

# IMO-3100, a Toll-Like Receptor (TLR) Antagonist, Suppresses TLR7- and TLR9-Mediated Immune Responses in Non-Human Primates

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## INTRODUCTION

Toll-like receptors (TLRs), belonging to the family of pattern recognition receptors (PRRs), act as primary sentinels of the innate immune system by recognizing a diverse array of exogenous, conserved microbial pathogen-associated molecular patterns (PAMPs) as well as endogenous, damage-associated molecular patterns (DAMPs) that are released from damaged or dying cells. Of the TLRs identified in humans, TLR3, 7, 8, and 9 are expressed in endosomal compartments and recognize nucleic acids (1). TLR3 senses viral double-stranded RNA, whereas TLR7 and 8 recognize viral single-stranded RNA. Bacterial and viral DNA containing unmethylated CpG motifs act as ligands for TLR9.

In autoimmune diseases such as systemic lupus erythematosus (SLE), psoriasis, rheumatoid arthritis and Sjögren's syndrome, circulating immune complexes containing host-derived nucleic acids act as ligands for TLR7 and TLR9 and trigger the secretion of pro-inflammatory cytokines and type I interferons (2-6).

We hypothesized that blocking TLR7- and TLR9-mediated immune responses using antagonists could be a novel approach for the treatment of autoimmune diseases, wherein TLR7 and TLR9 have been implicated. Use of TLR7 and TLR9 antagonists permits specific blocking of the immune responses mediated through these two receptors while retaining the functions of other TLRs and not affecting constituent levels of cytokines.

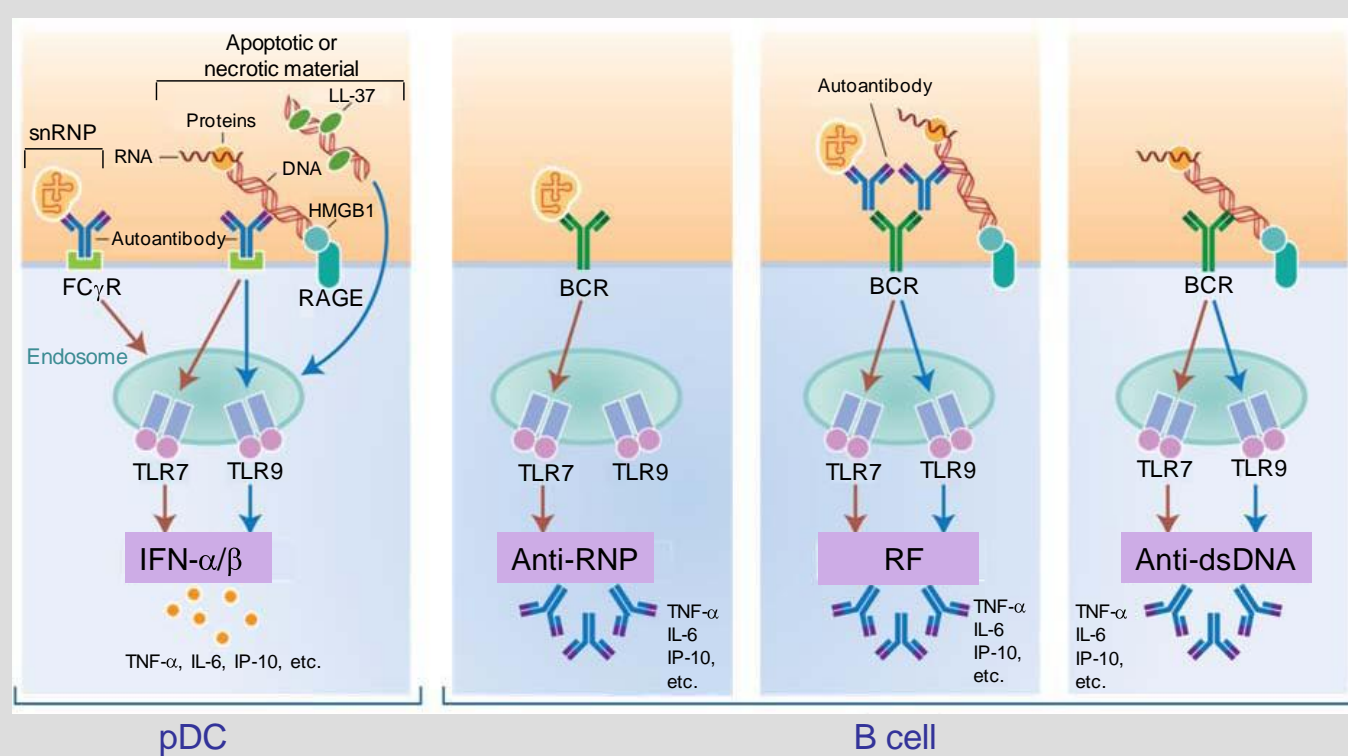
We have selected a DNA-based antagonist candidate, referred to as IMO-3100, for clinical development. In previous studies, IMO-3100 and its prototypes have been shown to inhibit TLR7- and 9-mediated immune responses in human cell-based assays and in preclinical models of lupus, collagen-induced arthritis, and psoriasis in mice (7-14).

