

# Treatment with IMO-3100, a Novel TLR7 and TLR9 Dual Antagonist, Inhibits Disease Development in a Mouse Model of Collagen Antibody-Induced Arthritis (CAIA)

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## INTRODUCTION

Toll-like receptors (TLR) are pattern recognition receptors that sense a wide range of pathogens or pathogen-associated molecular patterns. Endosomal TLRs recognize a variety of nucleic acids including unmethylated CpG dinucleotide-containing bacterial and viral DNA (TLR9), single stranded viral RNA (TLR7/8), and double stranded viral RNA (TLR3). These receptors play a critical role in the activation of innate immunity against bacterial and viral infection.

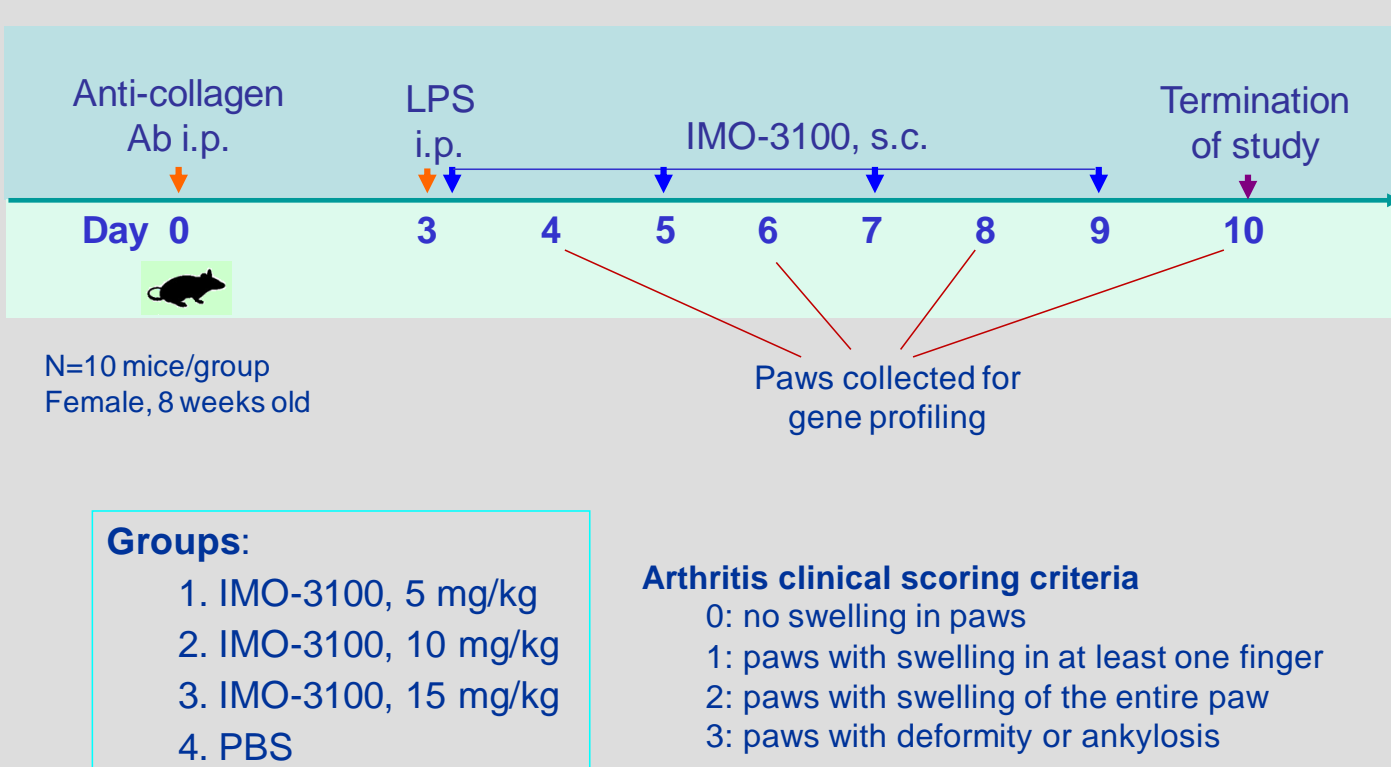
Uncontrolled TLR7- or TLR9-mediated activation of the immune system may trigger events that lead to the development of autoimmune diseases, such as rheumatoid arthritis (RA), lupus, psoriasis, and multiple sclerosis (1 - 4).

We have identified a novel class of DNA-based compounds that act as antagonists of TLR7 and TLR9 and inhibit immune responses induced by these receptors in mice and in human cells (5 - 7). In our earlier studies, these antagonists have shown potent activity in disease models of collagen-induced arthritis, lupus, multiple sclerosis, and psoriasis (8-13). Based on the preclinical data, IMO-3100 has been selected as a lead candidate for clinical development.

Mechanism of action studies in non-human primates and healthy human volunteers have demonstrated that administration of IMO-3100 results in target engagement of TLR7 and TLR9 and in transient inhibition of immune responses driven by these receptors.

