CR10049, THE FIRST OA-TARGETED KINASE INHIBITOR, AS A TREATMENT FOR THE INFLAMMATORY OA PHENOTYPE

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Background

- Osteoarthritis (OA) is a heterogeneous disease of the **entire joint** with multiple clinical phenotypes and disease mechanisms. **Inflammatory OA** is a common phenotype characterized by marked **synovitis**, **pain** and a faster rate of disease progression. So far, targeting a single mechanism to prevent or delay the progression of OA while controlling the disease symptoms, was not successful and efforts to develop effective disease-modifying OA therapies against single major molecular targets failed. We designed an OA-targeted kinase inhibitor, CR10049, that inhibits multiple OA-specific kinases involved in synovial inflammation and fibrosis, cartilage degradation, bone remodeling and chronic pain, as a valid therapeutic strategy to treat OA patients with an inflammatory OA phenotype. The aim of this study was to characterize the effects of this **novel small molecule** in in vitro and in vivo, including chemical (MIA, monoiodoacetate) and surgical (MMT, medial meniscal tear) OA models.

Methods

- **In vitro:** A panel assay for **159 human kinases** was used to evaluate the binding affinity of CR10049. **Human fibroblast-like synoviocytes** (HFLS) were stimulated with IL-1β 10ng/ml to mimic the inflammatory microenvironment of OA joint tissue, and gene expression analysis was performed.
- **MIA (chemical OA) model** (Inotiv; Boulder, USA) was induced in Sprague Dawley rats (n=10/group, n=6 naïve; 175-225 g) by injecting 2 mg MIA into the right knee. Animals were randomly treated intraarticularly (IA, 30 µl) on days 3 and 17 with vehicle or CR10049 at 250, 25 or 2.5 µg/knee. An active control group received triamcinolone acetonide (TCA) 60 µg/knee on day 3. Pain was evaluated by electronic von Frey testing and dynamic weight bearing.
- **MMT (surgical OA) model** (Inotiv; Boulder, USA) Lewis rats (n=12/group, n=6 naïve; 275-300 g) underwent medial meniscal tear (MMT) surgery into the right knee. Animals were IA (30 µl) injected on days 14 and 28 with vehicle or CR10049 at 250, 25 or 2.5 µg/knee. An active control group received fibroblast growth factor-18 (FGF-18) 5 µg/knee on day 7, 21 and 35.

Results

CR10049 INHIBITS OA-INVOLVED KINASE FAMILIES

CR10049 is an extremely potent inhibitor of Src family (cSrc, Lck, Fyn, Fgr, Frk) kinases, that have a major role in cartilage and bone pathological alteration and in pain[1, 2]. Concurrently, CR10049 inhibits also three other OA-targeted kinase families in a nanomolar range (IC50 < 20 nM).

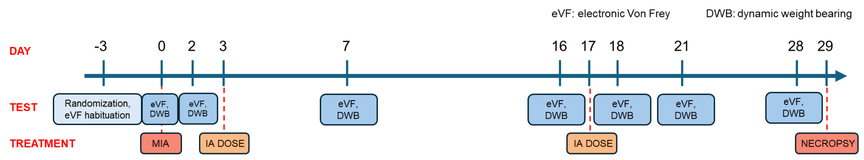
Kinase family	Src (cSrc, Fyn, Lck)	Growth factor receptors (VEGFR, FGFR1)	Ephrin receptor (EPHA3, EPHA5)	TRK members (TRKB, TRKC)
IC_{50} range (nM)	0,04-8 nM	3-7 nM	5-13 nM	7-16 nM

CR10049 INHIBITS INFLAMMATION IN A HUMAN SYNOVIOCYTE MODEL

Inhibitory effect of CR10049 on gene expression of pro-inflammatory and matrix-degradative enzymes in a synovial inflammation model.