In our recently reported single dose study in non-human primates (NHP), IMO-3100 administration inhibited ex-vivo immune responses mediated through TLR7 and TLR9 (15). In the study, PBMCs were isolated from blood collected pre-dose (0 hrs) through 168 hrs following a single dose of either 1.5 or 6 mg/kg of IMO-3100, stimulated with TLR agonists and cytokine secretion was monitored as an index of TLR activation/inhibition. IMO-3100 inhibited TLR7- and TLR9- but not TLR4- or TLR8-mediated cytokine secretion from ex-vivo stimulated PBMCs following in vivo administration in non-human primates (15).

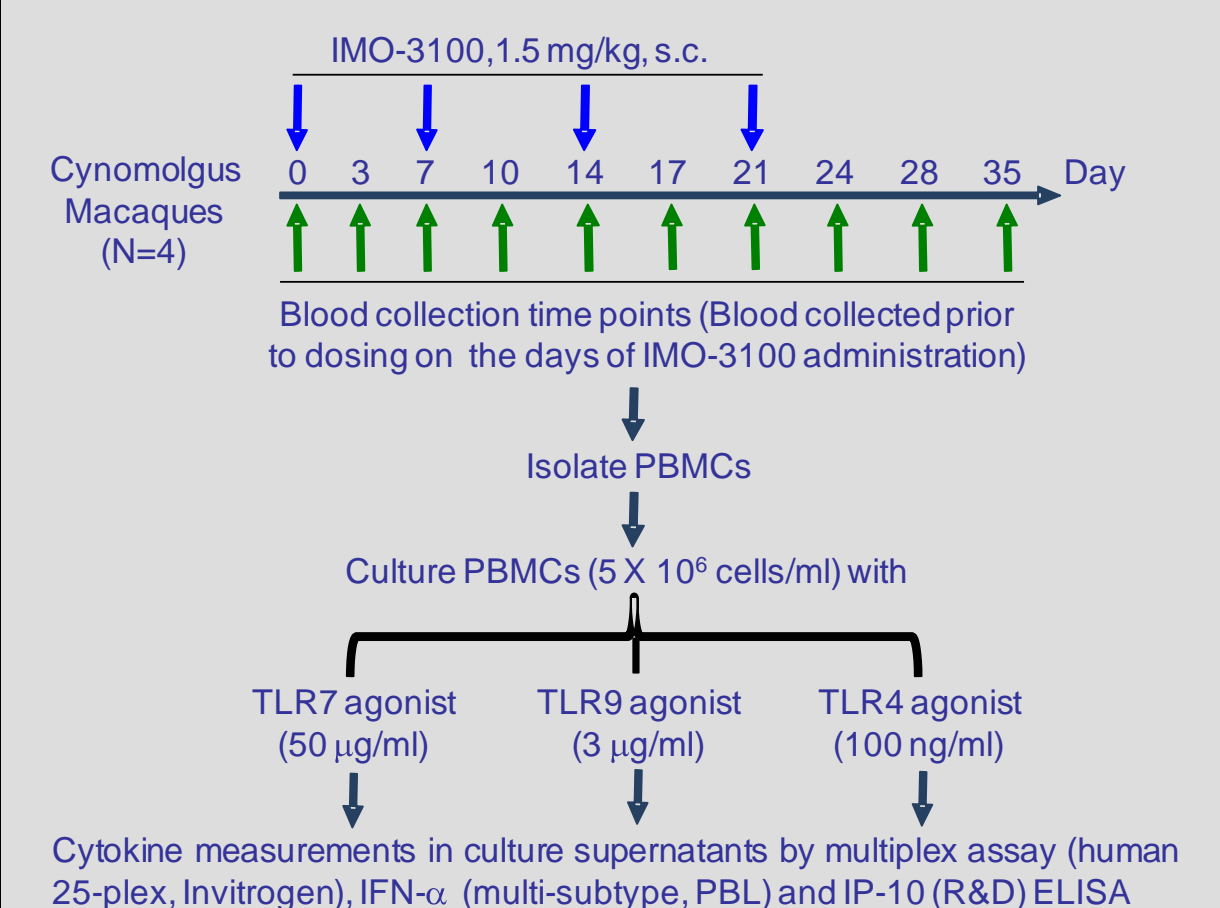
Also, in our first-in-human rising single dose Phase I clinical trial, IMO-3100 was well tolerated up to a dose of 0.64 mg/kg (16). PBMCs isolated from healthy subjects given IMO-3100 showed suppressed induction of several Th1 cytokines induced by TLR7 or TLR9 agonists in ex-vivo assays (16).

In the present study, we have further evaluated the pharmacodynamic mechanism of action of IMO-3100 in NHP to see if a multi-dose regimen of IMO-3100 results in sustained suppression of TLR7- and TLR9-mediated immune responses.

