

# Differential Binding of Sarilumab and Tocilizumab to IL-6R $\alpha$ and Effects of Receptor Occupancy on Clinical Parameters

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### **Acknowledgments**

The authors would like to thank Ching-Ha (Vicki) Lai for providing support for the bioanalytical analyses of PK data. Medical writing assistance was provided under the direction of the authors by Peter Tran, PhD, at Adelphi Communications Ltd (Macclesfield, UK) in accordance with Good Publication Practice (GPP3) guidelines.

*Funding:* The studies described herein were sponsored by Sanofi and Regeneron Pharmaceuticals, Inc. Funding for medical writing assistance with this manuscript was provided by Sanofi and Regeneron Pharmaceuticals, Inc.

*Competing interests:* C. Xu, P. Nolain, Q. Lu, M. Iglesias-Rodriguez, and V. Kanamaluru are employees of Sanofi Genzyme, and may hold stock and/or stock options in the company; A. Paccaly, A. Rafique, T. Potocky, G. St John, M. C. Nivens, and J. Fairhurst are employees of Regeneron Pharmaceuticals, Inc., and may hold stock and/or stock options in the company; R. Maldonado has received research grants or consulting fees from Aelis, Almirall, Boehringer Ingelheim, BrainCo, Esteve, Ferrer, GlaxoSmithKline, Grünenthal, GW Pharmaceuticals, Janus, Lundbeck, Pharmaleads, Phytplant, Rhodes, Sanofi, Spherium, Union de Pharmacologie Scientifique Appliquée, Upjohn, and Uriach; T. Ishii has received research grants or consulting fees, or participated in speakers' bureaus, from/for AbbVie, Asahi, Astellas, Chugai, Daiichi Sankyo, Eisai, GlaxoSmithKline, Janssen, Kasei Pharma,

Mitsubishi Tanabe, Ono, Pfizer, Sanofi, Takeda, Teijin, and UCB; E. Choy has received research grants or consulting fees, or participated in advisory boards or speakers' bureaus, from/for AbbVie, Amgen, AstraZeneca, Bio-Cancer, Biogen, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Chelsea Therapeutics, Chugai Pharma, Daiichi Sankyo, Eli Lilly, Ferring Pharmaceuticals, GlaxoSmithKline, Hospira, Ionis, Janssen, Jazz Pharmaceuticals, MedImmune, Merck Sharp & Dohme, Merrimack Pharmaceuticals, Napp, Novartis, Novimmune, ObsEva, Pfizer, R-Pharm, Regeneron Pharmaceuticals, Inc., Roche, Sanofi-Aventis, Sanofi Genzyme, SynAct Pharma, Tonix, and UCB; and P. Emery has received grant/research support or consulting fees from/for AbbVie, Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Pfizer, Roche, and UCB.

*Availability of data and materials:* Qualified researchers may request access to patient-level data and related study documents, including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and data set specifications. Patient-level data will be anonymized and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi's data-sharing criteria, eligible studies, and process for requesting access can be found at: <https://www.clinicalstudydatarequest.com>.

Word count: 3790

Total Figures and Tables: 6

References: 25

## Abstract

We evaluated interleukin (IL)-6R $\alpha$  signaling inhibition with sarilumab and tocilizumab, the association between IL-6R $\alpha$  receptor occupancy (RO) and C-reactive protein (CRP), and potential clinical relevance of any differences. For this, we measured IL-6R $\alpha$  binding and signaling inhibition with sarilumab and tocilizumab in vitro, simulated soluble (s)IL-6R $\alpha$  RO over time for approved sarilumab SC and tocilizumab IV and SC doses, and assessed associations between calculated RO and CRP reduction, DAS28-CRP, and ACR20/50/70 from clinical data. Sarilumab binds IL-6R $\alpha$  in vitro with 15–22-fold higher affinity than tocilizumab, and inhibits IL-6-mediated classical and *trans* signaling via membrane-bound and sIL-6R $\alpha$ . Sarilumab 200 mg and 150 mg SC Q2W achieved >90% RO after first and second doses, respectively, maintained throughout the treatment period. At steady-state trough, RO was greater with sarilumab 200 mg (98%) and 150 mg SC Q2W (94%), and tocilizumab 162 mg SC QW (>99%) and 8 mg/kg IV Q4W (99%), vs tocilizumab 162 mg SC Q2W (84%) and 4 mg/kg IV Q4W (60%). Higher RO was associated with greater CRP reduction and DAS28-CRP reduction, and more sarilumab patients achieving ACR20/50/70. Greatest reduction in CRP levels was observed with sarilumab (both doses) and tocilizumab 8 mg/kg IV Q4W (reductions proportionally smaller with 4 mg/kg IV Q4W). Higher IL-6R $\alpha$  binding affinity translated into higher RO with sarilumab vs tocilizumab 4 mg/kg Q4W or 162 mg Q2W; tocilizumab required the higher dose or increased frequency to maintain the same degree of RO and CRP reduction. Higher RO was associated with clinical parameter improvements.

## Keywords

rheumatoid arthritis, sarilumab, tocilizumab, receptors, interleukin-6, C-reactive protein

## Introduction

Rheumatoid arthritis (RA) is a chronic and debilitating autoimmune disease, and interleukin-6 (IL-6) is a pleiotropic cytokine that acts as a critical signaling node in the complex proinflammatory cytokine network that underpins RA.<sup>1,2</sup> IL-6 elevations have been noted in sera and synovial fluid from patients with RA, and correlate with RA disease activity and joint destruction.<sup>3</sup> IL-6 may contribute to comorbidities associated with RA, including mood disorders, cardiovascular disease, diabetes, and osteoporosis.<sup>4-6</sup>

IL-6 effects are mediated through interaction with the IL-6 receptor alpha subunit (IL-6R $\alpha$ ). IL-6 activates classical (*cis*) signaling through membrane-bound IL-6R $\alpha$  (mIL-6R $\alpha$ ), expressed on the surface of hepatocytes and hematopoietic cells, and *trans* signaling through soluble IL-6R $\alpha$  (sIL-6R $\alpha$ ), found in serum and synovial fluid. Signaling with sIL-6R $\alpha$  occurs after the IL-6/sIL-6R $\alpha$  complex binds to the ubiquitously expressed glycoprotein (gp)130 receptor, thus greatly expanding the spectrum of IL-6-responsive cells.<sup>5,7,8</sup> Pharmacodynamic (PD) effects of IL-6R blockade include decreased production of inflammatory acute-phase reactants. C-reactive protein (CRP) is one such example, which can also be considered a surrogate marker of efficacy.

Sarilumab and tocilizumab are monoclonal antibodies (mAbs) that block IL-6 binding to sIL-6R $\alpha$  and mIL-6R $\alpha$ , thereby inhibiting IL-6 signaling through this pathway.<sup>9-12</sup> Sarilumab (human mAb) and tocilizumab (humanized mAb) are approved for the treatment of adults with moderately to severely active RA and inadequate responses to disease-modifying antirheumatic drugs. Sarilumab is administered subcutaneously (SC; also subcutaneous) at 200 mg once every 2 weeks (Q2W), with reduction to 150 mg Q2W if required to manage laboratory abnormalities.<sup>11,12</sup> In the USA, the recommended tocilizumab dose is 4 mg/kg every 4 weeks (Q4W) for intravenous (IV; also intravenously) administration or 162 mg Q2W for SC administration. Up-titration to 8 mg/kg IV Q4W or 162 mg SC

weekly (QW) (if clinical response is inadequate) is recommended for IV and SC administration, respectively. In the European Union, higher tocilizumab doses of 8 mg/kg IV Q4W and 162 mg SC QW are recommended, with down-titration if required to manage laboratory abnormalities.<sup>9,10</sup>

The objectives of this analysis were to evaluate differences in IL-6R $\alpha$  binding profiles in vitro and the resultant functional activities of sarilumab and tocilizumab, and then to explore, using a pharmacokinetic (PK)/PD modeling approach, how binding translates in vivo to receptor occupancy (RO) following recommended dosing of sarilumab and tocilizumab. The association between RO and subsequent changes in clinical efficacy parameters (CRP reduction, 28-joint Disease Activity Score based on CRP [DAS28-CRP], and 20%/50%/70% improvement in American College of Rheumatology [ACR] responses) observed in a dose-ranging study in patients with RA, were also evaluated.

## Methods

Studies were conducted in accordance with Good Clinical Practice and the principles embodied in the Declaration of Helsinki, protocols and patient information were approved by relevant institutional review boards, and all patients provided written informed consent.

### In Vitro IL-6 Binding and Signaling

#### *Kinetic Binding Analysis*

Binding kinetics of sarilumab and tocilizumab to IL-6R $\alpha$  were measured using Surface Plasmon Resonance (SPR; Biacore<sup>TM</sup> T200). Further details are included in the Supplemental Appendix. Binding kinetics calculated were association rate constant ( $K_{on}$ ), dissociation rate constant ( $K_{off}$ ), and half-life ( $t_{1/2}$ ). The overall equilibrium dissociation constant ( $K_D$ ) was calculated from the ratio of  $K_{off}$  to  $K_{on}$ . Further details are included in the Supplemental Appendix.

### *Blockade of Dimeric Human IL-6R $\alpha$ Binding to IL-6*

Human (h)IL-6R $\alpha$  binding to IL-6 was assessed using an enzyme-linked immunosorbent assay (ELISA) competition assay. Further details are included in the Supplemental Appendix. Values for the inhibitory concentration at 50% activity (IC<sub>50</sub>) and effective concentration at 50% activity (EC<sub>50</sub>) were calculated for human (h)IL-6, sarilumab, and tocilizumab. Further details are included in the Supplemental Appendix.

### *Inhibition of Classical IL-6R $\alpha$ Signaling*

The activities of sarilumab and tocilizumab in blocking classical IL-6R $\alpha$  signaling were compared in vitro in cell proliferation assays using:

- DS-1: a human B lymphocyte cell line that proliferates in response to exogenous hIL-6, and endogenously expresses IL-6R $\alpha$  and gp130
- HepG2: a hepatocytic cell line endogenously expressing IL-6R $\alpha$  and gp130

Further details are included in the Supplemental Appendix.

### *Inhibition of Trans IL-6R $\alpha$ Signaling*

The ability of sarilumab to block *trans* signaling stimulated by the IL-6/sIL-6R $\alpha$  complex was assessed in a functional cell-based luciferase assay using a gp130-overexpressing human embryonic kidney/signal transducer and activator of transcription 3 (STAT3)/luciferase reporter cell line. Further details are included in the Supplemental Appendix.

