, double-blind, randomized, multi-center, placebo-controlled, single-dose escalation safety study consisting of 4 dosing cohorts in participants with knee OA. Each cohort was to consist of 8 treated subjects, with treatment allocation in a 3:1 active (of intra-articular OP-1 (also called BMP-7)) to placebo ratio. Eligible subjects were persons with symptomatic radiographic knee OA aged over 40 years. The primary objective of this study was to determine the safety and tolerability including laboratory assessments, immunogenicity data and radiographic assessments. Secondary objectives were to determine the proportion of subjects with a 20%, 50%, and 70% improvement in the WOMAC pain and function subscales at 4, 8, 12, and 24 weeks. Other secondary outcomes included the change from baseline to 4, 8, 12, and 24 weeks for the subscales of the Knee and Osteoarthritis Outcome Score (KOOS) survey, patient global, physician global, quality of life from the SF-36 and the OARSI responder criteria.

Results: The mean age of participants was 60 years and 73% were female. All 33 subjects who were enrolled completed the study, most adverse events were mild or moderate and were similar in placebo and OP-1 groups. The 1mg OP-1 group showed a higher frequency of injection site pain and there was no ectopic bone formation seen on plain x-rays. Most subjects in both the OP-1 and placebo groups experienced a 20% improvement in pain and by 12 Weeks, the OP-1 group was similar to placebo. In the participants who received 0.1mg and 0.3mg OP-1, there was a trend toward greater symptomatic improvement than placebo. The other secondary endpoints showed similar trends including the OARSI responder criteria for which the OP-1 groups had more responders than placebo (see table).

<table>
<thead>
<tr>
<th>Number (%) of Responders</th>
<th>0.03mg (N=7)</th>
<th>0.1mg (N=6)</th>
<th>0.3mg (N=6)</th>
<th>1mg (N=6)</th>
<th>All (N=25)</th>
<th>Placebo (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 4</td>
<td>3 (42.9%)</td>
<td>4 (66.7%)</td>
<td>1 (16.7%)</td>
<td>3 (33.3%)</td>
<td>10 (40%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Week 8</td>
<td>3 (42.9%)</td>
<td>4 (66.7%)</td>
<td>2 (33.3%)</td>
<td>2 (33.3%)</td>
<td>11 (44%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Week 12</td>
<td>1 (14.3%)</td>
<td>4 (66.7%)</td>
<td>2 (33.3%)</td>
<td>1 (16.7%)</td>
<td>8 (32%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Week 24</td>
<td>1 (14.3%)</td>
<td>5 (83.3%)</td>
<td>2 (33.3%)</td>
<td>1 (20%)</td>
<td>9 (37.5%)</td>
<td>1 (12.5%)</td>
</tr>
</tbody>
</table>

Conclusions: There was no dose limiting toxicity identified in this study. The overall trend for OP-1 symptom response was better than placebo with the 0.1 and 0.3 mg groups. The bell shaped dose response curve is consistent with pharmacodynamic studies in pre-clinical models. This symptom response, together with the lack of dose limiting toxicity provide further support for the continued development of this product for treatment of osteoarthritis.

501

RELATIVE EFFICACY OF HYALURONIC ACID VERSUS CORTICOSTEROIDS IN THE TREATMENT OF KNEE OSTEOARTHRITIS: META-ANALYSIS


1 Tufts Med. Ctr., Boston, MA; 2 Tufts Univ. Sch. of Med., Boston, MA

Purpose: Hyaluronic acid (HA) injections are widely used to treat knee osteoarthritis (OA). Current evidence on their efficacy, based on individual trials and meta-analyses, is inconsistent. In the face