In the present study, we examined the efficacy of IMO-3100 in a mouse RA model, collagen antibody-induced arthritis (CAIA).

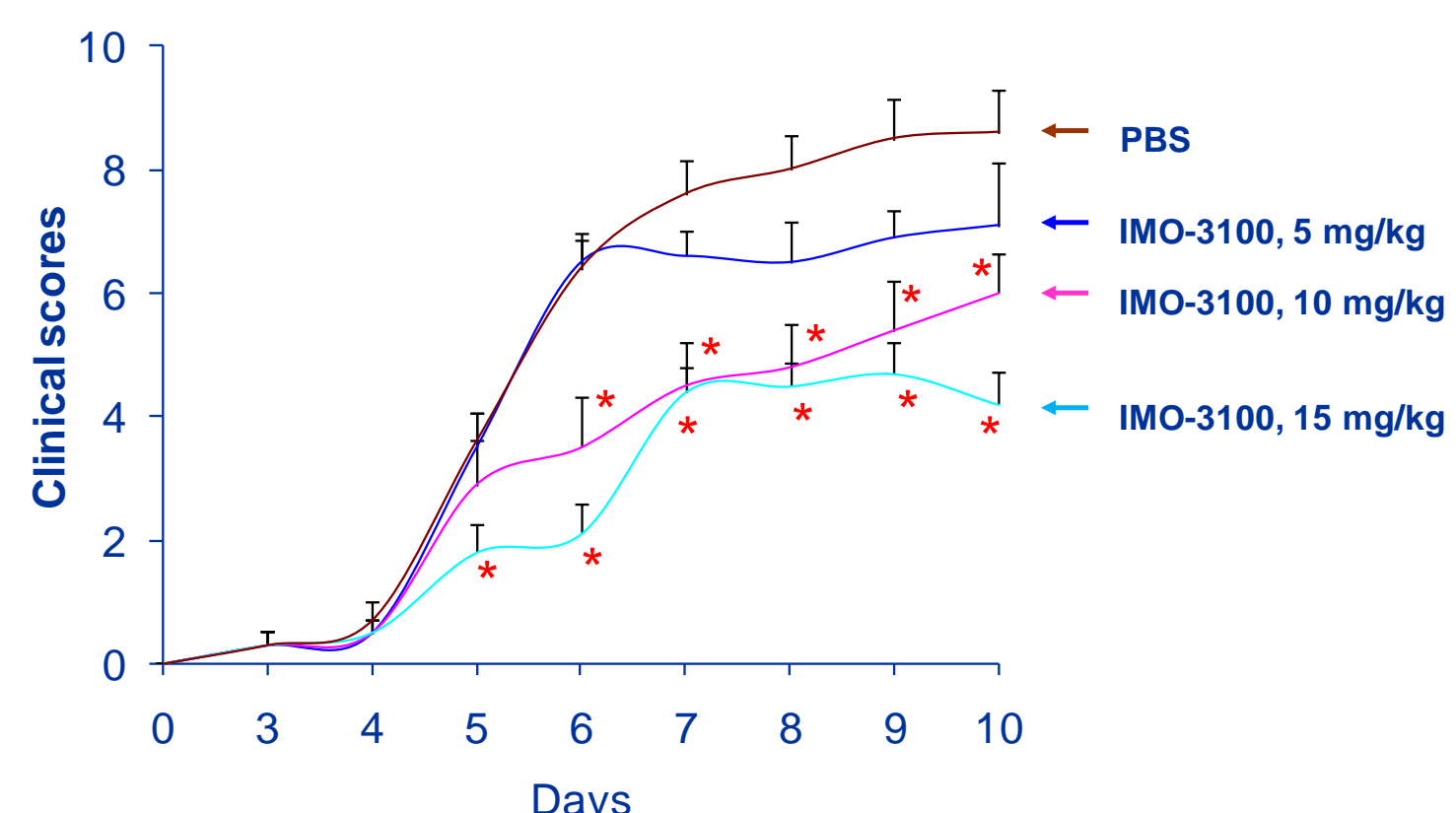
### Experimental Design for Evaluation of the Effects of IMO-3100 on CAIA in BALB/c Mice



Separate studies were conducted to analyze the gene profile in the paws of BALB/c mice treated with IMO-3100 at 15 mg/kg or PBS (n=10).