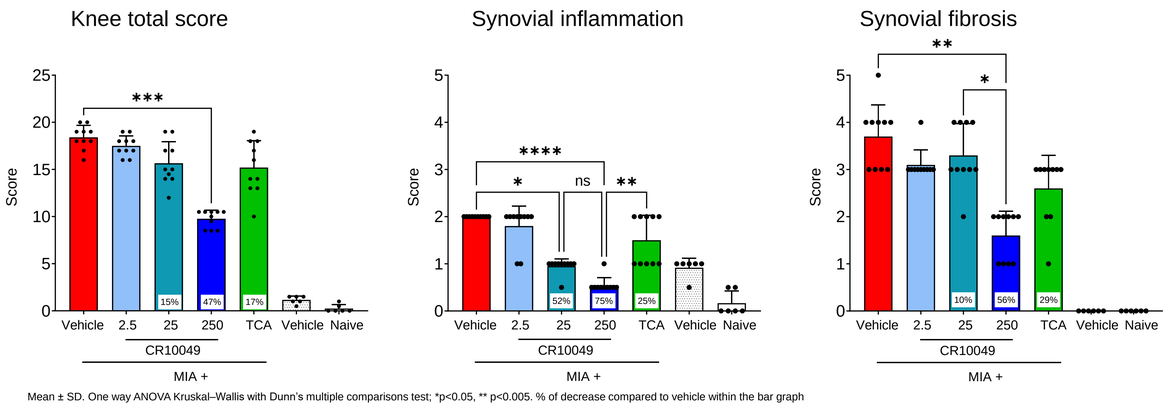
Marker	COX2	IL-6	MMP13	MMP3
IC_{50} (nM)	23	38	31	72

CR10049 IMPROVES JOINT STRUCTURE AND PAIN IN THE RAT MIA MODEL



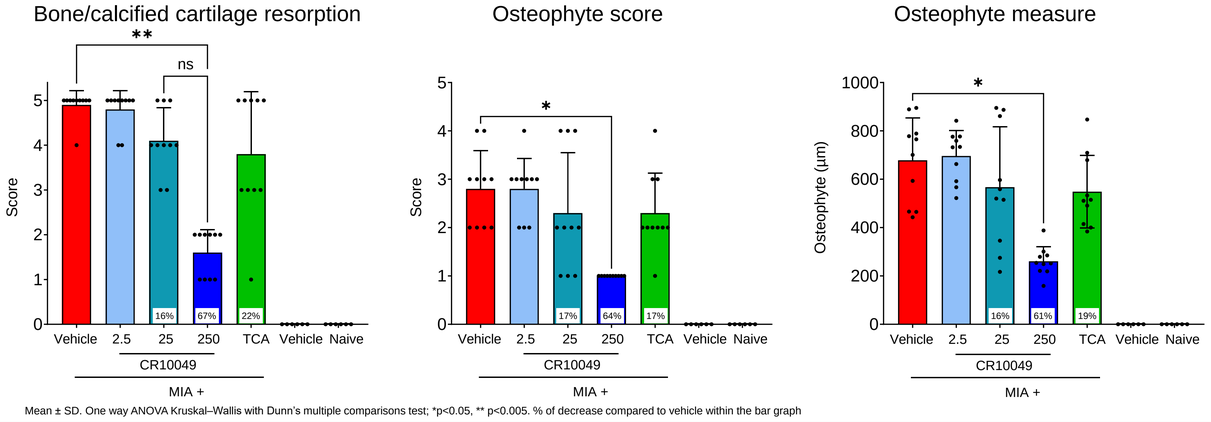
No sign of toxicity was seen as no changes in body weight were observed.

REDUCES SYNOVIAL INFLAMMATION AND FIBROSIS



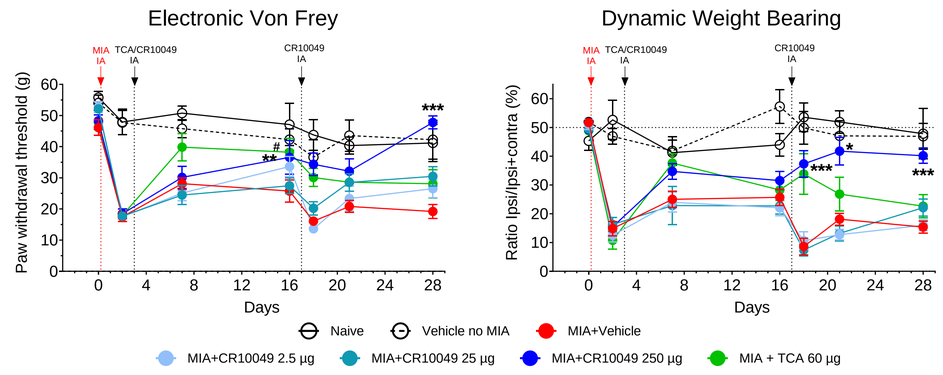
Mean ± SD. One way ANOVA Kruskal–Wallis with Dunn's multiple comparisons test; *p<0.05, ** p<0.005. % of decrease compared to vehicle within the bar graph

