

# EFFICACY OF XOMA 052 ANTI-IL-1 $\beta$ ANTIBODY IN COLLAGEN-INDUCED ARTHRITIS AND A MODEL OF ACUTE GOUT

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## ABSTRACT

The cytokine interleukin-1 $\beta$  (IL-1 $\beta$ ) is a key initiator of the inflammatory cascade involved in the pathology of arthritis and other inflammatory diseases, and its neutralization shows efficacy in both animal and human disease. The Human Engineered™ anti-IL-1 $\beta$  antibody, XOMA 052, was developed for treatment of inflammatory diseases and has a very high affinity ( $K_D = 0.3$  pM) to human IL-1 $\beta$  (hIL-1 $\beta$ ). XOMA 052 also has an affinity to mouse IL-1 $\beta$  (mIL-1 $\beta$ ) of 3.0 nM. In the work described here, XOMA 052 was tested for efficacy in collagen-induced arthritis (CIA). In the CIA model, XOMA 052 administration starting at immunization significantly suppressed the mean arthritic score throughout the course of disease. XOMA 052 administered after disease onset also suppressed the mean disease score, demonstrating clinically relevant therapeutic intervention. In addition, evaluation of high resolution radiographs showed that XOMA 052 prevented the bone destruction in the joints that is caused by arthritis. Data describing the suppressive effect of XOMA 052 on downstream biomarkers such as interleukin-6 are also described. Furthermore, we present results showing that XOMA 052 can block peritonitis in a mouse model of acute gout. These data demonstrate the species cross-reactivity and efficacy of XOMA 052 in mouse disease, supporting its clinical evaluation in rheumatoid arthritis and other inflammatory diseases. This antibody is currently in Phase 1 clinical trials for the treatment of Type 2 diabetes.

## INTRODUCTION

IL-1 is a pro-inflammatory cytokine secreted by a number of different cell types, including monocytes, macrophages, and pancreatic cells. The IL-1 gene family comprises the agonist cytokines, IL-1 $\alpha$  and IL-1 $\beta$ , the natural IL-1 receptor antagonist (IL-1Ra), and a number of different proteins that directly regulate IL-1 activity. Both IL-1 $\alpha$  and IL-1 $\beta$  are synthesized as cytoplasmic precursors, and although IL-1 $\alpha$  remains inside the cell, IL-1 $\beta$  is efficiently processed and secreted, making it an attractive target for therapeutic antibodies.

Using XOMA's Human Engineering™ technology, we have developed a potent anti-IL-1 $\beta$  antibody, XOMA 052. The affinity of XOMA 052 for human IL-1 $\beta$  (hIL-1 $\beta$ ) is 0.3 pM, while the affinity for mouse IL-1 $\beta$  (mIL-1 $\beta$ ) is 3 nM. Thus, there is a 10,000 fold difference in affinities for the two species. For some applications, an affinity in the single-digit nanomolar range is considered sufficient for a therapeutic effect. Thus, we wished to determine if XOMA 052 could inhibit the activity of mIL-1 $\beta$  in vitro and in vivo. This would facilitate the use of mouse models that are available for assessing efficacy of XOMA 052 in various disease indications.

## MATERIALS & METHODS

**Collagen-induced arthritis (CIA).** Male DBA/1 mice were immunized s.c. with 100  $\mu$ g bovine collagen type II (Chondrex) emulsified in Complete Freund's Adjuvant. At Day 21, mice were boosted s.c. with 50  $\mu$ g bovine collagen in Incomplete Freund's Adjuvant. Starting at Day -1 for prophylactic treatment, mice were treated with the indicated doses of isotype control (anti-KLH IgG2) or XOMA 052 antibody, twice a week. For the therapeutic dosing experiments, antibody was administered twice a week after a mean arthritic index of 1-2 was reached. Disease progression was monitored twice a week. A disease score of 0-4 was given to each paw using the following criteria: 0 = normal, non-arthritic paw; 1 = erythema with swelling confined to the mid-foot and/or involvement of one or more digits; 2 = moderate erythema and swelling of the mid-foot up to the tarsal region and/or involvement of one or more digits; 3 = substantial erythema and swelling of the mid-foot and the tarsal region and/or involvement of one or more digits; 4 = severe erythema and severe swelling encompassing the mid-foot, tarsal region and extending beyond (proximal to) the

ankle and/or involvement of one or more digits. An arthritic index of 0-16 was given to each animal by adding the disease score of all four paws.