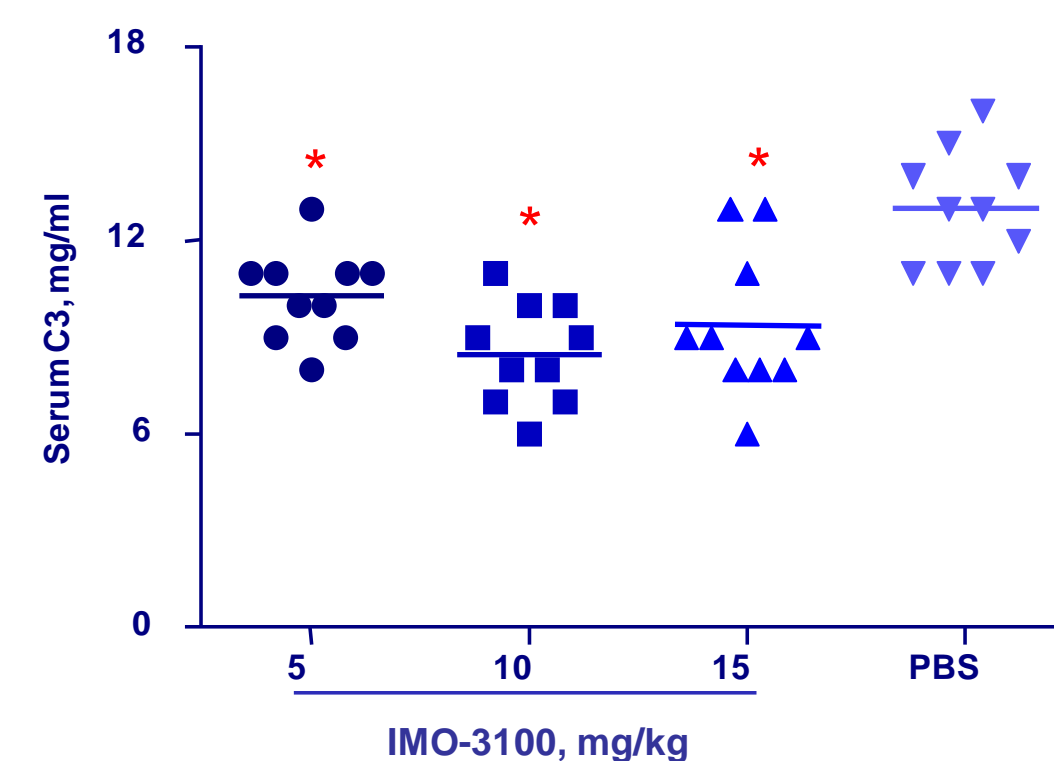
## RESULTS

### IMO-3100 Suppresses CAIA Development in Mice in a Dose Dependent Fashion



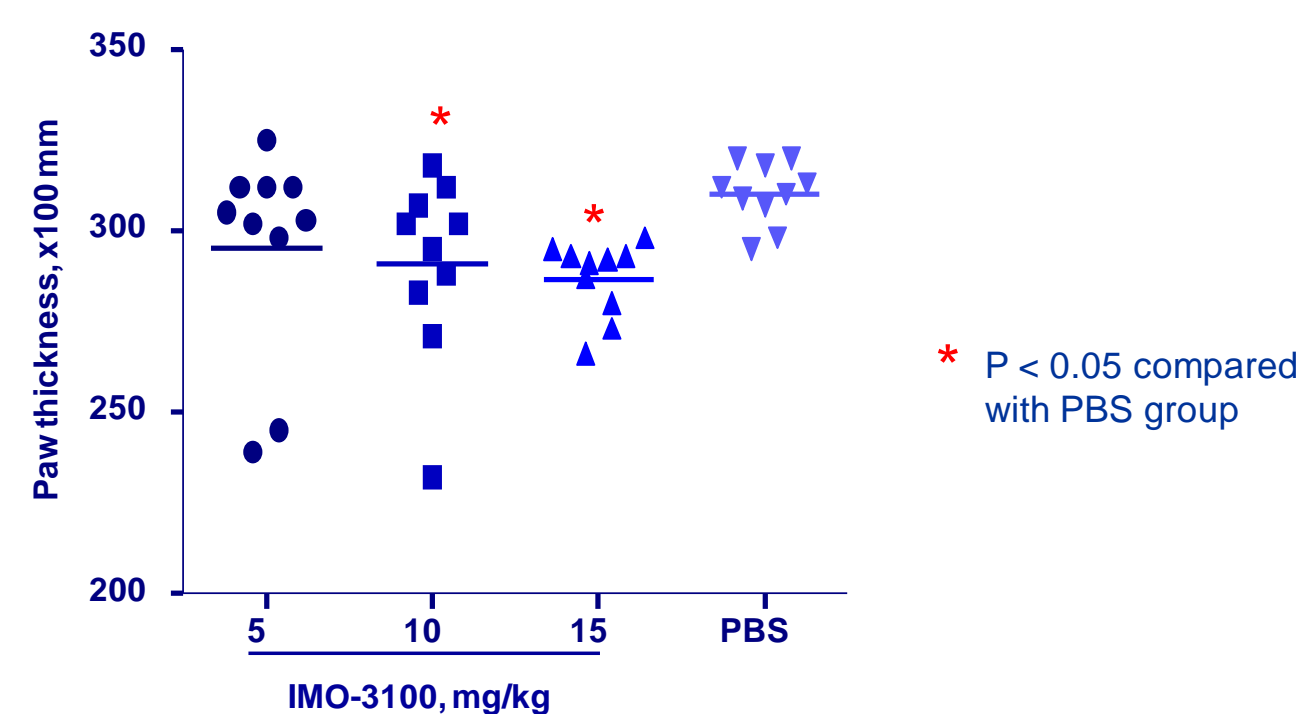
\* P < 0.05 compared with PBS group