## PK/PD Modeling of RO and Effects on CRP Reduction, DAS28-CRP, and ACR20/50/70

### *PK Model*

The PK framework for the sIL-6R $\alpha$  PK/PD models was provided by population PK (PopPK) models for sarilumab SC,<sup>13</sup> and tocilizumab IV<sup>14</sup> and SC.<sup>15,16</sup> These models described the PKs of sarilumab and tocilizumab using a two-compartment model with parallel linear and non-linear Michaelis–Menten elimination, and with first-order absorption for sarilumab and tocilizumab SC.<sup>13-15</sup>

### *sIL-6R $\alpha$ PK/PD Model Development*

Tocilizumab binding to sIL-6R $\alpha$  was described by a PK/PD model previously developed using data from studies evaluating tocilizumab IV (at 4 or 8 mg/kg Q4W) for 24 weeks in patients with RA.<sup>14</sup> Given the similarity of tocilizumab and sarilumab binding to sIL-6R $\alpha$  and mIL-6R $\alpha$ , the same structural model was used to develop the PK/PD model to describe sarilumab binding to sIL-6R $\alpha$  following SC dosing, using data from MOBILITY Part A (NCT01061736). MOBILITY Part A was a phase 2, double-blind, placebo-controlled, dose-ranging study in 306 patients with RA, evaluating 5 sarilumab SC regimens (100 mg Q2W, 150 mg Q2W, 100 mg QW, 200 mg Q2W, and 150 mg QW) over 12 weeks.<sup>17</sup>

The quasi-steady-state target-mediated drug disposition (TMDD) models describing PK/PD relationships to total sIL-6R $\alpha$  for sarilumab and tocilizumab, including the PD model of inhibiting sIL-6R $\alpha$  elimination, are summarized in Supplemental Figure S1. The PK/PD model for binding to sIL-6R $\alpha$  was used to predict the time course of free sIL-6R $\alpha$  concentrations for sarilumab and tocilizumab. Only sIL-6R $\alpha$  (not mIL-6R $\alpha$ ) was considered in these analyses. The sIL-6R $\alpha$  PK/PD model analysis was performed using NONMEM 7.2.0 (ICON plc, Dublin, Ireland). The quality of the PK/PD model was extensively evaluated using standard goodness-of-fit (GOF) criteria (observations vs individual and population predictions, and weighted residuals), as well as by the condition number. The final PK/PD model was evaluated using a visual predictive check (VPC) to test the robustness of the model and its predictive performance using the parameter estimates.

### *Simulation of sIL-6R $\alpha$ Occupancy by Sarilumab and Tocilizumab*

Literature-reported PK and PK/PD models of tocilizumab, and developed PK and PK/PD models of sarilumab, were used to profile the time courses of tocilizumab or sarilumab concentrations in serum, and estimate binding to sIL-6R $\alpha$  and free sIL-6R $\alpha$  concentrations for the approved dosage regimens of sarilumab SC (200 mg and 150 mg Q2W), tocilizumab



IV (8 and 4 mg/kg Q4W), and tocilizumab SC (162 mg QW and Q2W). sIL-6R $\alpha$  RO dynamic profiles (percentage RO over time) were calculated based on unbound sIL-6R concentrations:

$$RO = 1 - (\text{free sIL-6R}_{\text{posttreatment}} / \text{free sIL-6R}_{\text{baseline}})$$

RO over 24 weeks was calculated following sarilumab SC and tocilizumab IV regimens from ASCERTAIN (NCT01768572), a phase 3 safety study in which 202 patients with RA were randomized to sarilumab 200 mg or 150 mg SC Q2W, or tocilizumab 4 mg/kg IV Q4W, for 24 weeks.<sup>18</sup> Patients were able to up-titrate their tocilizumab IV dosage to 8 mg/kg in cases of inadequate clinical response (61% required up-titration). Additional simulation of RO for tocilizumab SC regimens was provided based on the phase 3 randomized, double-blind SUMMACTA and BREVACTA 24-week studies,<sup>15</sup> evaluating tocilizumab 162 mg SC QW vs IV 8 mg/kg Q4W (N = 1262) and tocilizumab 162 mg SC Q2W vs placebo (N = 656), respectively.<sup>19,20</sup>

#### *Association Between sIL-6R $\alpha$ RO and CRP, DAS28-CRP, and ACR20/50/70 Responses*

The association of median RO calculated from observed concentrations of free sIL-6R $\alpha$  measured in MOBILITY Part A<sup>17</sup> was plotted against median levels of CRP reduction, median DAS28-CRP reduction, and ACR20/50/70 responses by treatment groups in patients randomized to receive sarilumab 100 mg Q2W ( $n = 51$ ), sarilumab 150 mg Q2W ( $n = 51$ ), sarilumab 100 mg QW ( $n = 50$ ), sarilumab 200 mg Q2W ( $n = 52$ ), sarilumab 150 mg QW ( $n = 50$ ), or placebo ( $n = 52$ ) for 12 weeks. IL-6R $\alpha$  RO profiles were compared visually with changes in observed mean CRP levels from ASCERTAIN, described above. To further verify the association observed in ASCERTAIN, sIL-6R $\alpha$  RO profiles were compared visually with changes in reported mean CRP levels from SUMMACTA and BREVACTA.

## Results

### In Vitro IL-6 Binding and Signaling

Sarilumab bound with high affinity to recombinant monomeric and dimeric hIL-6R $\alpha$  in SPR assays, with  $K_D$  values of 61.9 pM and 12.8 pM, respectively (Table 1, Supplemental Figure S2A). Sarilumab showed 15–22-fold higher affinity than tocilizumab in binding to monomeric and dimeric hIL-6R $\alpha$  forms (Table 1, Supplemental Figure S2B).

In the ELISA competition assay, sarilumab directly blocked the binding of hIL-6R $\alpha$ -fragment crystallizable (Fc) to plate-coated hIL-6 with an  $IC_{50}$  of 108 pM (achieving complete blockade to baseline levels), whereas the immunoglobulin G1 isotype control showed no blocking activity under the same conditions (Supplemental Figure S3B). A constant concentration of 100 pM IL-6R $\alpha$ -human Fc was used in the assay, which bound to hIL-6 with an  $EC_{50}$  of 255 pM. At the time, only sarilumab was available for evaluation in this assay.

In the in vitro proliferation assay, both sarilumab and tocilizumab inhibited IL-6-mediated proliferation of DS-1 cells (classical signaling, Figure 1A), with  $IC_{50}$  values approximately 3.6-fold more potent for sarilumab than tocilizumab (226 pM vs 812 pM, in the presence of 1.0 pM IL-6). hIL-6 had an  $EC_{50}$  value of 0.5 pM in this assay. Sarilumab and tocilizumab inhibited IL-6-mediated luciferase activity in the HepG2 cell luciferase reporter assay, indicating inhibition of classical IL-6R $\alpha$  signaling via the STAT3 pathway (Figure 1B). Sarilumab was approximately 3.4-fold more potent than tocilizumab with an  $IC_{50}$  of 146 pM vs 496 pM (in the presence of 50 pM IL-6). hIL-6 had an  $EC_{50}$  value of 59 pM in this assay.

In the HEK293 cell line, IL-6 was shown to activate gp130 receptor *trans* signaling in the presence of 1 nM or 10 nM sIL-6R $\alpha$ -myc-myc-hexahistidine (mmH) with  $EC_{50}$  values of 1.8 nM and 0.7 nM, respectively; signaling could not be activated by IL-6 alone (Figure 1C and 1D). Sarilumab blocked *trans* signaling with an  $IC_{50}$  of 0.9 nM in the presence of 1 nM

sIL-6R $\alpha$ -mmH and 10 nM IL-6, and an IC<sub>50</sub> of 8.9 nM in the presence of 10 nM sIL-6R $\alpha$ -mmH and 10 nM IL-6 (Figure 1C and 1D). Again, at the time, only sarilumab was available for evaluation in this assay.

#### PK Modeling, PK/PD Modeling of RO and Effects on Clinical Efficacy Parameters

Parameter estimates of the sarilumab PK model are presented in Supplemental Table S1.

Parameter estimates of the sIL-6R $\alpha$  PK/PD models are presented in Supplemental Table S2.

GOF evaluation indicated that the final sIL-6R $\alpha$  PK/PD model was consistent with the observed data (Supplemental Figure S4) and the VPC showed that the time-course profiles with the observed concentrations (2.5<sup>th</sup>, 50<sup>th</sup>, and 97.5<sup>th</sup> percentiles) fitted the predicted parameters well (Supplemental Figure S5).

#### *Simulation of sIL-6R $\alpha$ Occupancy by Sarilumab and Tocilizumab*

Simulated sIL-6R $\alpha$  RO profiles over 24 weeks for sarilumab SC vs tocilizumab IV and sarilumab SC vs tocilizumab SC are shown in Figure 2A and 2B. Sarilumab 200 mg SC Q2W achieved >90% RO after the first dose, maintained over the dosing interval and throughout the 24-week simulated treatment period (Figure 2A). Simulated RO for sarilumab 150 mg SC Q2W decreased to 74% towards the end of the first dosing interval, but from the third dose onward was maintained at >90% over the full dosing interval.

The tocilizumab 8 mg/kg IV Q4W and the 162 mg SC QW dose regimens achieved >90% simulated RO from the first dose, maintained over the dosing interval and throughout the 24-week simulated treatment period (Figure 2A and 2B). In contrast, with the tocilizumab 4 mg/kg IV Q4W and the 162 mg SC Q2W dose regimens, trough RO values below the 90% threshold were predicted by the end of each dosing interval over the 24-week period (Figure 2A and 2B).

At week 24, steady-state trough IL-6R $\alpha$  RO levels were 98% and 94% for sarilumab 200 mg and 150 mg SC Q2W regimens, respectively, 99% and 60% for tocilizumab 8 mg/kg and

4 mg/kg IV Q4W regimens, respectively (Figure 2A), and >99% (99.6%) and 84% for tocilizumab 162 mg SC QW and Q2W regimens, respectively (Figure 2B).

#### *Association Between sIL-6R $\alpha$ Occupancy and Clinical Efficacy Parameters*

In MOBILITY Part A, the association of sIL-6R $\alpha$  RO was assessed for the following clinical efficacy parameters: percentage CRP reduction, DAS28-CRP score, and ACR20/50/70.

Week 12 RO was estimated based on the observed free sIL-6R $\alpha$  data in MOBILITY Part A for placebo, sarilumab 100 and 150 mg QW, and sarilumab 100, 150, and 200 mg Q2W, and plotted against Week 12 efficacy data. Higher RO was associated with greater CRP reduction and consequently with larger reductions in DAS28-CRP scores (Figure 3A and 3B). There was also an apparent association between higher RO and ACR20/50/70 responses (Figure 3C).