## Role of TLR7 and TLR9 in Autoimmune Diseases



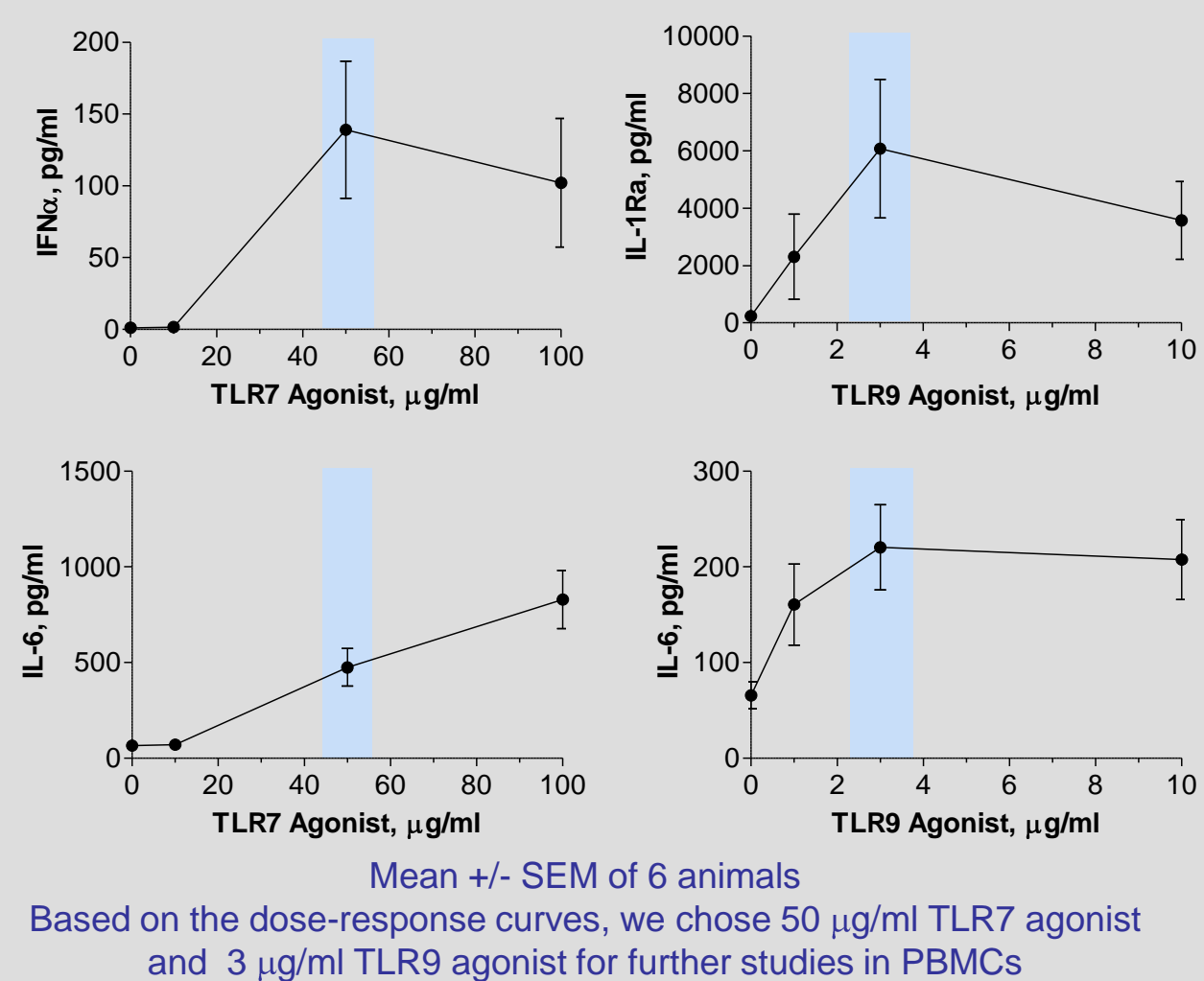
## EXPERIMENTAL PROTOCOL



**PBMC cultures:** Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation method. PBMCs ( $1 \times 10^6$  cells/0.2 ml/well in 96 well plates) were incubated with TLR7 (RNA-based, 50 μg/ml) (17), TLR9 (DNA-based, 3 μg/ml) (18), or TLR4 (LPS, 100 ng/ml) agonists for 24 hrs. Supernatants were then harvested and stored frozen until assay of cytokines/chemokines. The TLR agonists used in the study served as surrogates of endogenous immune complexes containing nucleic acids.