### IMO-3100 Reduces Complement C3 Levels in the Serum



\* P < 0.05 compared with PBS group

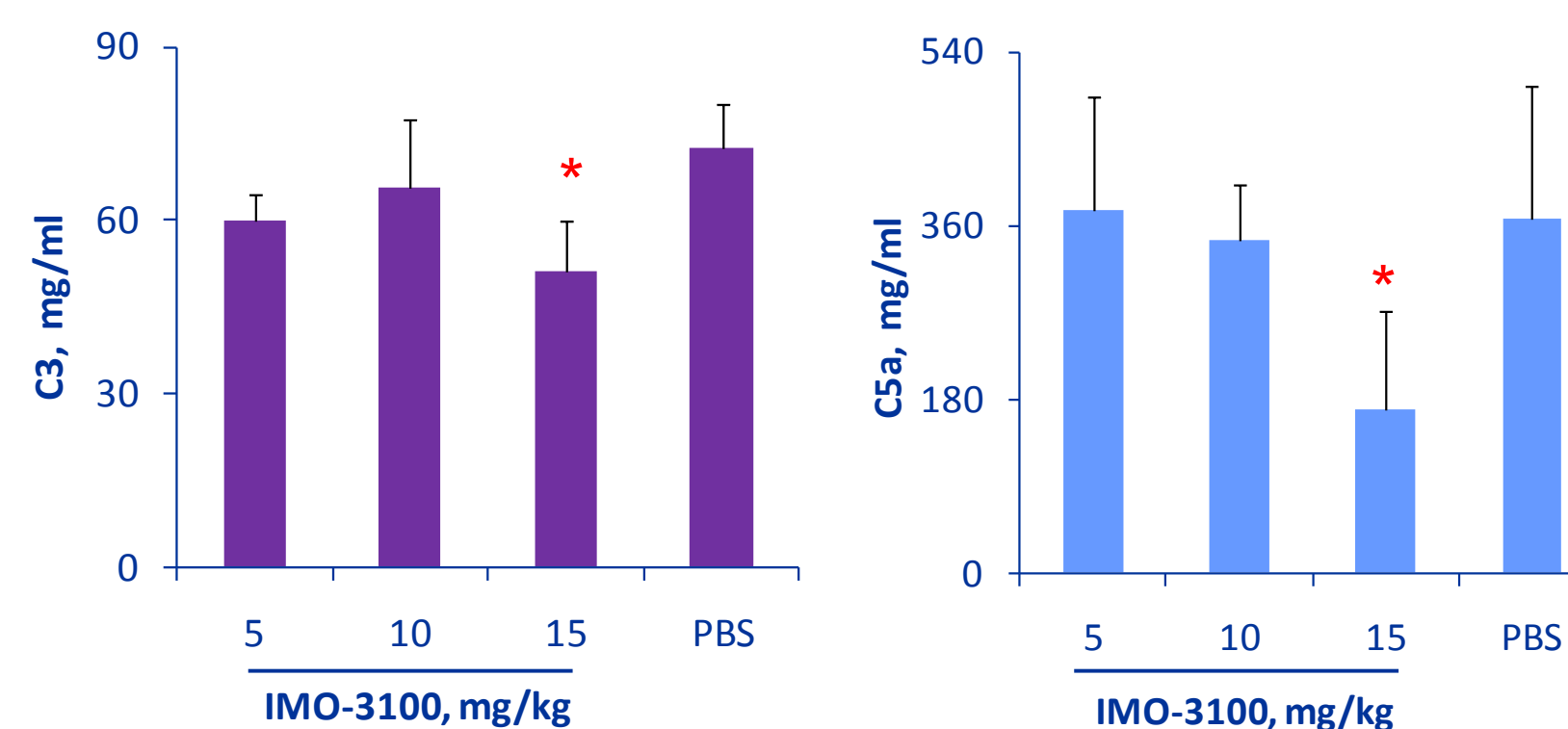
### IMO-3100 Reduces Paw Thickness Increase in Mice Affected with CAIA