In ASCERTAIN, where patients were randomized to sarilumab or tocilizumab, sarilumab induced rapid (from week 4: first assessment after first dose) and sustained reduction of CRP throughout the dosing period. At week 24, the greatest reduction in CRP levels was observed in patients receiving sarilumab SC (at either dose), or in patients who escalated their tocilizumab dose to 8 mg/kg IV Q4W (Figure 4). The magnitude of CRP reduction was lower in patients who remained on tocilizumab 4 mg/kg IV Q4W. Comparing observed CRP levels for SC QW and Q2W tocilizumab from SUMMACTA and BREVACTA (Figure 5), the inverse relationship between sIL-6R $\alpha$  RO and CRP appeared to hold true for these SC tocilizumab regimens.

## **Discussion**

In vitro experiments reported here demonstrate that sarilumab has a higher relative binding affinity to sIL-6R $\alpha$  compared with tocilizumab, and inhibits IL-6-induced cellular responses (ie, cell proliferation and STAT3 signaling) with higher potency and at lower concentrations than tocilizumab. Consistently, PK/PD modeling, using data from phase 2 and 3 studies in

patients with RA, indicated higher and more sustained IL-6R $\alpha$  RO with sarilumab SC Q2W dose regimens than with tocilizumab 4 mg/kg IV Q4W or 162 mg SC Q2W. Higher RO was associated with better clinical parameters.

IL-6 activates cells via a signaling mechanism that requires 2 receptor components: IL-6R $\alpha$  and gp130. IL-6 forms a heterodimer with IL-6R $\alpha$  that subsequently binds with high affinity to gp130, forming a heterotrimeric complex.<sup>5,7,8</sup> IL-6R $\alpha$  exists in both membrane-bound and soluble forms, with sIL-6R $\alpha$  generated through cleavage of mIL-6R $\alpha$  or alternative splicing. Classical signaling through mIL-6R $\alpha$  is limited to the few cell types that express mIL-6R $\alpha$ , that is hepatocytes, monocytes/macrophages, neutrophils, and some T cell subsets. *Trans* signaling through sIL-6R $\alpha$  may occur in virtually any nucleated cell type (including those that lack IL-6R $\alpha$  expression) because of the ubiquitous expression of membrane gp130.<sup>8</sup>

Previously, Mihara and colleagues showed that tocilizumab bound to sIL-6R $\alpha$  inhibited IL-6 binding in a dose-dependent manner, and dissociated IL-6 and sIL-6R $\alpha$  from their preformed complex. Tocilizumab suppressed the IL-6/sIL-6R $\alpha$  complex-induced proliferation of human gp130-transfected cells (BAF-h130) and bound hIL-6R-expressing COS-7 cells. It also suppressed growth of the human IL-6-dependent myeloma cell line KPMM2.<sup>21</sup> In the present in vitro studies, sarilumab was shown to bind directly to IL-6R $\alpha$ , but with a binding affinity for both monomeric and dimeric IL-6R $\alpha$  approximately 15–22-fold higher than that of tocilizumab. The studies confirm the ability of sarilumab to block both classical- and *trans*-mediated signaling under in vitro conditions. Sarilumab blocked IL-6-induced growth of the human B cell line DS-1 and inhibited IL-6-induced STAT3 signaling in the human hepatocyte cell line HepG2 at concentrations approximately 3-fold lower than tocilizumab. Sarilumab was found to completely inhibit activation in a *trans* signaling assay in which cells expressing gp130 were stimulated by the sIL-6R $\alpha$  complex.

Additional factors can influence binding affinity *in vivo*, including baseline receptor concentration, receptor turnover, receptor distribution, antibody concentration, and antibody distribution.<sup>22</sup> Therefore, a PK/PD model was developed that incorporated these parameters to profile sIL-6R $\alpha$  RO for approved dose regimens of sarilumab SC (200 mg and 150 mg Q2W) and tocilizumab IV (4 and 8 mg/kg Q4W) and SC (162 mg QW and Q2W). This model used previously published and validated PopPK parameters to describe sarilumab and tocilizumab drug concentrations in sera for the dosing regimens tested. Sarilumab and tocilizumab are both eliminated by parallel linear and non-linear pathways, with the linear, non-saturable proteolytic pathway predominating at higher concentrations.<sup>9,12</sup>

Sarilumab and tocilizumab bind to both the sIL-6R $\alpha$  and mIL-6R $\alpha$  forms. It was shown that the TMDD system with 2 targets can be approximated by equations that describe both sarilumab or tocilizumab and sIL-6R $\alpha$  concentrations, and include 2 target-mediated elimination terms (with different maximum elimination rate [ $V_{\max}$ ] and Michaelis–Menten constant [ $K_m$ ] parameters). However, mIL-6R $\alpha$  was not measured in the clinical studies and insufficient data did not allow for separation of the 2 different  $V_{\max}$  and  $K_m$  parameters. The *in vitro*  $K_D$  values for tocilizumab–sIL-6R (0.11  $\mu\text{g/mL}$  [0.75 nmol/L]) and tocilizumab–mIL-6R binding (0.38–0.43  $\mu\text{g/mL}$  [2.5–2.9 nmol/L]) suggest a similar range of binding affinity for the 2 forms of the target.<sup>14</sup>

RO assays applied in both non-clinical and clinical studies provided an insight into PK/PD relationships for binding to receptors. RO on circulating cells has been used as a PD biomarker for nivolumab and etrolizumab.<sup>23</sup> RO simulations generated from the PK/PD models in the current study indicate that sarilumab 200 mg SC dosing regimens, and the tocilizumab 8 mg/kg IV Q4W and 162 mg SC QW regimens, are able to achieve and maintain high levels of target sIL-6R $\alpha$  RO for the entire dosage interval over a 24-week treatment period (for sarilumab 150 mg SC dosing at Week 4 onwards). For the lower

tocilizumab dose of 4 mg/kg IV Q4W and the less-frequent 162 mg SC Q2W regimen, which comprise the USA-recommended tocilizumab starting doses, RO fell to lower levels towards the end of each dosing period. It is conceivable that the lower RO contributed to the lesser decrease in CRP levels with the tocilizumab 4 mg/kg IV Q4W dose regimen relative to the sarilumab treatment regimens and the tocilizumab 8 mg/kg IV Q4W dose regimen. These RO findings are consistent with the higher sIL-6R $\alpha$  binding affinity and slower dissociation kinetics of sarilumab compared with tocilizumab observed in earlier in vitro assays, and with the serum tocilizumab trough concentration being 134-fold lower with the 4 vs 8 mg/kg IV regimen, and 10.5-fold lower with the Q2W vs QW regime.<sup>9,15</sup>

Besides being of scientific interest, the importance of the current findings becomes apparent with the associations between RO, and PD and clinical parameters. Concentrations of sIL-6R $\alpha$  in sera were measured in MOBILITY Part A, and the dose-ranging portion of that study indicated an association between the degree of RO and the degree of CRP reduction, DAS28-CRP improvement, and/or ACR20/50/70 response at Week 12. MOBILITY Part A was a Phase 2 study with N=37–49 patients for each arm.<sup>17</sup> The median percentage reduction of free sIL-6R $\alpha$  represented the central tendency of highly variable data. Similarly, observed CRP and DAS28-CRP data were also highly variable in this relatively small sample size study, eg, coefficient of variance of CRP reduction ranged from 130% to 380%. The mean values of CRP and DAS28-CRP data were in general consistent with median values, and median values were used in Figure 3. Notwithstanding the limitation of a small sample size and highly variable data, Figure 3 supports a qualitative association of RO and clinical parameters. The clinical efficacy of two doses of sarilumab (150 mg q2w and 200 mg q2w) were evaluated in the large Phase 3 study, MOBILITY Part B.<sup>24</sup> Both doses provided sustained clinical efficacy vs placebo, with the greatest improvements observed for patients treated with the sarilumab 200 mg q2w dose. ASCERTAIN was the first double-blind,

multiple-dose safety study in patients with RA to include sarilumab SC and tocilizumab IV dosing regimens within the same study,<sup>25</sup> allowing PK/PD comparisons to be made. The IL-6R $\alpha$  RO results generated with the present models are inversely associated with observed changes in CRP levels over the 24-week treatment period of ASCERTAIN, confirming the expected association between RO and clinically relevant PD markers. Rapid dose-related reduction of CRP sustained over 24 weeks was noted for sarilumab SC 200 and 150 mg Q2W regimens, whereas little or no CRP reduction was evident at week 4 in the tocilizumab 4 mg/kg IV Q4W group. As anticipated based on RO simulations, patients on the 4 mg/kg IV Q4W dose who up-titrated their tocilizumab dose to 8 mg/kg IV Q4W because of insufficient clinical response experienced reductions in CRP levels similar to those observed in the sarilumab groups.

It is not possible to directly compare SC regimens of sarilumab and tocilizumab in terms of the association between sIL-6R $\alpha$  occupancy and CRP, as ASCERTAIN did not include SC tocilizumab as it was not available at that time. When the RO results generated for the QW and Q2W tocilizumab regimens were evaluated, the inverse relationship between sIL-6R $\alpha$  occupancy and CRP appeared to hold true for the SC tocilizumab regimens, in addition to the observed CRP data for these regimens in SUMMACTA and BREVACTA.<sup>15</sup> PK/PD analysis of these 2 studies showed a more gradual decline of PD responses (both CRP and erythrocyte sedimentation rate) over time for the SC Q2W regimen, compared with the tocilizumab IV Q4W and SC QW regimens. Other limitations include the possible effects on CRP by a number of other factors, most notably infection. Also, the findings presented here are restricted to the sIL-6R $\alpha$  isoform, because mIL-6-R $\alpha$  was not measured in either the tocilizumab or sarilumab trials.

These findings on the effects of RO on clinical efficacy parameters support selection of dosing regimens of sarilumab (150 mg and 200 mg SC Q2W) as providing the required



occupancy of IL-6R $\alpha$  to elicit optimal reduction of clinical markers of IL-6 activity (CRP). These findings might have important clinical implications because they suggest that, for tocilizumab, the lower 4 mg/kg IV Q4W and 162 mg SC Q2W dosing regimens may not provide adequate occupancy of IL-6R $\alpha$  to elicit the desired clinical effect. This is consistent with in vitro experiments that show that sarilumab has a higher relative binding affinity for IL-6R $\alpha$  and is more potent at inhibiting IL-6-mediated signaling at lower concentrations in serum than tocilizumab.

### **Conclusions**

In summary, sarilumab demonstrated higher sIL-6R $\alpha$  binding affinity and potency compared with tocilizumab. Higher and more sustained RO with IL-6R $\alpha$  was achieved with sarilumab SC Q2W and tocilizumab IV 8 mg/kg dose regimens compared with tocilizumab 4 mg/kg IV Q4W or 162 mg SC Q2W. The dosing regimens with higher RO (sarilumab 150 mg SC Q2W and 200 mg SC Q2W and tocilizumab 8 mg/kg IV Q4W and 162 mg SC QW) were associated with a greater reduction in CRP, a measure of the acute phase response and a potential surrogate clinical efficacy parameter in patients with high levels of CRP and disease activity.