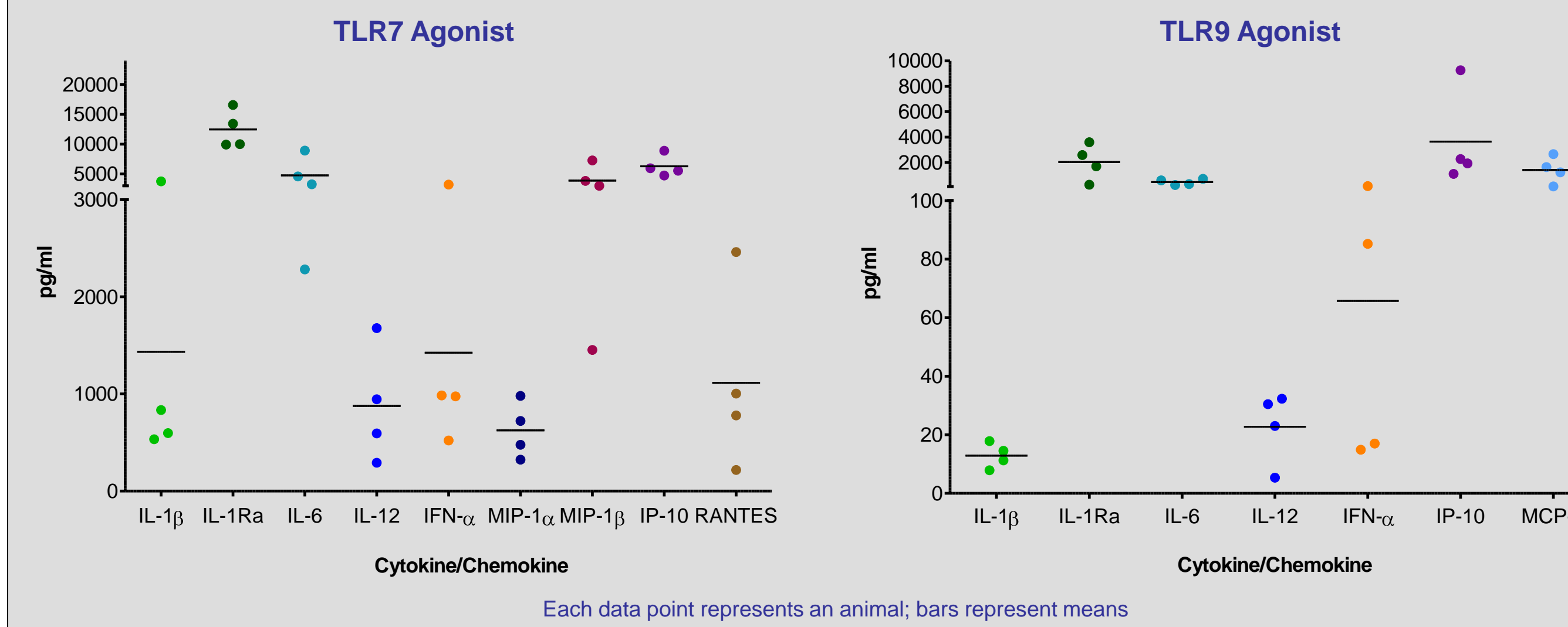
Cytokine levels in culture supernatants were determined on a Luminex platform using human 25-plex cytokine antibody bead kits. The following cytokines could be detected consistently using the human multiplex kit: IL-1β, IL-1Ra, IL-6, IL-8, IL-12, MIP-1α, MIP-1β, RANTES and MCP-1. IFN-α and IP-10 levels were measured by ELISA.

## Agonist-Induced Cytokine Dose-Response Curves in NHP PBMCs



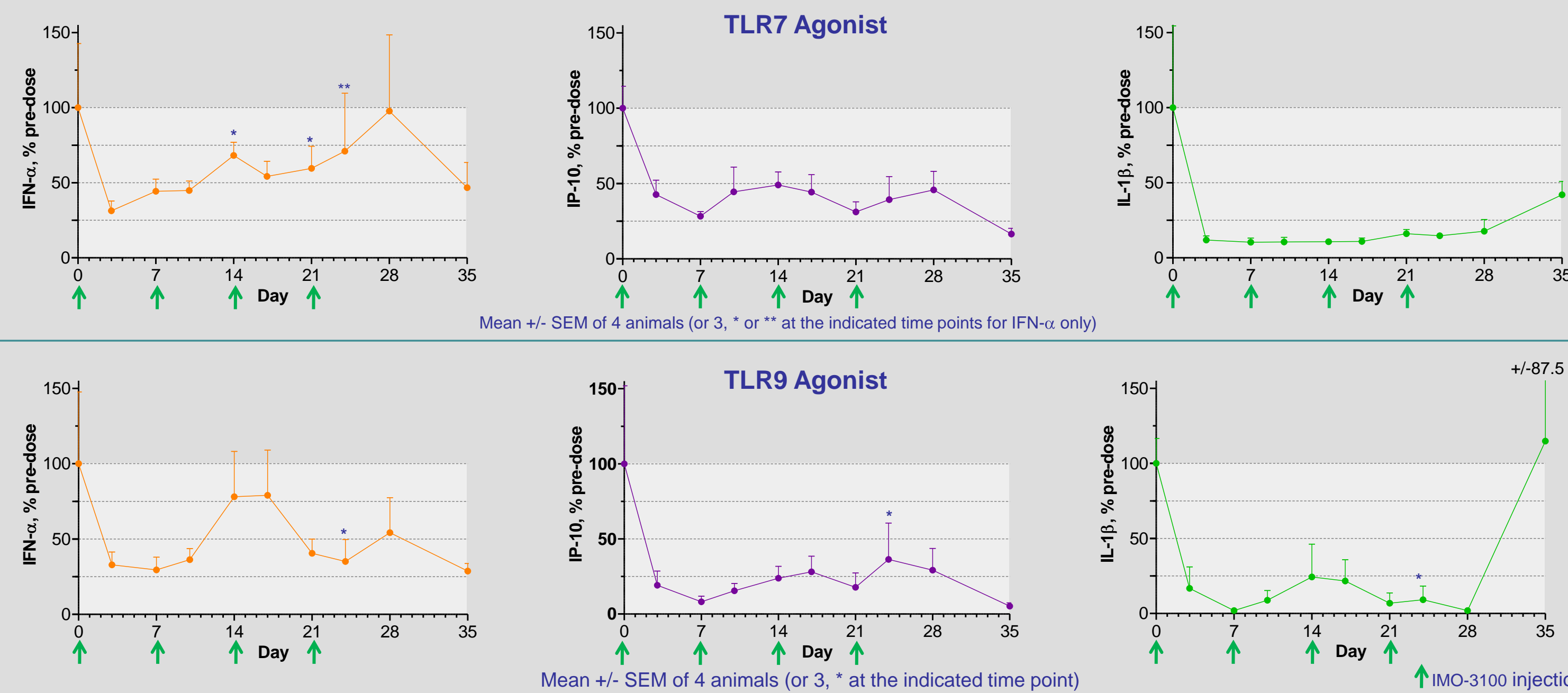
Mean +/- SEM of 6 animals  
Based on the dose-response curves, we chose 50 μg/ml TLR7 agonist and 3 μg/ml TLR9 agonist for further studies in PBMCs