\* P < 0.05 compared with PBS group

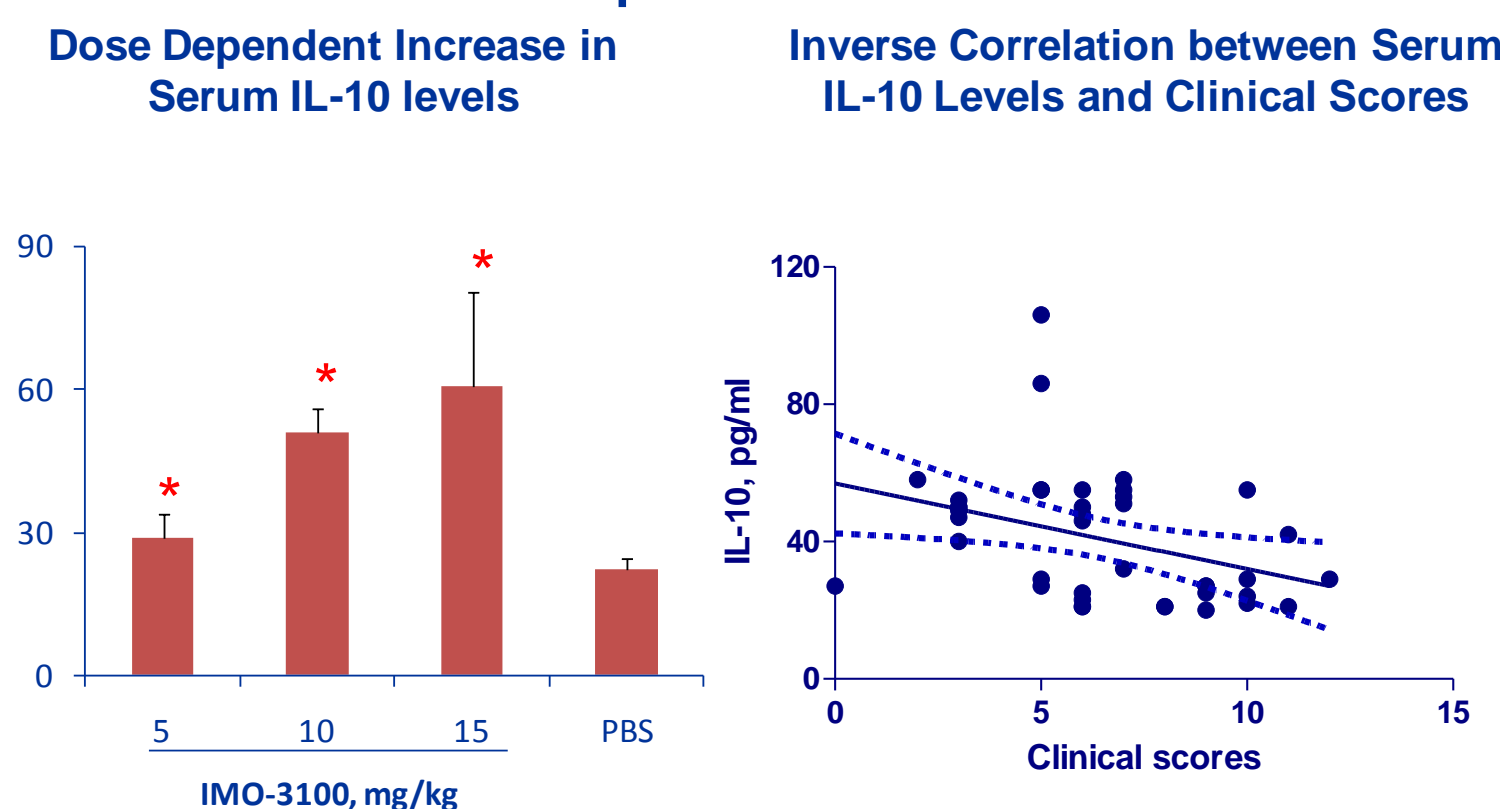
Paw thickness in the hind feet was measured daily for mice affected with CAIA and treated with different doses of IMO-3100 or PBS from day 0 to 10. Data above pertain to the hind paw thickness at the end of the study (day10).