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## Figures and Tables

**Figure 1.** Blockade of classical IL-6R $\alpha$  signaling by sarilumab and tocilizumab in (A) proliferation assay in DS-1 cells and (B) STAT3 signaling in HepG2/STAT3-Luc cells, and sarilumab blockade of *trans* IL-6R $\alpha$  signaling in HEK293/gp130/STAT3-Luc cells exposed to 10 nM hIL-6 and (C) 1 nM hIL-6R $\alpha$  or (D) 10 nM hIL-6R $\alpha$ . Error bars indicate standard deviation.

**Figure 2.** Simulated receptor occupancy profile through week 24 for (A) sarilumab SC vs tocilizumab IV and (B) sarilumab SC vs tocilizumab SC.

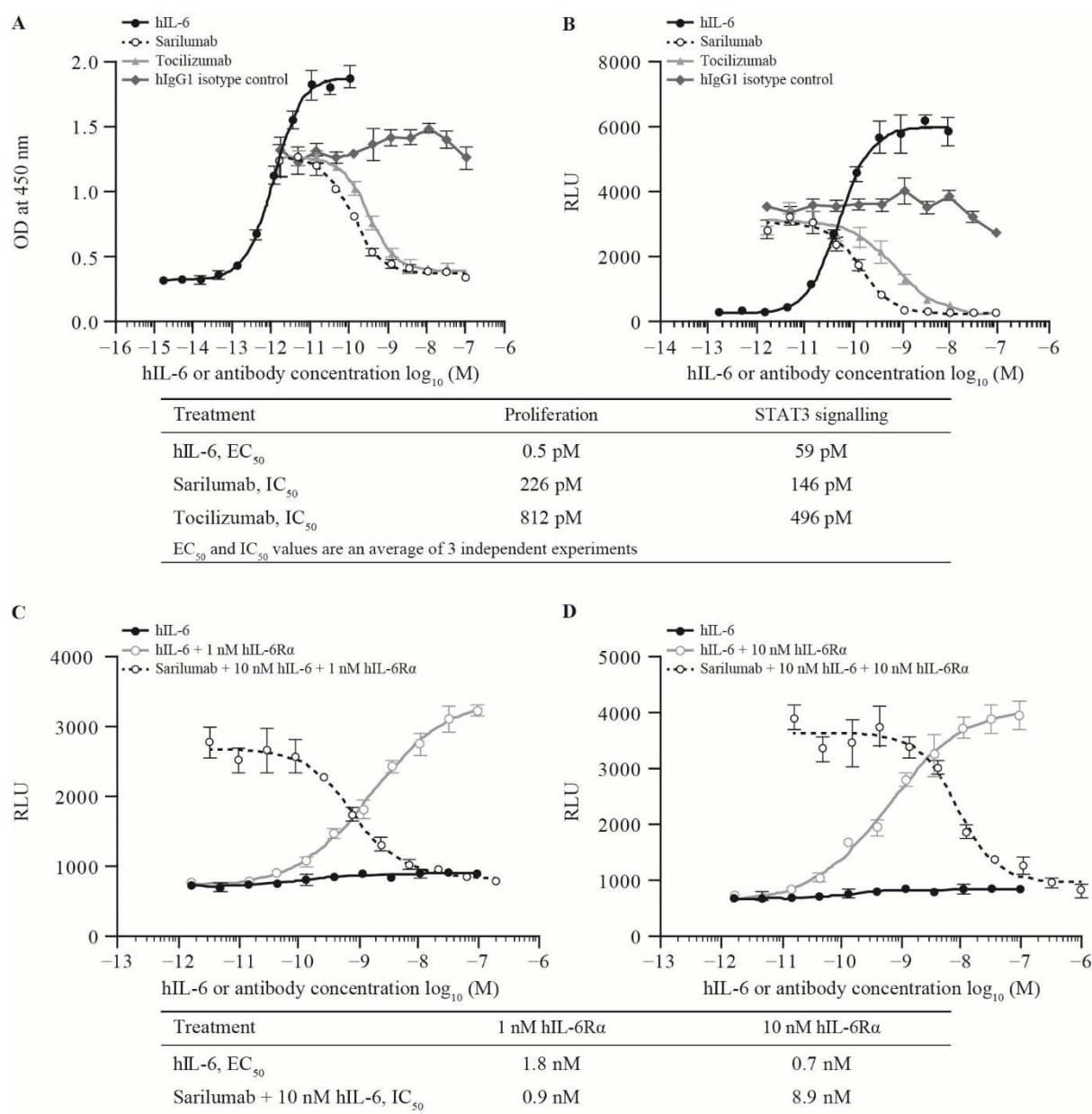
**Figure 3.** Relationship between receptor occupancy and (A) CRP reduction, (B) DAS28-CRP reduction, and (C) ACR20/50/70 responses with placebo and sarilumab (MOBILITY Part A).

**Figure 4.** Observed mean CRP levels ( $\pm$  SE) in patients with RA treated with sarilumab SC or tocilizumab IV (ASCERTAIN).

**Figure 5.** Mean changes from baseline in CRP for patients treated with tocilizumab SC and IV (SUMMACTA, BREVACTA), reprinted under CC BY-NC-ND 4.0 from Figure 3 by *The Journal of Clinical Pharmacology*.

**Table 1.** Pharmacokinetic and binding parameters for sarilumab and tocilizumab to monomeric (mmH-tagged) and dimeric (mFc-tagged) human IL-6R $\alpha$  proteins.

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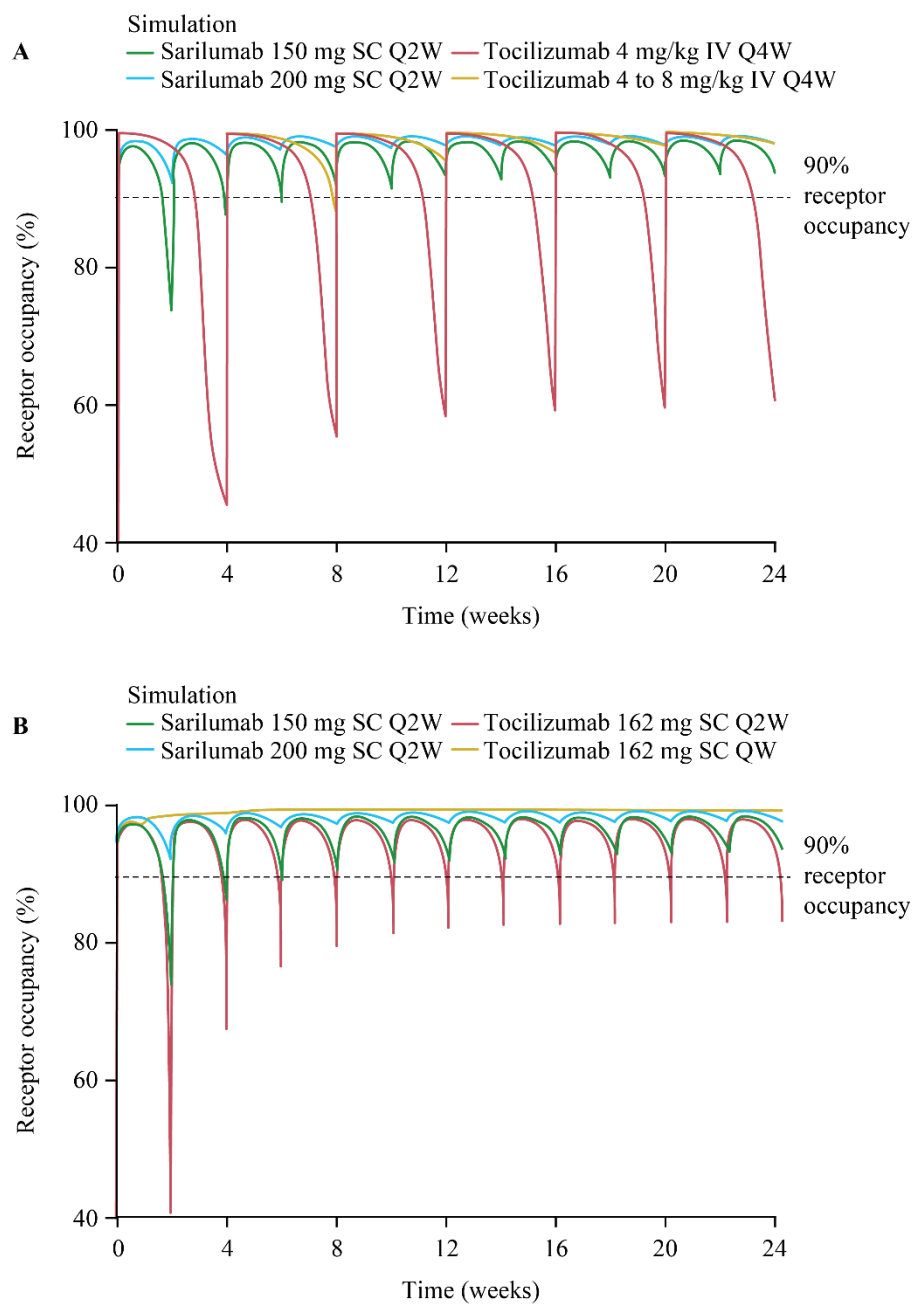
EC<sub>50</sub>, effective concentration at 50% activity; gp, glycoprotein; hIgG1, human

immunoglobulin G1; hIL-6, human interleukin-6; hIL-6R $\alpha$ , human interleukin-6 receptor

alpha subunit; IC, inhibitory concentration; IC<sub>50</sub>, inhibitory concentration at 50% activity;