IMPROVES BONE REMODELING



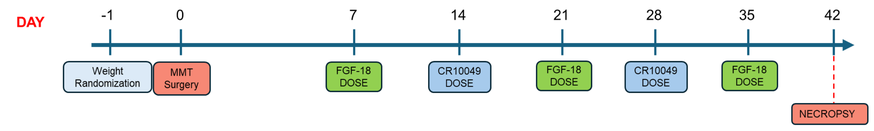
Mean ± SD. One way ANOVA Kruskal–Wallis with Dunn's multiple comparisons test; *p<0.05, ** p<0.005. % of decrease compared to vehicle within the bar graph

IMPROVES MECHANICAL ALLODYNIA AND WEIGHT-BEARING IMBALANCE IN MIA



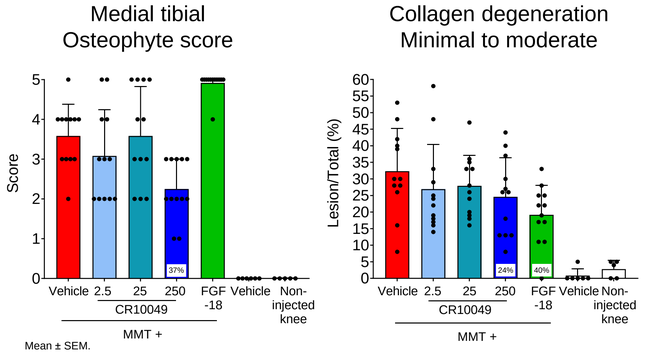
Mean ± SEM. Two-way ANOVA with Tukey's multiple comparisons test. * or # p<0.05, ** p<0.01, *** p<0.001 vs Vehicle

CR10049 IMPROVES JOINT STRUCTURE IN THE RAT MMT MODEL

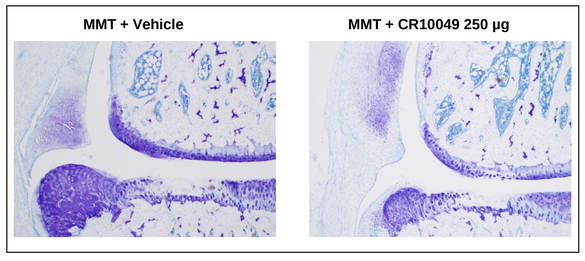


No sign of toxicity was seen as no changes in body weight were observed.

REDUCES OSTEOPHYTE SCORE AND COLLAGEN DEGENERATION



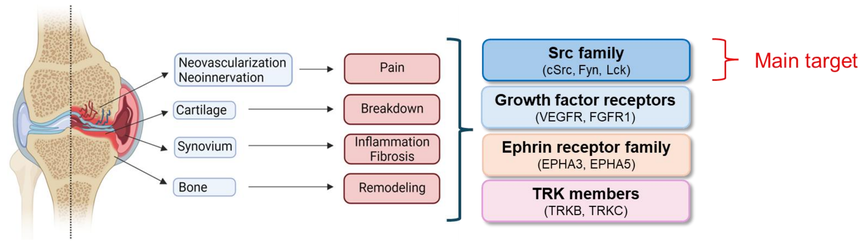
Mean ± SEM.



Histopathology analysis showed evidence of repair in the medial collateral ligament (MCL) and meniscus area

Conclusions

CR10049 targets a group of kinases with a crucial role in the OA disease. Their inhibition resulted in a strong reduction of inflammation leading to a significant **improvement in pain behaviour** as well as in the **overall joint structure**.



CR10049, improving symptoms and structure, is the first OA-targeted kinase inhibitor for the treatment of the OA inflammatory phenotype.

References

[1] Li, K. et al. Tyrosine kinase Fyn promotes osteoarthritis by activating the β-catenin pathway. Ann. Rheum. Dis. (2018). [2] Novikov, F. N. et al. Inhibition of SYK and cSrc kinases can protect bone and cartilage in preclinical models of osteoarthritis and rheumatoid arthritis. Sci Rep (2022).