**Analysis of bone pathology.** Hind paws were collected from mice and preserved in 10% formalin. Samples were sent to Charles River Preclinical Services Montreal for further processing and analysis. High resolution radiographs of the right and left hindpaws (Tibi-tarsal joint, metatarsal and phalangeal joints) were obtained using a tabletop cabinet high resolution digital x-ray machine (FAXITRON). A semi-quantitative evaluation of the radiographs was performed using the following scoring scheme. Scoring was limited to bone erosion and periosteal bone proliferation where a score of 0 denotes no damage/within normal/no bone changes; 1 = minor bone changes; 2 = slight bone changes; 3 = moderate bone changes; and 4 = severe bone changes. The sum of all radiographic scores per paw was used to generate the cumulative individual animal radiographical score (range 0-16).

**Histology.** After radiography, hind paws were decalcified in formic acid, dehydrated through graded alcohols, cleared with xylene and embedded in paraffin. Two sections were cut. The first section was stained with Hematoxylin-Phloxine-Safran (HPS) and the other one with Safranin O. HPS stain was used to enhance osteoclast detection.

**Measurement of IL-6 in situ.** Paws were collected at the end of CIA studies and immediately frozen in liquid nitrogen. Frozen paws were pulverized with the Bessman Tissue Pulverizer and transferred into lysis buffer containing phosphatase and protease inhibitors. After grinding with a tissue homogenizer, samples were centrifuged. Supernatants were tested for protein content using the Pierce BCA Assay. Cytokine levels were measured in duplicate using the Meso Scale Discovery (MSD) Sector 6000 R and Multi-Spot plates.

**Monosodium urate (MSU) crystal-induced acute peritonitis.** Studies performed at Bio-Quant (San Diego, CA). Peritonitis was induced by injecting 0.5 mg of MSU crystals into the peritoneal space of Balb/c mice. Mice were treated 2 hours earlier with intraperitoneal injection of isotype control antibody or XOMA 052. For comparison, one group of mice received IL-1Ra (Anakinra) at the same time as MSU injection. After 6 hours, peritoneal lavage was performed and the lavage fluid was centrifuged to collect cells. Cells were counted and a fraction was used for cytosin and leukocyte differential counts. Peritonitis was measured by calculating the number of neutrophils in the lavage. The number of neutrophils is determined by multiplying the total cell count in the lavage by the percentage of neutrophils in the differential count. The same method was used to calculate the number of macrophages in the lavage fluid.

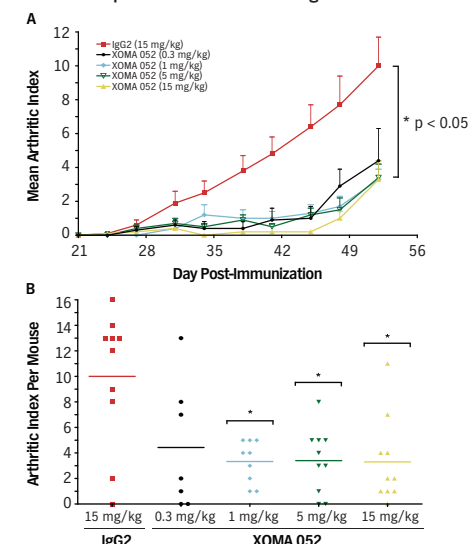
## RESULTS

We have previously demonstrated that XOMA 052, which is specific for human IL-1 $\beta$  (hIL-1 $\beta$ ), can also neutralize mouse IL-1 $\beta$  (mIL-1 $\beta$ ), although with a potency 1,000 times less than that against human. In the D10.G4.1 (D10) proliferation assay, XOMA 052 had an  $IC_{50}$  against hIL-1 $\beta$  in the low pM range ( $2.5 \pm 0.4$  pM) and an  $IC_{50}$  of  $2.6 \pm 0.4$  nM against mIL-1 $\beta$  activity (data not shown).