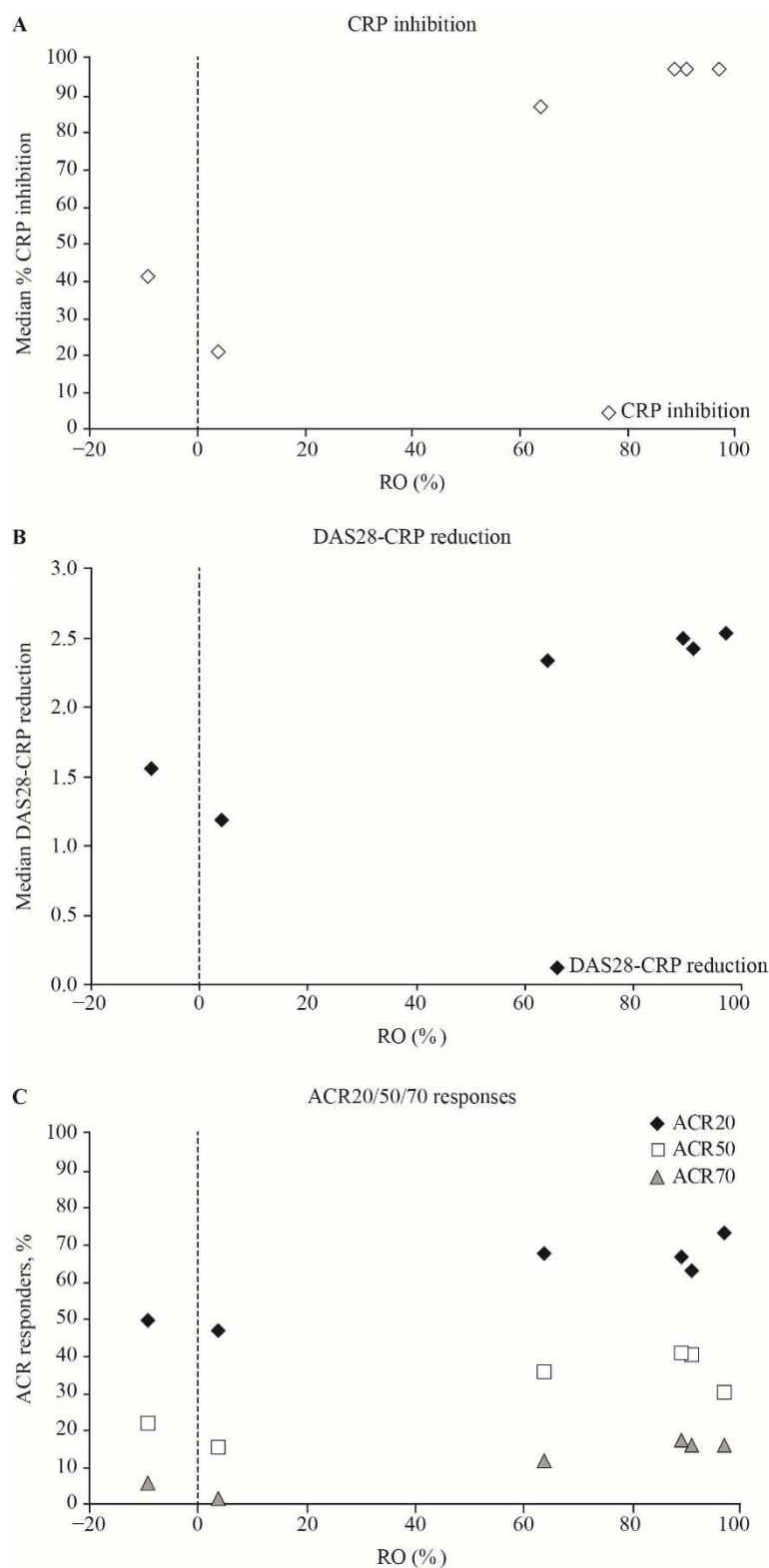
IL-6R $\alpha$ , interleukin-6 receptor alpha subunit; Luc, luciferase; OD, optical density; RLU, relative luminescence unit; STAT3, signal transducer and activator of transcription 3.

**Figure 2.** Simulated receptor occupancy profile through week 24 for (A) sarilumab SC vs tocilizumab IV and (B) sarilumab SC vs tocilizumab SC.



IV, intravenous; Q2W, every 2 weeks; Q4W, every 4 weeks; SC, subcutaneous.

**Figure 3.** Relationship between RO and (A) CRP reduction, (B) DAS28-CRP reduction, and (C) ACR20/50/70 responses with placebo and sarilumab (MOBILITY Part A [NCT01061736]).

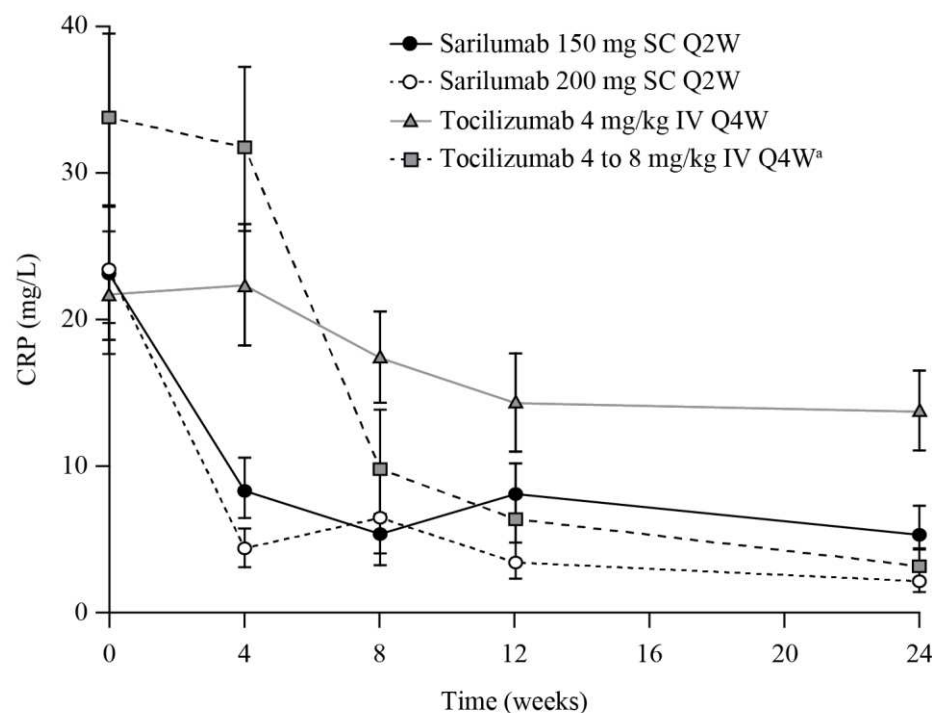




Patients received placebo ( $n = 52$ ) or sarilumab 100 mg Q2W ( $n = 51$ ), 150 mg Q2W ( $n = 51$ ), 200 mg Q2W ( $n = 52$ ), 100 mg QW ( $n = 50$ ), or 150 mg QW ( $n = 50$ ) in MOBILITY Part A (NCT01061736). Changes in free sIL-6R $\alpha$  levels were used to calculate receptor occupancy and associations were assessed against clinical parameters. Receptor occupancy and pharmacodynamic endpoints were calculated and plotted at Week 12 trough.

ACR, American College of Rheumatology; ACR20/50/70, 20%/50%/70% improvement in American College of Rheumatology criteria; CRP, C-reactive protein; DAS28-CRP, 28-joint Disease Activity Score based on C-reactive protein; Q2W, every 2 weeks; QW, weekly; RO, receptor occupancy; sIL-6R $\alpha$ , soluble interleukin-6 receptor alpha subunit.

**Figure 4.** Observed mean CRP levels ( $\pm$  SE) in patients with RA treated with sarilumab SC or tocilizumab IV (ASCERTAIN).

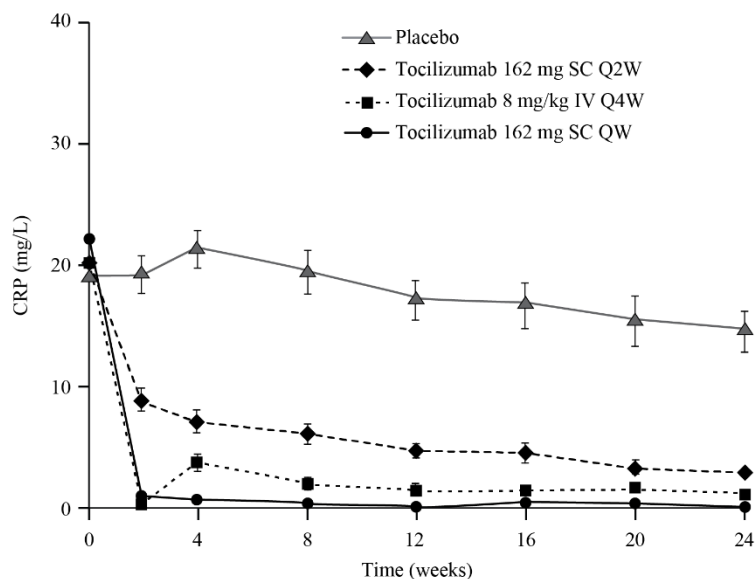


Number of patients	0	4	8	12	24
Sarilumab 150 mg	49	47	45	44	39
Sarilumab 200 mg	51	47	51	43	39
Tocilizumab 4 mg/kg	40	37	37	37	35
Tocilizumab 4 to 8 mg/kg	39	39	39	39	39

<sup>a</sup>Patients started treatment at 4 mg/kg IV Q4W followed by an increase to 8 mg/kg IV Q4W, based on clinical response; ASCERTAIN (NCT01768572).

CRP, C-reactive protein; IV, intravenous; Q2W, every 2 weeks; Q4W, every 4 weeks; RA, rheumatoid arthritis; SC, subcutaneous; SE, standard error.

**Figure 5.** Mean changes from baseline in CRP for patients treated with tocilizumab SC and IV (SUMMACTA and BREVACTA), reprinted under CC BY-NC-ND 4.0 from Figure 3 by *The Journal of Clinical Pharmacology*.



Number of patients

Placebo	—	217	217	217	210	209	145	128	124
Tocilizumab 162 mg SC Q2W	- - -	428	426	423	413	419	366	353	345
Tocilizumab 8 mg/kg IV Q4W	· · ·	536	522	532	519	518	511	507	498
Tocilizumab 162 mg SC QW	—	557	549	616	543	538	527	524	516

Mean CRP levels following treatment with SC or IV tocilizumab from Figure 3 by *The Journal of Clinical Pharmacology* is licensed under CC BY-NC-ND 4.0; SUMMACTA (NCT01194414); BREVACTA (NCT1232569).

CRP, C-reactive protein; IV, intravenous; Q2W, every 2 weeks; Q4W, every 4 weeks; QW, every week; SC, subcutaneous.

**Table 1.** Pharmacokinetic and binding parameters for sarilumab and tocilizumab to monomeric (mmH-tagged) and dimeric (mFc-tagged) human IL-6R $\alpha$  proteins.

Antigen	Antibody	$K_{on}$ (1/Ms)	$K_{off}$ (1/s)	$K_D$ (M)	$t_{1/2}$ (h)
Human IL-6R $\alpha$ monomeric	Sarilumab	$8.56e^5$	$5.30e^{-5}$	$6.19e^{-11}$	3.6
	Tocilizumab	$1.60e^5$	$2.14e^{-4}$	$1.34e^{-9}$	0.9
Human IL-6R $\alpha$ dimeric	Sarilumab	$4.02e^5$	$5.16e^{-6}$	$1.28e^{-11}$	37.3
	Tocilizumab	$7.48e^4$	$1.47e^5$	$1.96e^{-10}$	13.1

IL-6R $\alpha$ , interleukin-6 receptor alpha subunit;  $K_D$ , overall equilibrium dissociation constant;  $K_{off}$ , dissociation rate constant;  $K_{on}$ , association rate constant; mFc, mouse fragment crystallizable; mmH, myc-myc-hexahistidine;  $t_{1/2}$ , half-life.