### IMO-3100 Reduces Complement C3 and C5a Deposit in the Paws



\* P < 0.05 compared with PBS group

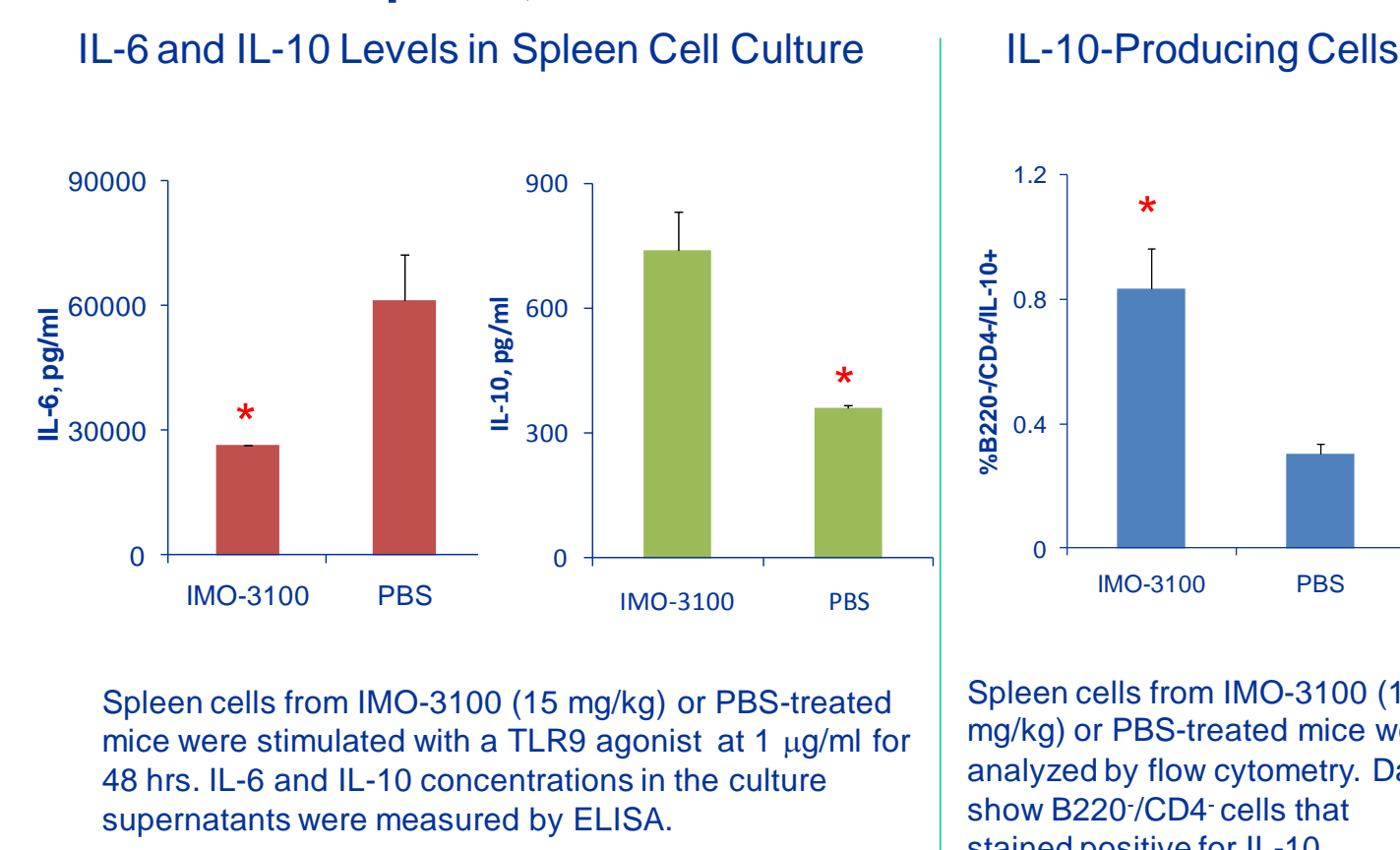
### IMO-3100 Induces Elevation of Serum IL-10 Levels in a Dose Dependent Manner