Nevertheless, XOMA 052 has demonstrated efficacy in the DBA/1 model of collagen-induced arthritis (CIA). At doses of 1, 5 and 15 mg/kg, XOMA 052 administration led to improvement in disease score (Figure 1). The effect was statistically significant at 5 mg/kg and 15 mg/kg from Day 21 through Day 52 (Figure 1A). At Day 52, where mean arthritic index of control IgG2 treated mice is greater than 10, XOMA 052 significantly improved disease scores by 66% at a dose of 1, 5, and 15 mg/kg (Figure 1B).

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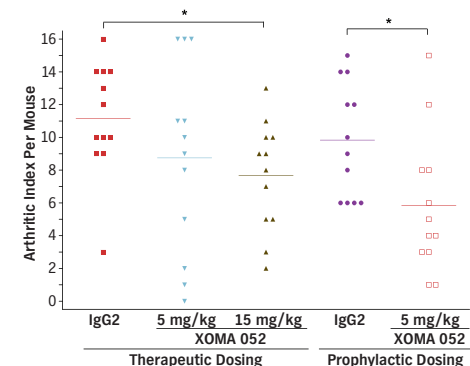
Figure 1. XOMA 052 protects mice from collagen-induced arthritis.



A, Mean arthritic index of all mice from boost until Day 52. Mice received XOMA 052 or isotype control beginning at Day -1. n = 7-10 mice/group. \*, p < 0.05 for 5 mg/kg and 15 mg/kg XOMA 052 versus IgG2 control, 1-way ANOVA and Tukey's test. B, Day 52 arthritic index per mouse. \*, p < 0.05 versus IgG2 control, 1-way ANOVA and Tukey's test.

In addition to preventing disease starting at Day -1 (prophylactic dosing), XOMA 052 also prevents arthritis in established CIA (therapeutic dosing). When XOMA 052 administration was delayed until the onset of symptoms, disease scores were still significantly reduced (Figure 2). The protective effect of XOMA 052 was statistically significant at 15 mg/kg.

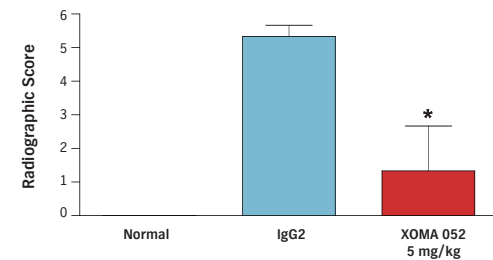
Figure 2. XOMA 052 protects mice from collagen-induced arthritis after disease onset.



Comparison of therapeutic versus prophylactic treatment. Arthritic scores of mice on Day 51 after immunization. n = 12 mice/group. \*, p < 0.05, Mann-Whitney test.

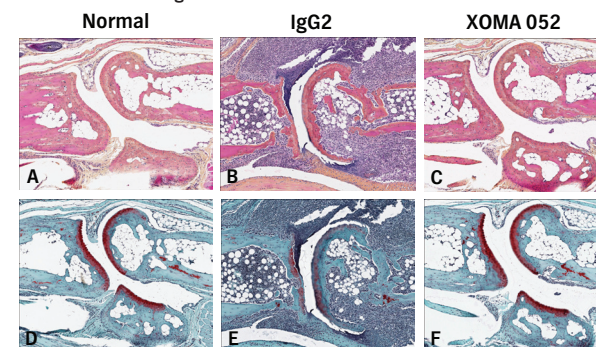
Inflammation in RA induces osteoclastogenesis and bone resorption. In order to study the effect of XOMA 052 on bone pathology, hind paws were collected from mice at the end of the prophylactic CIA study. Analysis of radiographic images showed that XOMA 052 could prevent the bone erosion and periosteal bone proliferation caused by CIA (Figure 3). The same paws were further processed, and sections were stained with Hematoxylin-Phloxine-Safran (HPS) or Safranin O (Figure 4). Paws from mice treated with isotype control antibody showed massive inflammatory infiltration (Figure 4B) and loss of cartilage (Figure 4E) in arthritic joints. This was largely prevented by treatment with XOMA 052 (Figure 4C, 4F).