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## Supplemental Appendix

Christine Xu et al. Differential Binding of Sarilumab and Tocilizumab to IL-6R $\alpha$  and Effects of Receptor Occupancy on Clinical Parameters

Supplemental Methodology

**Supplemental Figure S1.** Sarilumab and tocilizumab sIL-6R $\alpha$  receptor occupancy models.

**Supplemental Figure S2.** Ligand-binding properties of sarilumab (107 RU) and tocilizumab (108 RU). Representative sensograms of (A) sarilumab binding to monomer human IL-6R $\alpha$ -mmH 10, 5, 2.5, 1.25, and 0.625 nM, and (B) tocilizumab binding to monomer human IL-6R $\alpha$ -mmH 20, 10, 5, 2.5, 1.25, and 0.625 nM are shown as black lines. The data were globally fitted to a 1:1 binding interaction model using T200 evaluation software 2.0. Kinetic fits from the analyses were overlaid on the binding data in red.

**Supplemental Figure S3.** Blockade of dimeric hIL-6R $\alpha$  binding to IL-6 (ELISA competition assay). (A) Dose–response of hIL-6R $\alpha$ -hFc binding to hIL-6 and (B) concentration-dependent blockade of hIL-6R $\alpha$ -hFc binding to hIL-6 by sarilumab.

**Supplemental Figure S4.** Basic goodness-of-fit plots with LOWESS (red lines) for the final model.

**Supplemental Figure. S5.** Final model visual predictive check after multiple doses of sarilumab 100 mg Q2W, 150 mg Q2W, 200 mg Q2W, 100 mg QW, or 150 mg QW.

**Supplemental Table S1.** Parameter estimates of the sarilumab population PK model

**Supplemental Table S2.** Parameter estimates of the sIL-6R population PK/PD model.

## Supplemental Methodology

### *Kinetic Binding Analysis*

The binding kinetics of sarilumab and tocilizumab to interleukin-6 receptor- $\alpha$  (IL-6R $\alpha$ ) were measured using Surface Plasmon Resonance (SPR; Biacore™ T200) at 25°C. Sarilumab and tocilizumab were captured on an anti-human Fc-coupled chip surface and human IL-6R $\alpha$  (hIL-6R $\alpha$ ) flowed across the surface (at concentrations of 20–1.25 nM, depending on the antigen used). Antigen-dependent changes in resonance units (reflecting binding to the captured antibody) were monitored, from which binding kinetics were calculated, including the association rate constant ( $K_{on}$ ), dissociation rate constant ( $K_{off}$ ), and half-life ( $t_{1/2}$ ). The overall equilibrium dissociation constant ( $K_D$ ) was calculated from the ratio of  $K_{off}$  to  $K_{on}$ .

### *Blockade of Dimeric hIL-6R $\alpha$ Binding to IL-6 (Competitive ELISA)*

A 3-fold dilution series of sarilumab or an isotype control immunoglobulin G1 (IgG1) antibody (30 nM to 0.5 pM) was preincubated for 1 hour with 100 pM of dimeric hIL-6R $\alpha$  with a C-terminal human IgG1 fragment crystallizable (Fc) tag (IL-6R $\alpha$ -hFc), after which the mixtures were transferred to 96-well microtiter plates onto which human IL-6 (hIL-6) (2  $\mu$ g/mL) had been immobilized. Bound IL-6R $\alpha$ -hFc/IL-6 complexes were detected with a horseradish peroxidase-conjugated anti-hIL-6 Fc antibody (Jackson ImmunoResearch). Plates were visualized with 3,3', 5,5' tetramethylbenzidine (BD Biosciences) and absorbance determined using a Victor multilabel counter (Perkin Elmer). Inhibitory concentration at 50% activity ( $IC_{50}$ ) and effective concentration at 50% activity ( $EC_{50}$ ) values, the concentrations of drug resulting in half-maximal inhibition or response, respectively, were determined (GraphPad Prism™ v6) using a four-parameter logistic model.

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*Inhibition of Classical IL-6R $\alpha$  Signaling (Cell Proliferation and Signal Transducer and Activator of Transcription 3 [STAT3] Response Element Activation Assays)*

The activities of sarilumab and tocilizumab in blocking classical IL-6R $\alpha$  signaling were compared in vitro in cell proliferation assays using:

- DS-1: a human B lymphocyte cell line that proliferates in response to exogenous hIL-6 and endogenously expresses IL-6R $\alpha$  and glycoprotein (gp)130
- HepG2: a hepatocytic cell line endogenously expressing IL-6R $\alpha$  and gp130

Serial dilutions of sarilumab (100 nM to 1.7 pM) and tocilizumab (100 nM to 1.7 pM) were added to DS-1 cells (American Type Culture Collection [ATCC], CRL-11102), followed by hIL-6 (1 pM). Plates were incubated (37°C, 5% CO<sub>2</sub> for 4 days) and then visualized using AlamarBlue (Biosource) or WST-8 (Dojindo).

In the second assay, HepG2 cells (ATCC, HB-8065) were transiently transfected with a signal transducer and activator of transcription 3 (STAT3)-luciferase reporter plasmid. Dilution series of sarilumab (100 nM to 1.7 pM) and tocilizumab (100 nM to 1.7 pM) were added to the transfected cells seeded in 96-well plates, followed by hIL-6 (50 pM). Plates were incubated (37°C, 5% CO<sub>2</sub> for 6 hours) and visualized using Steady-Glo or One-Glo luciferase substrate.

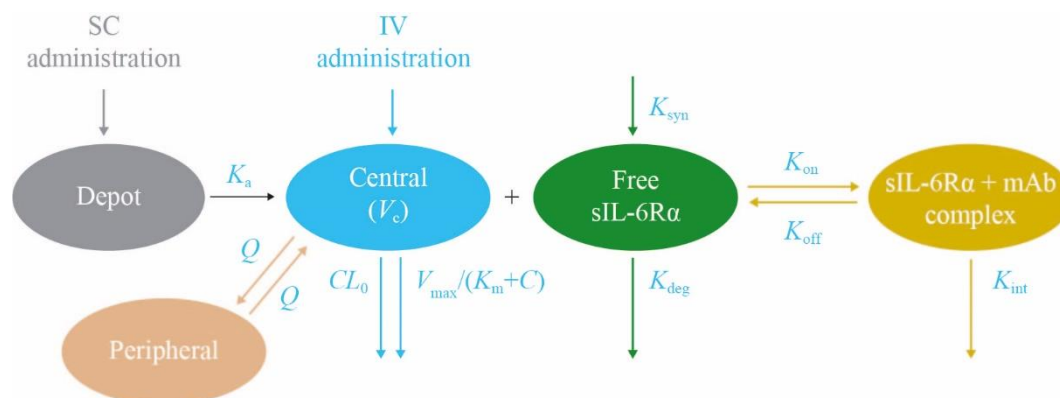
In both assays, plates were read on a Victor X5 multilabel counter, and EC<sub>50</sub> and IC<sub>50</sub> values calculated as described above.

*Inhibition of Trans IL-6R $\alpha$  Signaling (Luciferase Assay)*

The ability of sarilumab to block *trans* signaling stimulated by a soluble complex of IL-6 and IL-6R $\alpha$  was assessed in a functional cell-based luciferase assay using a gp130-overexpressing human embryonic kidney/STAT3/luciferase reporter cell line. Serial dilutions of sarilumab (200 nM to 3.4 pM) were preincubated with 1 nM of monomeric soluble (s)IL-6R $\alpha$  with a C-terminal myc-myc-hexahistidine tag (IL-6R $\alpha$ -mmH), and transferred together with hIL-6

(12.5 nM) to 96-well plates seeded with HEK293/gp130/STAT3-Luc cells (ATCC CRL-1573). For the dose–response curve, hIL-6 concentrations ranging from 15 nM to 2.5 pM were used along with human monomeric sIL-6R $\alpha$ -mmH (1 nM). Following incubation (37°C, 5% CO<sub>2</sub> for 5 hours), response was measured using One-Glo luciferase substrate (Promega) and read on a Victor X5 multilabel counter.



**Supplemental Figure S1.** Sarilumab and tocilizumab sIL-6R $\alpha$  receptor occupancy models.

-----2 compartment model with parallel linear and Michael-Menten elimination

$A1$  = amount in depot,  $A2$  = amount in central,  $A3$  = amount in peripheral

Tocilizumab concentrations for IV administration:

$$dA2/dt = Q/V_p * A3 - (CL_0 + Q)/V_c * A2 - V_{max} * C / (K_m + C)$$

$$dA3/dt = Q/V_c * A2 - Q/V_p * A3$$

Tocilizumab or sarilumab concentrations for SC administration:

$$dA1/dt = -K_a * A1$$

$$dA2/dt = K_a * A1 + (Q/V_p) * A3 - ((CL_0 + Q)/V_c * A2 - (V_{max} * C) / (K_m + C))$$

$$dA3/dt = (Q/V_c) * A2 - (Q/V_p) * A3$$

Where  $t$  is the time,  $C$  is the drug (sarilumab or tocilizumab) concentration,  $A1$ ,  $A2$  and  $A3$  are the amount of drug in the depot, central and peripheral compartments,  $V_{max}$  is the maximum target-specific elimination rate,  $K_M$  is the Michaelis–Menten constant,  $CL_0$  is the linear clearance, and  $Q$  is the intercompartment clearance.

Total sIL-6R $\alpha$  concentrations:

$$dR_{tot}/dt = K_{syn} - K_{deg} * R_{tot} * (1 - I_{max} * (C / (K_{ss} + C)))$$

$$I_{\max} = 1 - K_{\text{int}}/K_{\text{deg}}$$

Where  $R_{\text{tot}}$  is the total sIL-6R concentration,  $K_{\text{syn}}$  is the sIL-6R production rate,  $K_{\text{deg}}$  and  $K_{\text{int}}$  are, elimination rates of the unbound sIL-6R $\alpha$  and mAb–sIL-6R $\alpha$  complex, respectively, and  $K_{\text{ss}}$  is the quasi–steady-state constant.