\* P < 0.05 compared with PBS group

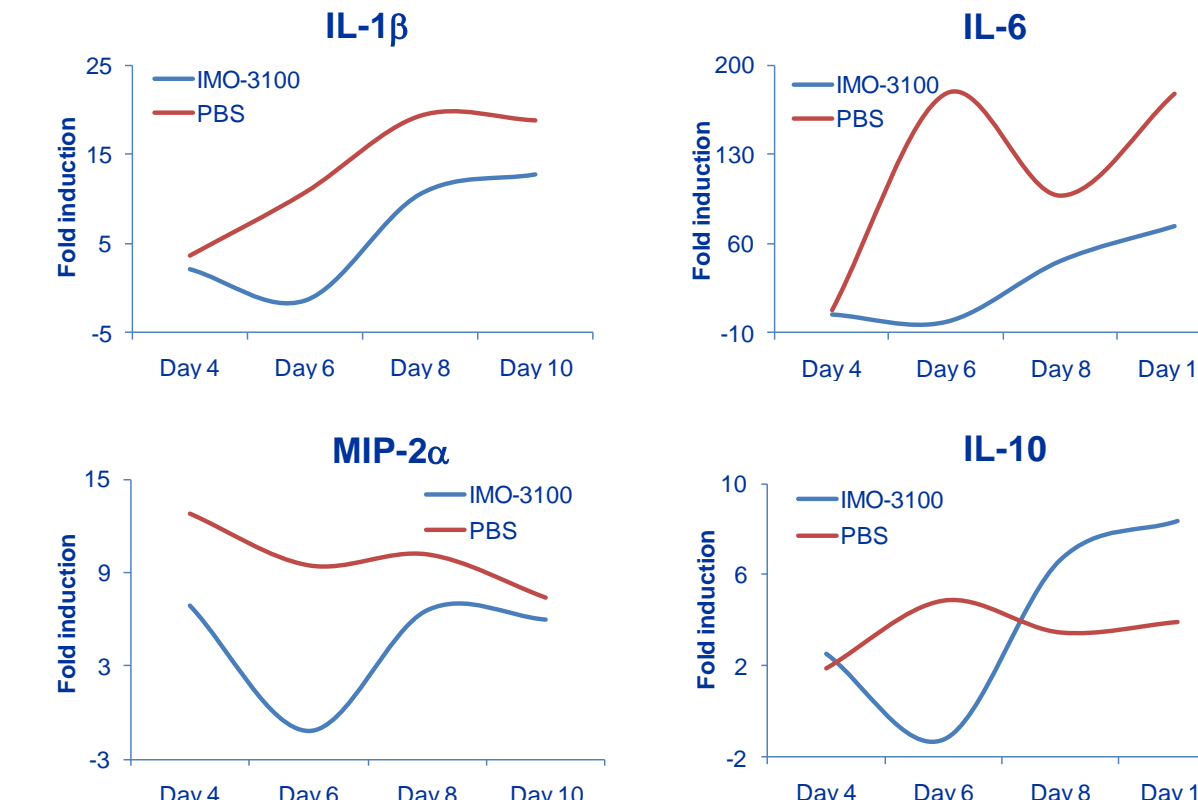
Spearman r = - 0.408, p = 0.009

### IMO-3100 Increases the Number of IL-10-Producing Cells in the Spleen, and Reduces IL-6 Production



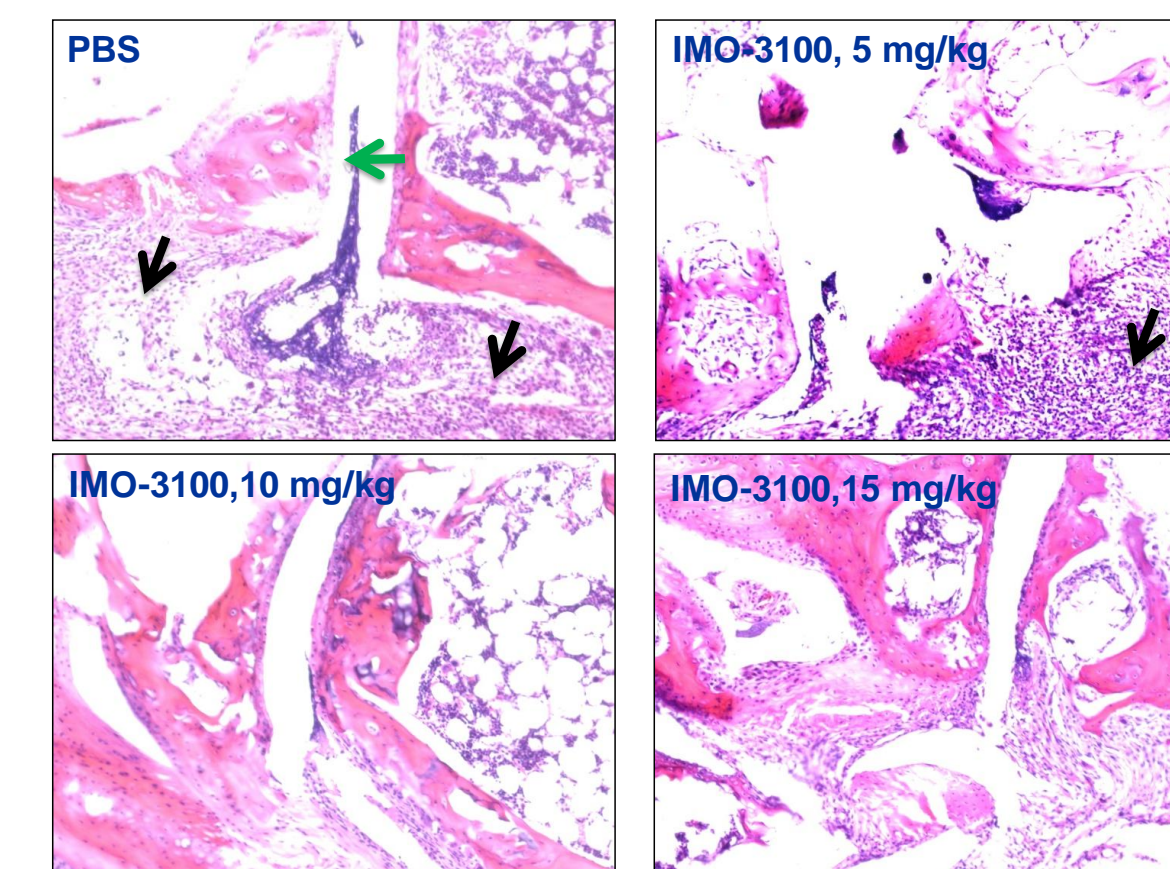
\* P < 0.05 compared with PBS group

### IMO-3100 Impacts Cytokine mRNA Expression in the Paws



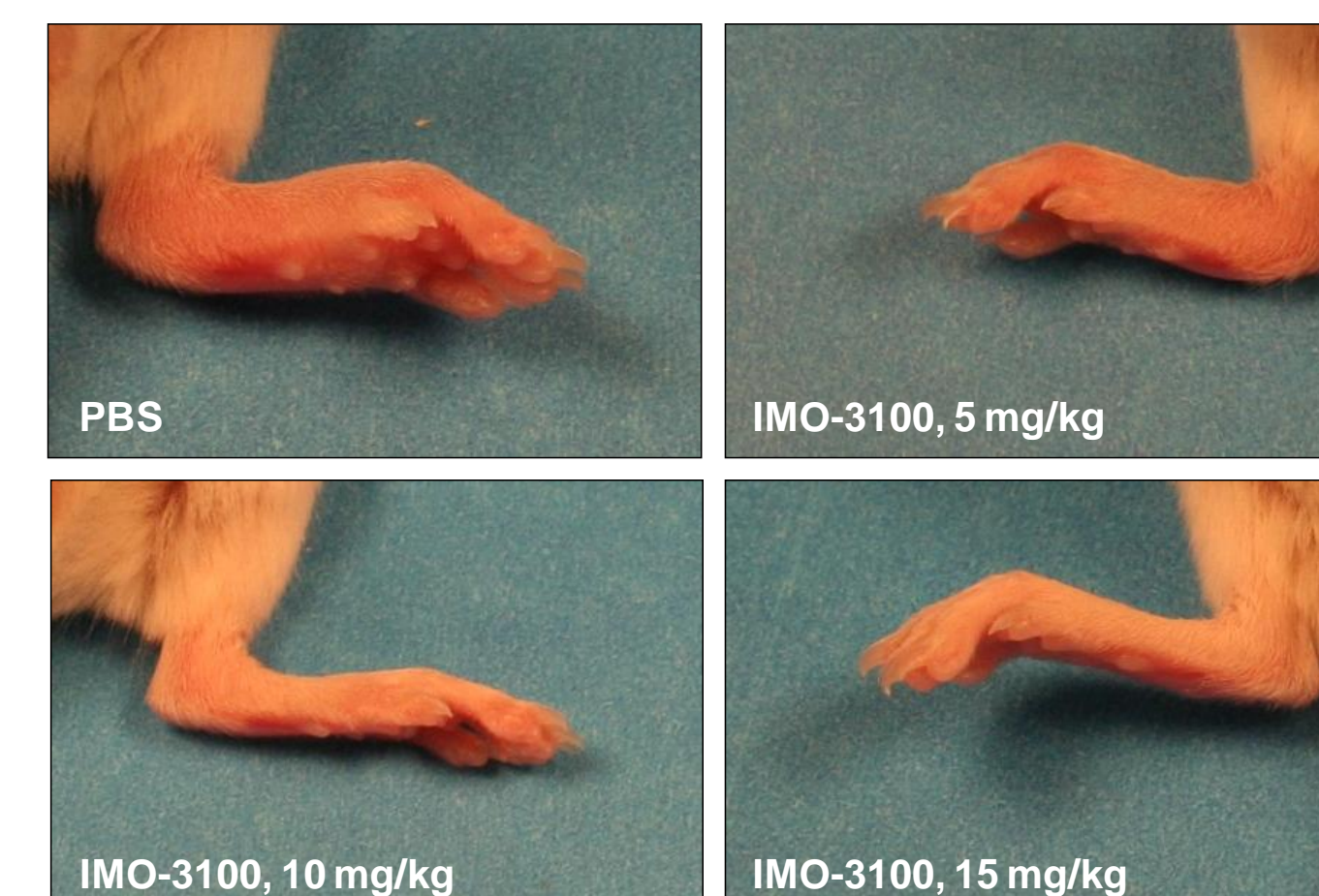
Total RNA was extracted from hind paws taken from IMO-3100 (15 mg/kg) or PBS treated mice with CAIA (n=4) at different time points and analyzed for the proinflammatory cytokines IL-1β, IL-6 and MIP-2α, and regulatory cytokine IL-10 by RT-PCR. Folds relative to the levels of naïve mice.

### IMO-3100 Reduces Inflammation and Bone Destruction in the Foot Joints of Mice with CAIA



Black arrows indicate leukocyte infiltration and green arrow indicates bone destruction. (HE stain, Magnification x 100)

### IMO-3100 Reduces Swelling in the Limbs of Mice with CAIA



## SUMMARY

IMO-3100 treatment of mice with CAIA resulted in:

- Reduction of arthritis symptoms (clinical scores)
- Decrease in complement C3 levels in the serum
- Decrease in C3 and C5a deposition in the paws
- Elevation of IL-10 levels in the serum
- Reduced mRNA expression of IL-1β, IL-6 and MIP-2α, and increased expression of IL-10 in the paws
- Suppression of IL-6 and enhancement of IL-10 production by spleen cells
- Increase in IL-10 producing cells in the spleens
- Reduction of inflammation and bone destruction in the joint tissues

These results indicate that the dual TLR7/9 antagonist IMO-3100 can effectively suppress disease development in this mouse RA model.

- IMO-3100 may have the potential for treatment of RA in humans.
- Phase I clinical studies of IMO-3100 in healthy subjects have been completed:
  - IMO-3100 is safe at the administered dose levels.
  - Target engagement of TLR7 and TLR9 has been demonstrated.

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