Figure 3. XOMA 052 prevents bone pathology in collagen-induced arthritis.



Scoring results based on radiographic images of hind paws from prophylactically treated mice. n = 3 mice/group. \*, p < 0.05 vs. IgG2 control, unpaired t test.

Figure 4. XOMA 052 prevents inflammation and bone and cartilage destruction in collagen-induced arthritis.

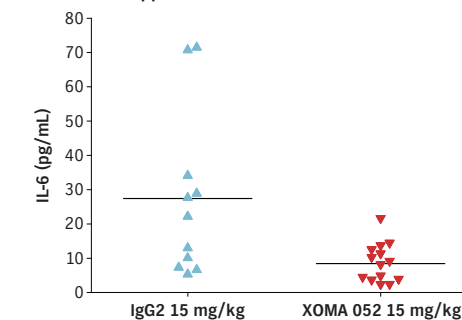


Hind paws from prophylactically treated mice were collected at end of study and preserved in 10% formalin. Paws were taken from non-immunized mice (A, D) or mice dosed with IgG2 (B, E) or 5 mg/kg XOMA 052 (C, F). Shown are longitudinal sections of metatarsal-phalangeal joints, stained with Hematoxylin-Phloxine-Safran (HPS, A-C) or with Safranin O (D-F).

Multiple cytokines are known to be involved in the etiology and pathology of rheumatoid arthritis. The literature has shown that the disease score in CIA correlates with the level of inflammatory cytokines. In particular, interleukin-6 (IL-6), which is a downstream marker of IL-1 $\beta$  activity, is important for T-cell mediated joint damage. IL-6 is secreted by monocytes, synovial fibroblasts, B cells, and T cells. To explore the effects of XOMA 052 on downstream markers of disease, we examined the expression of IL-6 and other cytokines in the serum and paws of arthritic mice.

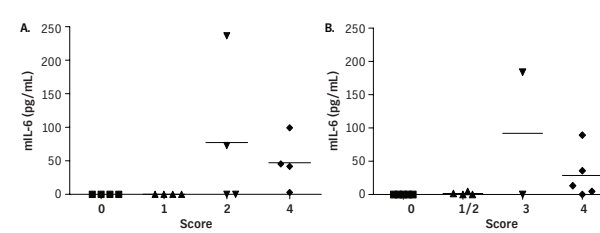
As previously shown (Figures 1 and 2), treatment of mice with XOMA 052 suppresses disease. In addition, XOMA 052 also suppresses the level of IL-6 in the serum (Figure 5). Thus, the ability of XOMA 052 to prevent arthritic disease correlates with its ability to modulate the expression of inflammatory cytokines known to be involved in this disease. Additionally, we verified in two separate experiments that clinical paw scores correlate with in situ levels of IL-6 (Figure 6). Current experiments are aimed at looking at the effect of XOMA 052 on IL-6 expression in the paws.

Figure 5. XOMA 052 suppresses IL-6 in the serum.



Levels of IL-6 in the serum of immunized mice treated therapeutically with isotype control or XOMA 052, measured via MSD. n = 11-14 mice/group. p = 0.0260 for XOMA 052 vs. IgG2. Horizontal bars represent the mean IL-6 values. Representative of 2 experiments.

Figure 6. Clinical paw scores correlate with in situ levels of IL-6.



