the reversible synovial hyperplasia seen in the toxicity studies and is expected from IA-injected particulate agents that lodge in the synovium. The cartilage damage improvements together with the gait data indicates that MM-II treatment was overall beneficial in the rat MMT model and has the potential to be a disease modifying OA therapy in addition to its pain lowering ability.

PRESENTATION NUMBER: 4

INTRAMUSCULAR GLUTEAL GLUCOCORTICOID INJECTION VERSUS INTRA-ARTICULAR GLUCOCORTICOID INJECTION IN KNEE OSTEOARTHRITIS: A 24-WEEK MULTICENTER RANDOMIZED CONTROLLED NON-INFERIORITY TRIAL

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Purpose: To assess whether intramuscular (IM) gluteal glucocorticoid injection is non-inferior to intra-articular (IA) glucocorticoid injection in reducing knee pain for patients with knee osteoarthritis (OA) in primary care.

Methods: The study was a pragmatic randomized controlled non-inferiority trial with two parallel groups. Patients ($\geq$ 45 years) with symptomatic knee OA were randomly (1:1) allocated to receive an injection of 40 mg triamcinolone acetonide either IA in the knee joint or IM in the ipsilateral ventrogluteal area. All patients were followed for 24 weeks after the injection. The primary outcome was the Knee injury and Osteoarthritis Outcome Score (KOOS) pain score (0-100, 0 indicates extreme pain) at 4 weeks, with a non-inferiority margin of 7. Secondary outcomes included the non-inferiority of KOOS pain scores at 2, 8, 12 and 24 weeks. Statistical analysis was based primarily on the per-protocol (PP), and secondary on the intention-to-treat (ITT) principle. Linear mixed models with repeated measurements were used for calculating group differences over time, adjusted for baseline KOOS pain score, sex, presence of depression and duration of knee OA. Minimally clinically important difference (MCID) for KOOS pain score = 7.

Results: Of a total of 145 patients included, 65% female, mean (SD) age 67 (10) years, mean (SD) baseline KOOS pain score 48 (17). 71 were randomized to the IA group and 74 to the IM group. In PP analysis, 138 patients were included with 66 in IA group and 72 in IM group. Patients reported a clinically relevant improvement in knee pain from 2 to 12 weeks after the injection in both groups (Fig. 1). At 4-week follow-up, the mean inter-group difference in KOOS pain score was not statistically significant, but its 95% confidence interval (CI) contained the pre-specified non-inferiority margin of 7 (IA minus IM: 3.4; 95CI, -3.3-10.1; $p_{superiority} = 0.320$) (Fig. 2). The differences of KOOS pain scores at 2, 8, 12 and 24 weeks were all not significant, and non-inferiority was shown at 8 and 24 weeks follow-up (Fig. 2). All the results were similar in ITT analysis.

Conclusions: KOOS pain score differences between the two groups were non-significant and smaller than MCID over the 24 weeks. However, IM glucocorticoid injection, compared to IA injection, could present inferior effectiveness in some cases at 4 weeks after injection. IM injection was non-inferior to IA injection only at 8 and 24 weeks.

Figure 1. KOOS pain scores over 24 weeks of patients in PP analysis. The shaded area indicates 95% CI. Higher KOOS pain score indicates less pain.

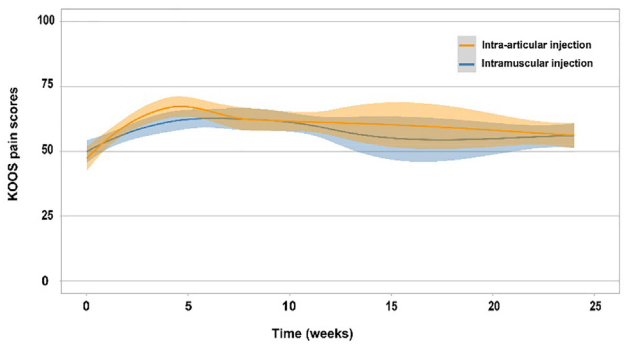
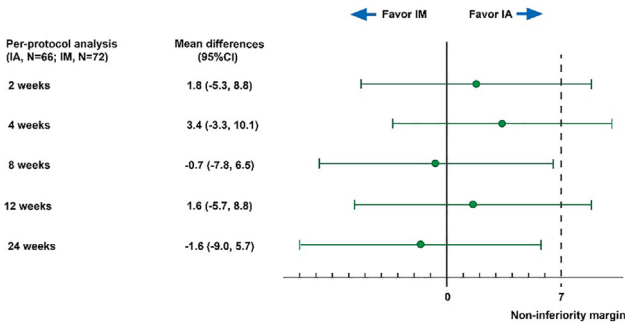


Figure 2. Forest plot for adjusted KOOS pain score differences (IA group minus IM group) and their 95% CIs.