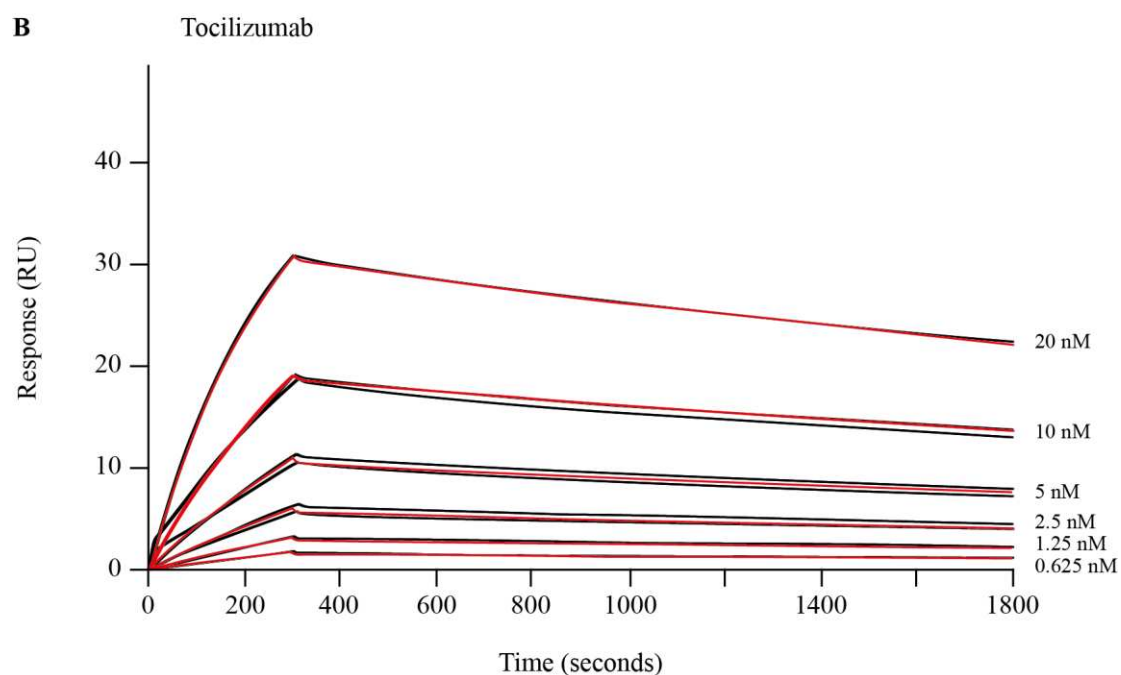
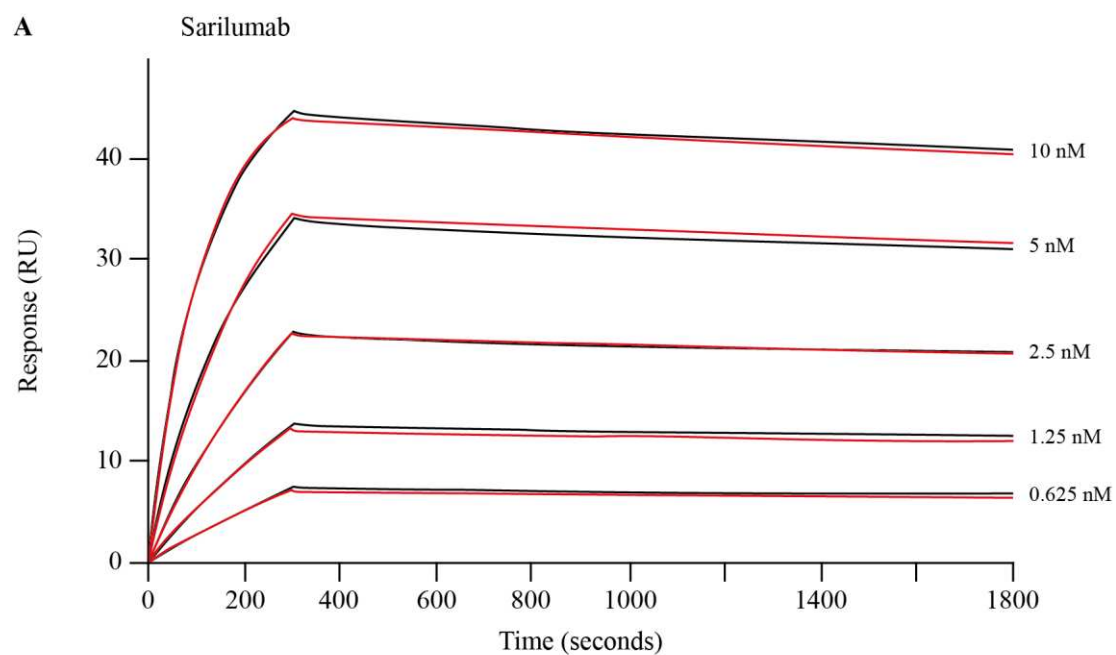
The unbound sIL-6R $\alpha$  concentration and concentration of sIL-6R $\alpha$  bound to tocilizumab:

$$R_{\text{unbound}} = R_{\text{tot}} * K_{\text{ss}} / (K_{\text{ss}} + C)$$

$$R_{\text{bound}} = R_{\text{ss}} * C / (K_{\text{ss}} + C)$$

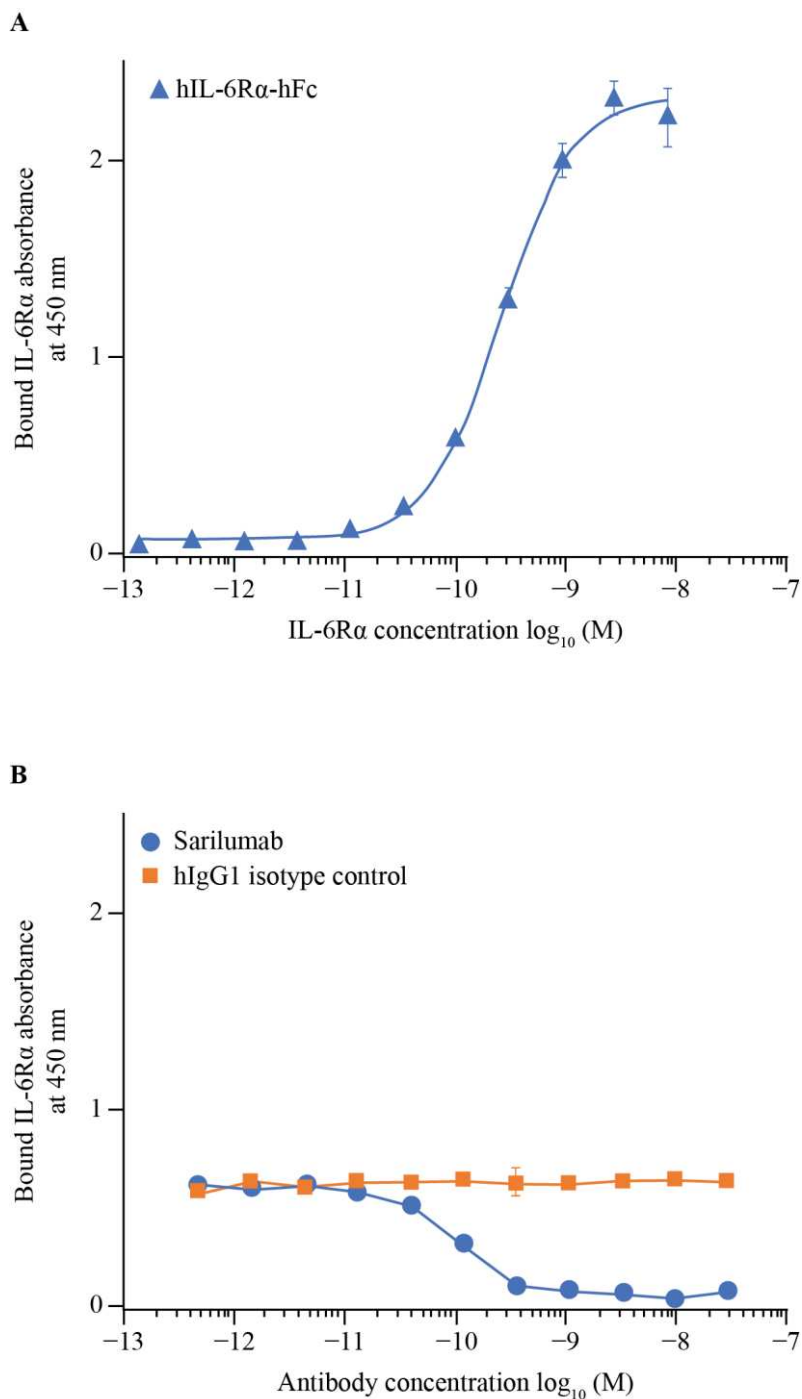
$C$ , concentration;  $CL_0$ , apparent clearance;  $F$ , bioavailability; IV, intravenous;  $K_{\text{deg}}$ , degradation rate constant;  $K_{\text{int}}$ , internalization rate constant;  $K_m$ , Michaelis–Menten constant;  $K_{\text{off}}$ , dissociation rate constant;  $K_{\text{on}}$ , association rate constant;  $K_{\text{syn}}$ , synthesis rate constant; mAb, monoclonal antibody; SC, subcutaneous; sIL-6R $\alpha$ , soluble interleukin-6 receptor alpha subunit;  $V_c$ , apparent volume of central compartment;  $V_{\text{max}}$ , maximum elimination rate.

**Supplemental Figure S2.** Ligand-binding properties of sarilumab (107 RU) and tocilizumab (108 RU). Representative sensograms of (A) sarilumab binding to monomer human IL-6R $\alpha$ -mmH 10, 5, 2.5, 1.25, and 0.625 nM, and (B) tocilizumab binding to monomer human IL-6R $\alpha$ -mmH 20, 10, 5, 2.5, 1.25, and 0.625 nM are shown as black lines. The data were globally fitted to a 1:1 binding interaction model using T200 evaluation software 2.0. Kinetic fits from the analyses were overlaid on the binding data in red.



Sensogram of binding kinetics of sarilumab binding to monomer IL-6R $\alpha$  proteins; sarilumab (107 RU) was captured on an anti-human Fc region-coupled chip surface. Human IL-6R $\alpha$  proteins were tested in duplicate in a 2-fold dilution series: the association phase of human IL-6R $\alpha$  was monitored at 50  $\mu$ l/minute for 5 minutes over each of the captured surfaces. Fc, fragment crystallizable; IL-6R $\alpha$ , interleukin-6 receptor alpha subunit; RU, resonance unit.