## Levels of Selected Cytokines Induced by TLR Agonists in Pre-Dose NHP PBMCs



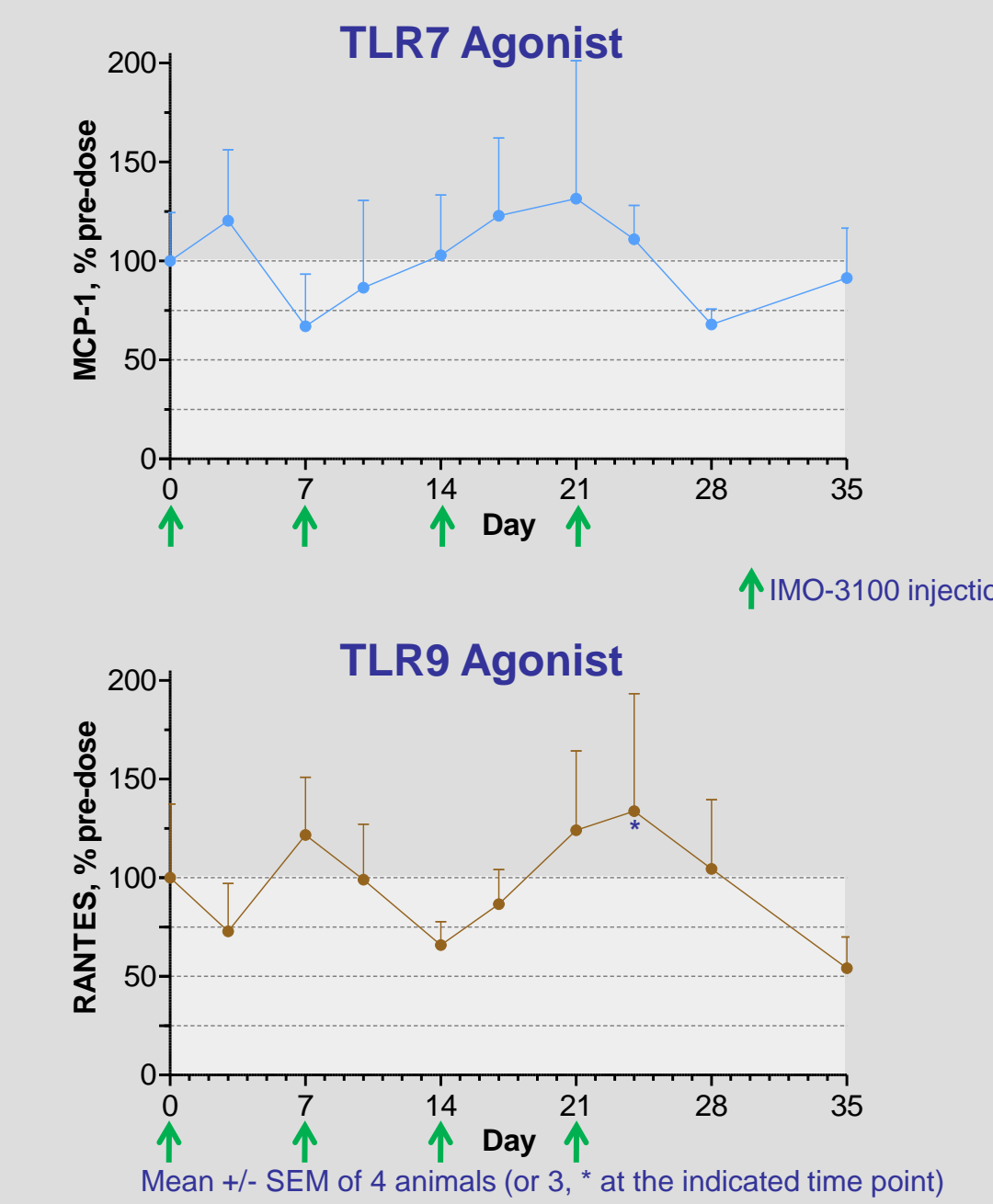
Each data point represents an animal; bars represent means