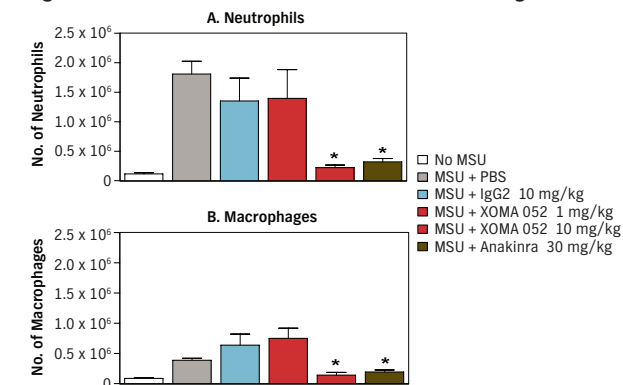
A, Levels of IL-6 in frozen paws from arthritic mice, measured via MSD. n = 4 paws/score. There is a correlation between increasing disease score and increasing IL-6 levels (Spearman correlation coefficient = 0.7385). B, Levels of IL-6 in frozen paws from a separate study, measured via MSD. n = 2-5 paws/score. Spearman correlation coefficient = 0.6453. Horizontal bars represent the mean IL-6 values.

In addition to rheumatoid arthritis, IL-1 $\beta$  is implicated in many inflammatory and autoimmune diseases, including sJIA (systemic-onset juvenile idiopathic arthritis), gout, and recently, Type 2 diabetes. We have previously shown that XOMA 052 can reduce hyperglycemia and preserve beta-cell function in the diet-induced obesity model of Type 2 diabetes<sup>1</sup>. We subsequently tested XOMA 052 in a mouse model of acute gout, monosodium urate (MSU) crystal-induced acute peritonitis. In this model, injection of MSU crystals leads to a rapid influx of inflammatory cells, in an IL-1-dependent manner<sup>2</sup>.

XOMA 052 administered at 10 mg/kg was able to block the neutrophil infiltration (Figure 7A). Thus, peritonitis induced by the MSU crystals was reduced by

84% relative to isotype control (p < 0.05, unpaired t test). In addition, XOMA 052 blocked the infiltration of macrophages (Figure 7B). Although the affinity of XOMA 052 for mouse IL-1 $\beta$  is 10,000-fold lower than against human, there was no significant difference between treatment with 10 mg/kg XOMA 052 and 30 mg/kg Anakinra. Notably, compared to previously described results with Anakinra, our results demonstrate that inhibition of IL-1 $\beta$  alone is sufficient to block the MSU crystal-induced inflammation.

Figure 7. XOMA 052 blocks inflammation in a model of acute gout.



A, Number of infiltrating neutrophils in the peritoneal lavage, 6 hours after MSU injection. B, Number of infiltrating macrophages in the peritoneal lavage, 6 hours after MSU injection. n = 6 mice/group. \*, p < 0.05 for XOMA 052 10 mg/kg vs. IgG2 control, or Anakinra vs. PBS, unpaired t test.

## CONCLUSIONS

Utilizing our integrated antibody discovery and biologics development capabilities, we have Human Engineered™ an anti-IL-1 $\beta$  antibody, XOMA 052, with very high affinity and potency against human IL-1 $\beta$ . This same antibody also has high affinity for mouse IL-1 $\beta$  and is potent against mIL-1 $\beta$  activity in vitro and in vivo. XOMA 052 was able to effectively prevent and treat disease in a mouse model of RA, and this effect correlated with the modulation of IL-6, another pro-inflammatory cytokine with a central role in joint damage. Moreover, XOMA 052 prevented the bone and cartilage destruction caused by CIA as assessed by radiography and histopathology. XOMA 052 can also block the induced peritonitis in an acute model of gout. These data demonstrate the species cross-reactivity and efficacy of XOMA 052 in mouse disease, supporting its clinical evaluation in RA and other inflammatory diseases.

## REFERENCES

- Owyang et al, Poster at American Diabetes Association 68th Scientific Sessions, June 9, 2008
- So et al, Arthritis Research & Therapy 2007, 9:R28

## ACKNOWLEDGEMENTS

Thanks to Hany Zayed for statistical analysis of IL-6 data.