PRESENTATION NUMBER: 5

INTRA-ARTICULAR DRUG DEPOTS FOR CONTROLLED RELEASE OF HEAVY CHAIN ONLY ANTIBODIES BLOCKING JOINT INFLAMMATION

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Purpose: In many inflammation-driven diseases, blocking IL-1 signaling can be a potential strategy to help restore normal homeostasis. The modified recombinant version of IL-1RA, known by the name Anakinra, which competes with IL-1β is seen as a potential disease modifying osteoarthritis drug (DMOAD). However, as with many therapeutic proteins it shows a short half-life and loss of bioactivity. Currently, this problem is addressed by frequent injections of high doses of therapeutic proteins that is associated with unwanted side effects and patient morbidity. Various ways were explored to formulate IL-1RA within a slow release system in order to be released over a prolonged period of time. Nevertheless, the problem of short-half life and loss of bioactivity remains in these slow release systems. Here we report an alternative for Anakinra, based on the variable domain of single chain heavy chain only antibodies (VHHs). These VHHs are easy to produce in yeast and are more cost effectively than conventional antibodies. In addition, their high stability at extreme temperatures and pH values makes them suitable to investigate whether they can be potential DMOADs. As a hypothesis, we believe that when incorporating VHHs that block inflammation in a slow release system, they could potentially solve the issue of short half-life and bioactivity for therapeutic protein delivery. As a slow release system, we want to use an injectable gel with excellent controlled release properties in the intra-articular space. The aim of this study is to provide a proof of concept for the controlled release of a bioactive VHH that could block IL-1 signaling in the intra-articular space.

Methods: After immunization, phage library construction and panning rounds, we obtained several clones of VHHs binding IL-1R with high specificity. Dose response ELISAs and surface plasmon resonance imaging (SPRi) were used to characterize the binding affinity of the VHH clones to IL-1R. Biological activity of the VHHs was measured using a HEK293t cell line stably transfected with an NFκB reporter construct after co-stimulation with IL-1β. As a positive control, we used a biosimilar of IL-1RA. In vitro release studies were carried out using an injectable sustained drug release system composed of end-capped PCLA-PEG-PCLA triblock copolymers in various compositions, which are suited for controlled release of proteins. The compositions were formulated with final loading of 2.8 and 28 μg/ml anti-IL-1R VHH for at least 28 days. The concentration is based on a respectively 1000-10,000 times excess of IL-1β concentration occurring in synovial fluid of OA patients.

Results: The characterization experiments showed that four of the IL-1R binding VHHs have an affinity in the low nanomolar range. In addition, in epitope binning experiments we saw that some combinations of VHH and IL-1RA show a possible additive or maybe even a

synergistic effect in blocking IL-1β. We determined the effect of the IL-1R binding VHHs in the presence or absence of IL-1β on the reporter cell line. Here, it was found that two anti-IL1R VHHs were able to block IL-1 signaling. Subsequently, we incorporated these two VHHs within PCLA-PEG-PCLA drug depots and assessed the release rate. The drug depots were liquid at room temperature ensuring easy injectability and formed a drug depot after injection. Over a time span of 28 days, we took several samples and observed sustained release in at least two out of three compositions, one with a release in the first 14 days and the other a stable release over at least 60 days. In all the compositions that showed release, biological activity was confirmed over time using both dose response ELISA and SPRi.

Conclusions: We have isolated two IL-1R binding VHHs that block IL-1 signaling. Furthermore, we showed that these VHHs can be incorporated within PCLA-PEG-PCLA drug depots and show a prolonged in vitro release and biological activity. VHHs blocking of IL-1R may constitute a cost-effective anti-inflammatory drug and an alternative DMOAD for recombinantly produced proteins or conventional antibodies.

PRESENTATION NUMBER: 6

THE "PLACEBO EFFECT" IN OSTEOARTHRITIS CLINICAL TRIALS: CHALLENGING THE NARRATIVE

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Purpose: The Results of randomized controlled trials of potential disease-modifying osteoarthritis drugs (DMOADs) often show a precipitous decline in the estimated mean pain score for the active treatment arm and the placebo arm during follow-up. The decrease in the mean pain score in the placebo arm is often attributed to the placebo effect, which has been defined as "a beneficial effect produced by a placebo drug or treatment, which cannot be attributed to the properties of the placebo itself, and must therefore be *due to the patient's belief in that treatment*". We offer an alternative explanation. Our objective is to illustrate conditions under which longitudinal outcome measures will reliably produce an apparent "placebo effect", using data from an observational study.

Methods: The Osteoarthritis Initiative is a longitudinal observational study of participants with or at risk of symptomatic knee OA. Participants completed the WOMAC questionnaire, underwent bilateral posteroanterior fixed-flexion weight-bearing knee radiography with joint space width measurement, and blood pressure measurement, at baseline and annual clinic visits through the four-year follow-up. We selected participants with Kellgren-Lawrence (KL) grade 2 or 3 in the right knee at baseline (n=1,837), grouped them according to baseline right knee WOMAC pain score (categories: 0, 1, 2-4, 5-7, and 8-20) and plotted the mean pain score for each group through the Y4 follow-up visit (Fig. 1a). Similarly, we selected participants with KL 2 or 3 in the right knee at Y1 (n=1,749) and grouped them according to the Y1 right knee WOMAC pain score, plotting group means through the Y4 follow-up visit (Fig. 1b); the same analysis was repeated at Y2 (n=1,662; Fig. 1c). A similar analysis was conducted for medial fixed JSW(x=0.250; fJSW) in KL 2,3 right knees (Fig. 2), and systolic blood pressure among all OAI participants (Y0 n=4,795, Y1 n=4,260, Y2 n=4,031; Fig. 3). For each outcome measure (WOMAC pain, medial fJSW, and systolic blood pressure) we used observations at baseline and Y1 to estimate the ratio of between-person variance to total variance (ρ), where total variance is the sum of between-person variance and within-person variance, and generated bootstrap 95% confidence intervals(CI).

Results: The group of participants with WOMAC pain scores ≥ 8 at baseline had a lower average pain score at the Y1 visit, followed by stable average pain scores at subsequent visits, while the participants with a WOMAC pain score of 0 at baseline had a greater average pain score at Y1 (Fig. 1a). Similar trends occurred when participants were