## IMO-3100 Inhibits Induction of IFN-α, IP-10 and IL-1β in NHP PBMCs Stimulated with TLR7 or TLR9 Agonist



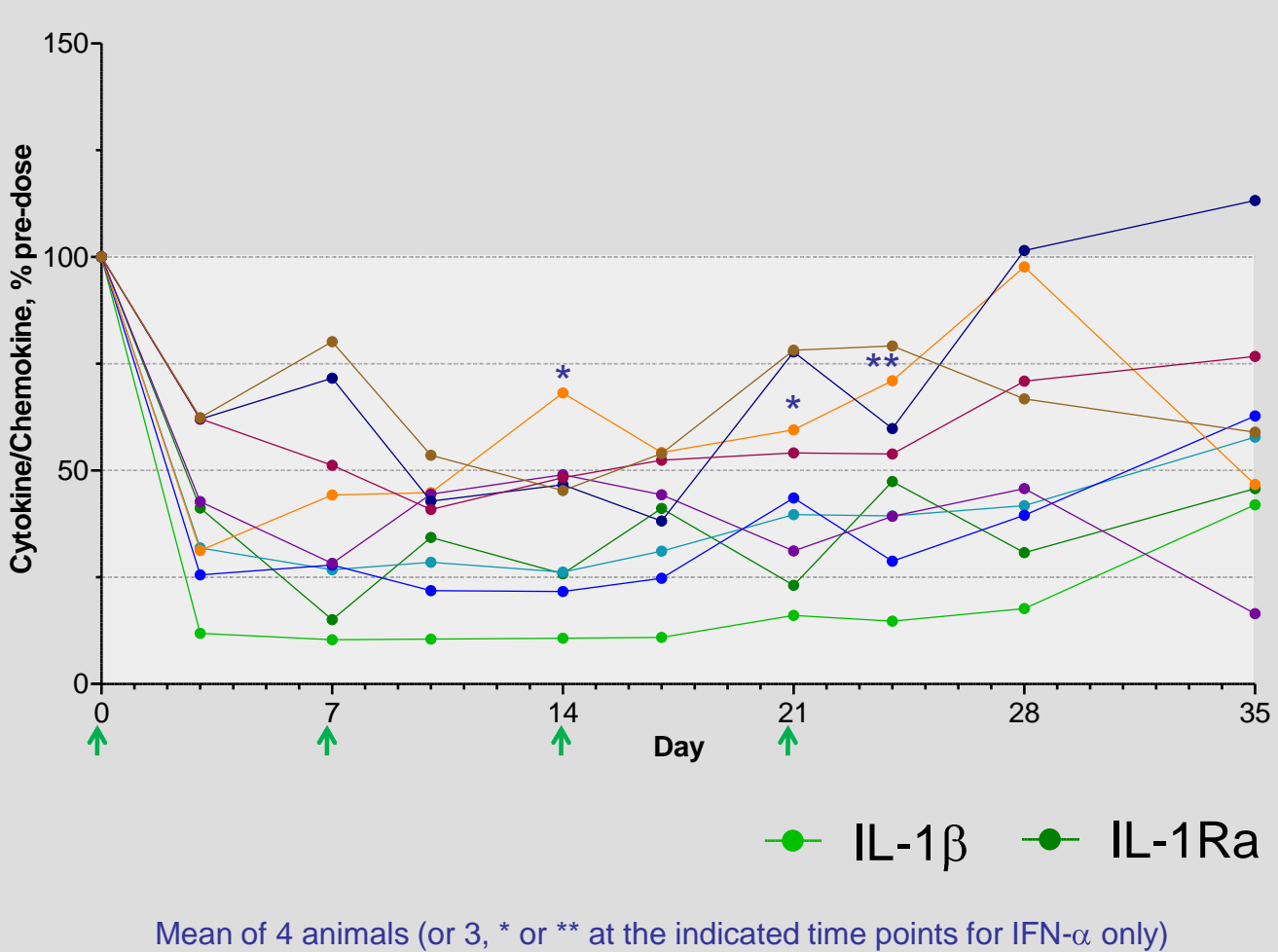
Mean +/- SEM of 4 animals (or 3, \* or \*\* at the indicated time points for IFN-α only)

## Cytokine Secretion not Suppressed by IMO-3100



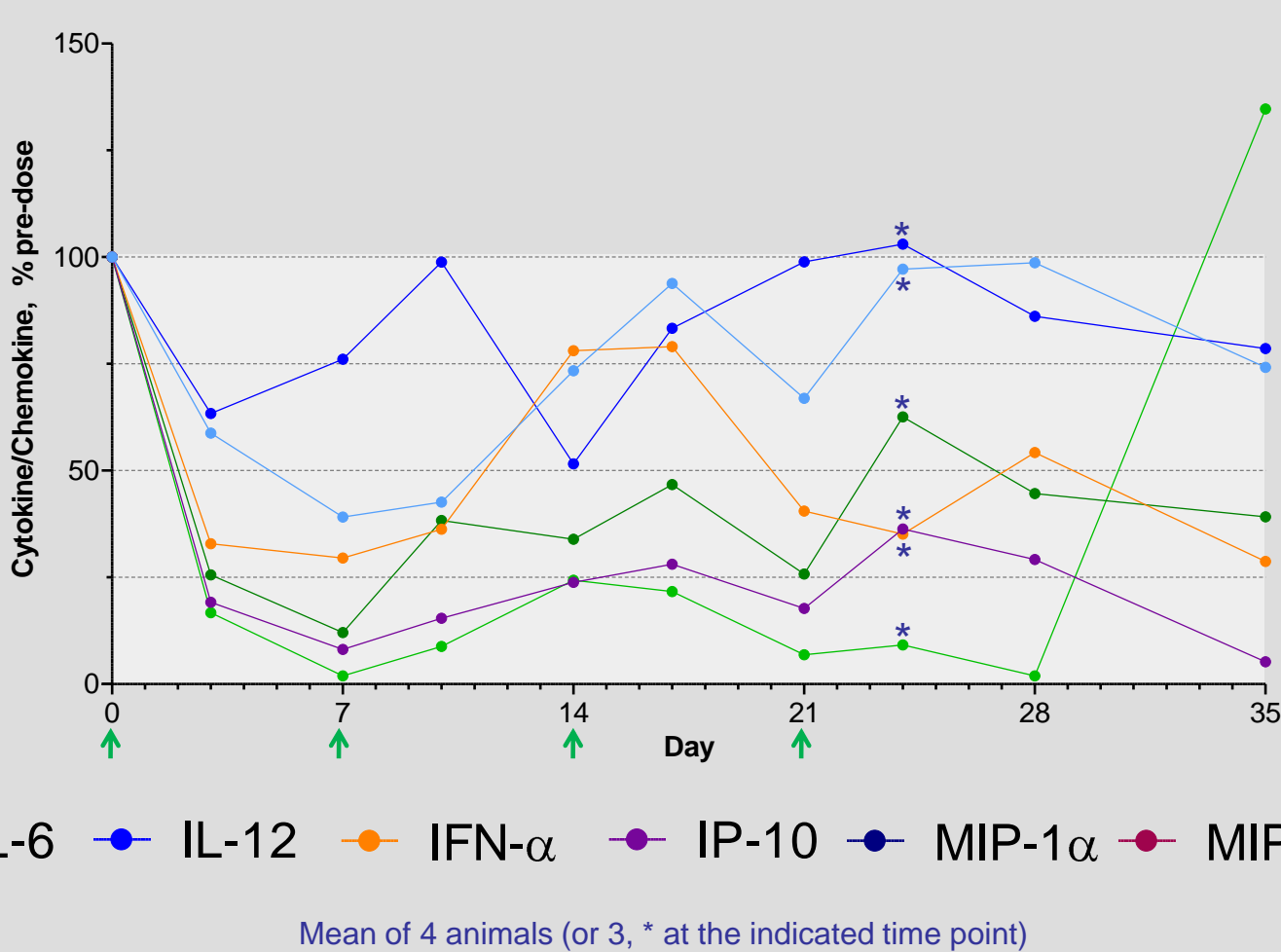
Mean +/- SEM of 4 animals (or 3, \* at the indicated time point)