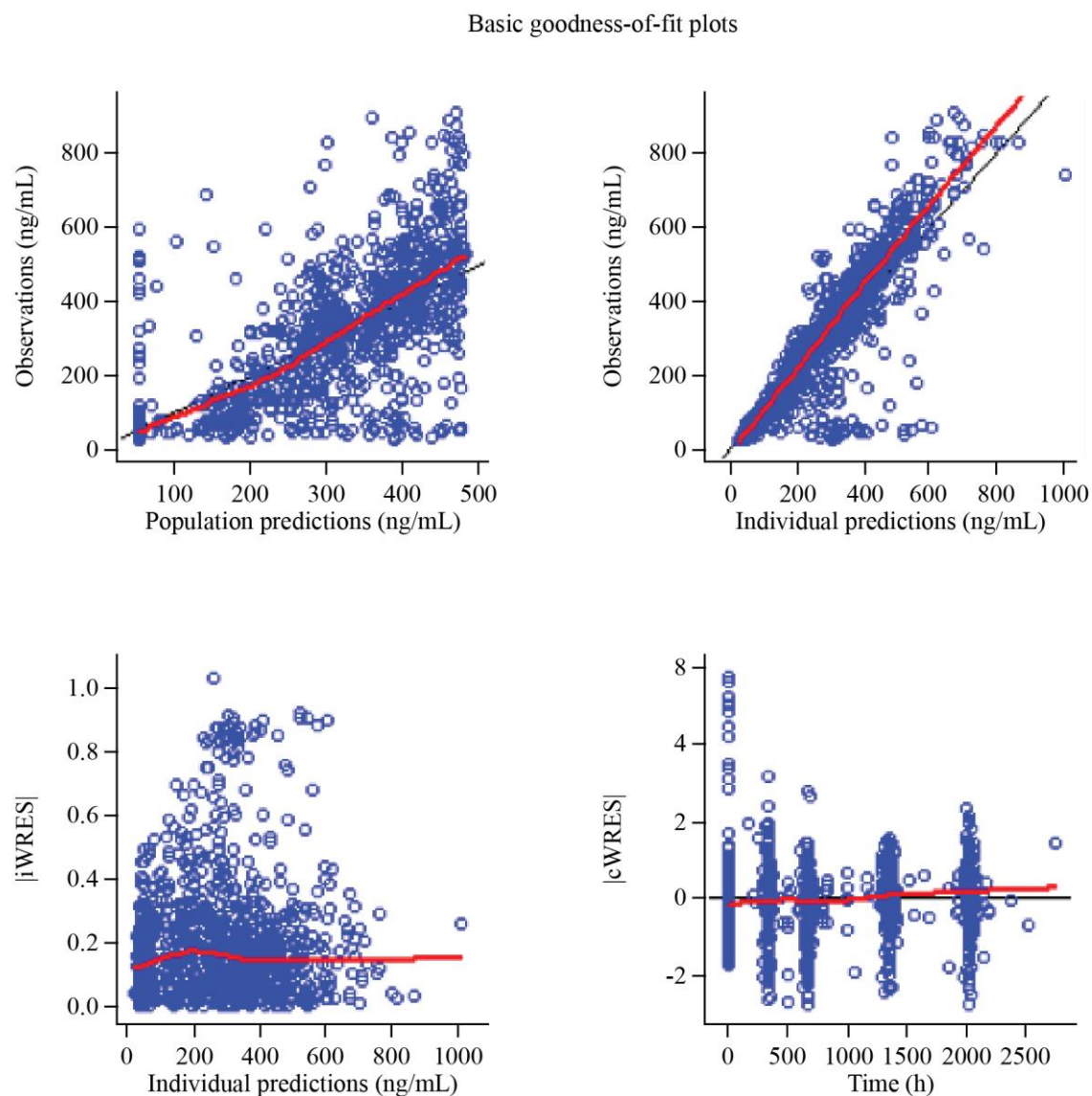
**Supplemental Figure S3.** Blockade of dimeric hIL-6R $\alpha$  binding to IL-6 (ELISA competition assay). (A) Dose–response of hIL-6R $\alpha$ -hFc binding to hIL-6 and (B) concentration-dependent blockade of hIL-6R $\alpha$ -hFc binding to hIL-6 by sarilumab.



ELISA, enzyme-linked immunosorbent assay; Fc, fragment crystallizable; hIgG1, human immunoglobulin G1; hIL-6, human interleukin-6; hIL-6R $\alpha$ , human interleukin-6 receptor

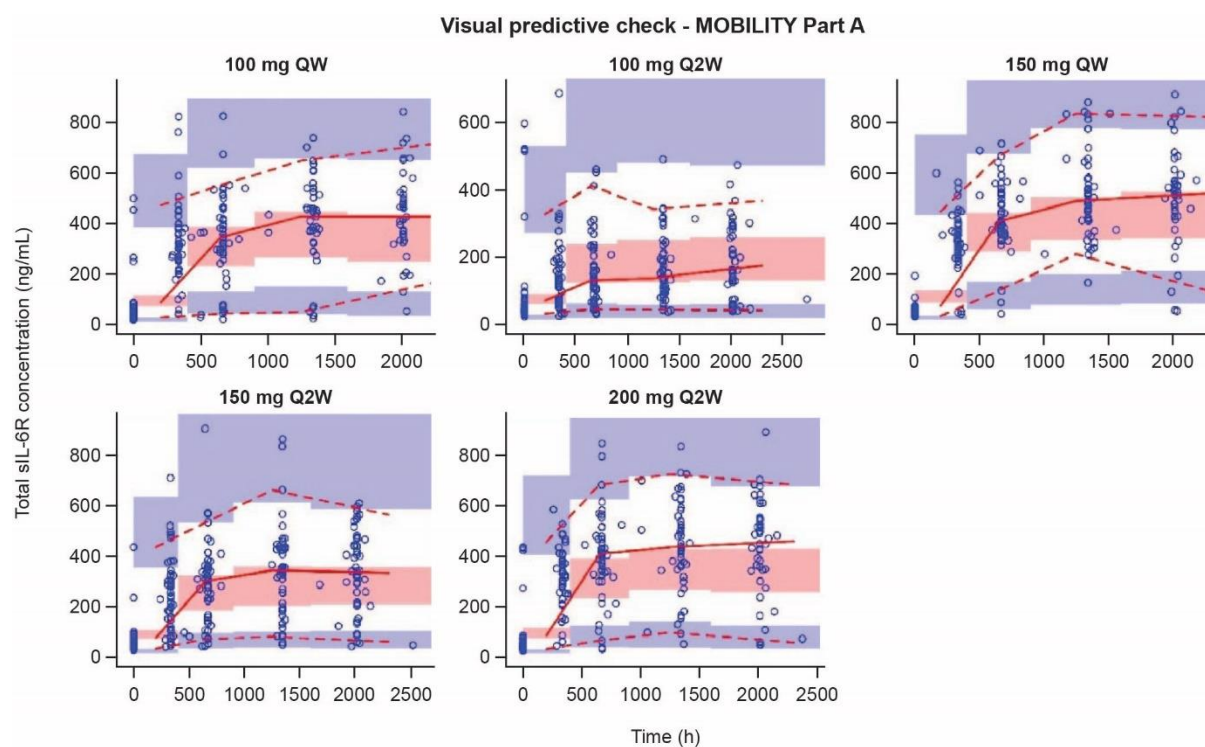
alpha subunit; hIL-6R $\alpha$ -hFc, recombinant extracellular domain of human IL-6R $\alpha$  generated with an N-terminal amino acid linker sequence comprising the Fc of human IgG1; IgG1, immunoglobulin G1; IL-6, interleukin-6; IL-6R $\alpha$ , interleukin-6 receptor alpha subunit.

**Supplemental Figure S4.** Basic goodness-of-fit plots with LOWESS (red lines) for the final model.



|cWRES|, conditional weighted residuals; |iWRES|, individual weighted residuals; LOWESS, locally weighted scatterplot smoothing.

**Supplemental Figure S5.** Final model visual predictive check after multiple doses of sarilumab 100 mg Q2W, 150 mg Q2W, 200 mg Q2W, 100 mg QW, or 150 mg QW.



Solid and dashed red lines connect the median and bounds (ie, 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles) of observed concentrations at each time point (blue circles). Blue and pink bands represent the 95% confidence intervals around predicted concentrations at each time point.

Q2W, every 2 weeks; QW, every week; sIL-6R, soluble interleukin-6 receptor.



**Supplemental Table S1.** Parameter estimates of the sarilumab population PK model.

Parameter	Estimate	RSE%	95% CI
$V_m$ (mg/d)	8.06	1.96	7.34–8.95
$K_m$ (mg/L)	0.939	4.38	0.751–1.12
$V_c/F$ (L)	2.08	3.20	1.81–2.48
$CL_0/F$ (L/d)	0.260	5.36	0.230–0.290
$K_a$ (d <sup>-1</sup> )	0.136	2.53	0.123–0.160
$Q/F$ (L/d)	0.156	3.87	0.138–0.252
$V_p/F$ (L)	5.23	5.98	2.60–10.1
Interindividual variability (CV%)			
$V_m^a$	32.4	6.07	27.2–35.2
$CL_0/F^a$	55.3	6.21	44.0–65.6
$V_c/F^a$	37.3	16.7	27.0–51.4
$K_a^a$	32.1	9.52	27.1–43.2
Block $V_m-CL_0/F^b$	-0.566	10.8	(-0.668)–(-0.405)
Residual variability (CV%)			
$\sigma^{2c}$	0.395	0.811	0.366–0.421

<sup>a</sup>Interindividual variability is expressed as % coefficient of variation (CV%).

<sup>b</sup>Estimate of covariance between two variances is expressed as correlation coefficient.

<sup>c</sup>Variance of residual error is based on the log-transformed dependent variable (ie, sarilumab concentration).

$CL_0/F$ , apparent linear clearance from central compartment;  $K_a$ , absorption rate constant;  $K_m$ , Michaelis–Menten constant;  $Q/F$ , apparent intercompartmental clearance;  $RSE$  (%), percentage of relative standard error ( $100 \times \text{Standard error/Estimate}$ );  $V_c/F$ , apparent volume of central compartment;  $V_m$ , maximum elimination rate;  $V_p/F$ , apparent peripheral volume of distribution;  $WT$ , body weight.

**Supplemental Table S2.** Parameter estimates of the sIL-6R population PK/PD model.

Parameter	Estimate	RSE%	95% CI
$K_{\text{syn}}$ (ng/mL/day)	40.1	7.8	33.8–46.3
$K_{\text{deg}}$ (1/h)	0.734	9.2	0.600–0.871
$I_{\text{max}}$	0.891	0.7	0.879–0.902
$K_{\text{ss}}$ ( $\mu\text{g/mL}$ )	0.264	22.4	0.146–0.381
Interindividual variability (CV%)			
$K_{\text{syn}}$	47.8	19.3	37.4–56.2
$K_{\text{ss}}$	158	49.3	186–223
Residual variability (CV%)			
Proportional	30.9	10.8	27.4–34.1

CI, confidence interval; CV, coefficient of variation;  $I_{\text{max}}$ , maximum intensity;  $K_{\text{deg}}$ , degradation rate constant;  $K_{\text{ss}}$ , steady-state constant;  $K_{\text{syn}}$ , synthesis rate constant; mAb, monoclonal antibody; PD, pharmacodynamic; PK, pharmacokinetic; RSE, relative standard error; sIL-6R, soluble interleukin-6 receptor.