## IMO-3100: Inhibition of TLR7 Agonist-Induced Cytokines



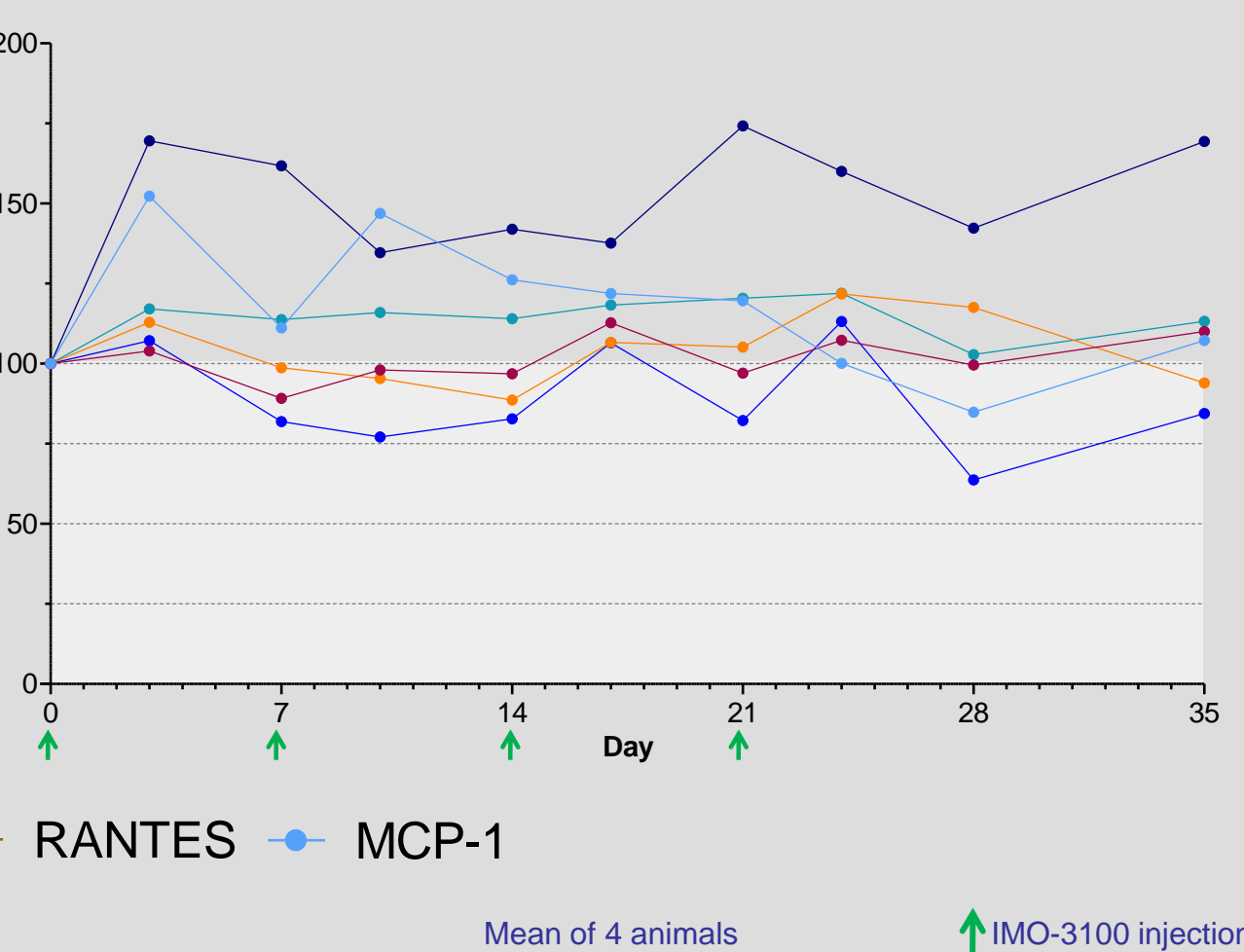
Mean of 4 animals (or 3, \* or \*\* at the indicated time points for IFN-α only)

## IMO-3100: Inhibition of TLR9 Agonist-Induced Cytokines



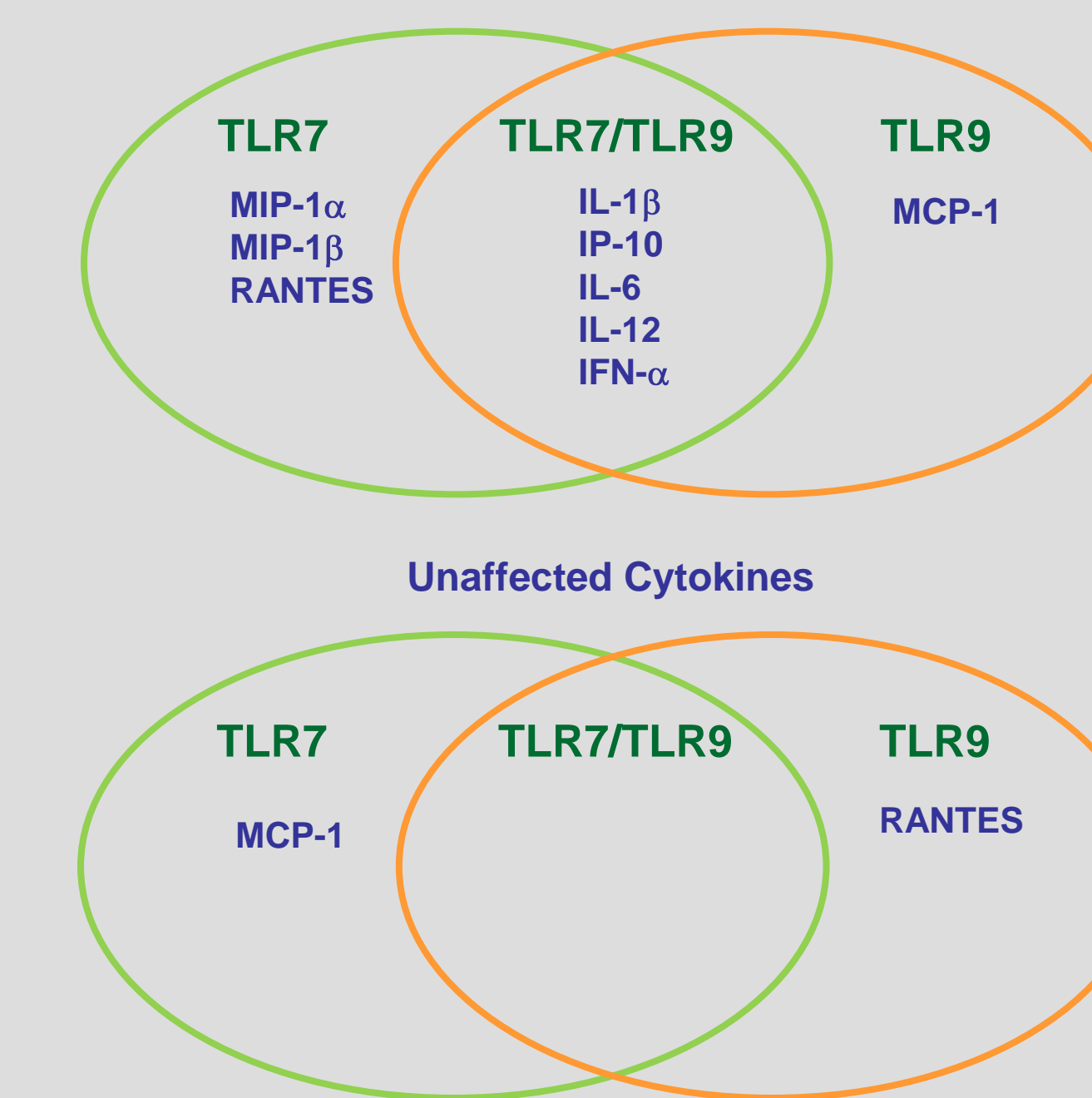
Mean of 4 animals (or 3, \* at the indicated time point)

## IMO-3100: No Effect on TLR4-Mediated Responses



Mean of 4 animals

## IMO-3100: NHP Multiple Dose Pharmacodynamic Activity Cytokines Inhibited



## SUMMARY

- Weekly administration of IMO-3100 to non-human primates for 4 weeks suppressed ex-vivo immune responses mediated through TLR7 and TLR9.
- Cytokines including IL-1β, IFN-α, IL-6, IP-10, IL-1Ra, MIP-1α, and MIP-1β showed a 25 to 95% reduction compared with pre-dose levels.
- Weekly administration of IMO-3100 led to continued suppression of cytokines up to day 28.
- By day 35, two weeks after the last dose of IMO-3100 administration, secretion of most cytokines started to rebound to pre-dose levels.
- IMO-3100 showed insignificant suppression of TLR4 agonist-induced cytokine responses.

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