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Differential Binding of Sarilumab and Tocilizumab to IL-6Rα and Effects of Receptor Occupancy on Clinical Parameters

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Abstract

We evaluated interleukin (IL)-6Rα signaling inhibition with sarilumab and tocilizumab, the association between IL-6Rα receptor occupancy (RO) and C-reactive protein (CRP), and potential clinical relevance of any differences. For this, we measured IL-6Rα binding and signaling inhibition with sarilumab and tocilizumab in vitro, simulated soluble (s)IL-6Rα 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-6Rα in vitro with 15–22-fold higher affinity than tocilizumab, and inhibits IL-6-mediated classical and *trans* signaling via membrane-bound and sIL-6Rα. 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-6Rα 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.[1,2] IL-6 elevations have been noted in sera and synovial fluid from patients with RA, and correlate with RA disease activity and joint destruction.[3] IL-6 may contribute to comorbidities associated with RA, including mood disorders, cardiovascular disease, diabetes, and osteoporosis.[4-6]

IL-6 effects are mediated through interaction with the IL-6 receptor alpha subunit (IL-6Rα). IL-6 activates classical (*cis*) signaling through membrane-bound IL-6Rα (mIL-6Rα), expressed on the surface of hepatocytes and hematopoietic cells, and *trans* signaling through soluble IL-6Rα (sIL-6Rα), found in serum and synovial fluid. Signaling with sIL-6Rα occurs after the IL-6/sIL-6Rα complex binds to the ubiquitously expressed glycoprotein (gp)130 receptor, thus greatly expanding the spectrum of IL-6-responsive cells.[5,7,8] 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-6Rα and mIL-6Rα, thereby inhibiting IL-6 signaling through this pathway.[9-12] 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.[11,12] 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.[9,10]

The objectives of this analysis were to evaluate differences in IL-6Rα 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-6Rα were measured using Surface Plasmon Resonance (SPR; Biacore™ 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-6Rα Binding to IL-6

Human (h)IL-6Rα 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_{50}) and effective concentration at 50% activity (EC_{50}) were calculated for human (h)IL-6, sarilumab, and tocilizumab. Further details are included in the Supplemental Appendix.

Inhibition of Classical IL-6Rα Signaling

The activities of sarilumab and tocilizumab in blocking classical IL-6Rα 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-6Rα and gp130
- HepG2: a hepatocytic cell line endogenously expressing IL-6Rα and gp130

Further details are included in the Supplemental Appendix.

Inhibition of Trans IL-6Rα Signaling

The ability of sarilumab to block *trans* signaling stimulated by the IL-6/sIL-6Rα 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-6Rα PK/PD models was provided by population PK (PopPK) models for sarilumab SC,[13] and tocilizumab IV[14] and SC.[15,16] 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.[13-15]

sIL-6Rα PK/PD Model Development

Tocilizumab binding to sIL-6Rα 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.[14] Given the similarity of tocilizumab and sarilumab binding to sIL-6Rα and mIL-6Rα, the same structural model was used to develop the PK/PD model to describe sarilumab binding to sIL-6Rα 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.[17]

The quasi-steady-state target-mediated drug disposition (TMDD) models describing PK/PD relationships to total sIL-6Rα for sarilumab and tocilizumab, including the PD model of inhibiting sIL-6Rα elimination, are summarized in Supplemental Figure S1. The PK/PD model for binding to sIL-6Rα was used to predict the time course of free sIL-6Rα concentrations for sarilumab and tocilizumab. Only sIL-6Rα (not mIL-6Rα) was considered in these analyses. The sIL-6Rα 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-6Rα 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-6Rα and free sIL-6Rα 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-6Rα RO dynamic profiles (percentage RO over time) were calculated based on unbound sIL-6R concentrations:

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

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.[18] 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,[15] 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.[19,20]

Association Between sIL-6Rα RO and CRP, DAS28-CRP, and ACR20/50/70 Responses

The association of median RO calculated from observed concentrations of free sIL-6Rα measured in MOBILITY Part A[17] 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-6Rα 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-6Rα 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-6Rα 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-6Rα forms (Table 1, Supplemental Figure S2B).

In the ELISA competition assay, sarilumab directly blocked the binding of hIL-6Rα-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-6Rα-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-6Rα 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-6Rα-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-6Rα-mmH and 10 nM IL-6, and an IC_{50} of 8.9 nM in the presence of 10 nM sIL-6Rα-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-6Rα PK/PD models are presented in Supplemental Table S2. GOF evaluation indicated that the final sIL-6Rα 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^{th}, 50^{th}, and 97.5^{th} percentiles) fitted the predicted parameters well (Supplemental Figure S5).

Simulation of sIL-6Rα Occupancy by Sarilumab and Tocilizumab

Simulated sIL-6Rα 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-6Rα 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-6Rα Occupancy and Clinical Efficacy Parameters

In MOBILITY Part A, the association of sIL-6Rα 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-6Rα 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-6Rα 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-6Rα 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-6Rα 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-6Rα and gp130. IL-6 forms a heterodimer with IL-6Rα that subsequently binds with high affinity to gp130, forming a heterotrimeric complex.[5,7,8] IL-6Rα exists in both membrane-bound and soluble forms, with sIL-6Rα generated through cleavage of mIL-6Rα or alternative splicing. Classical signaling through mIL-6Rα is limited to the few cell types that express mIL-6Rα, that is hepatocytes, monocytes/macrophages, neutrophils, and some T cell subsets. *Trans* signaling through sIL-6Rα may occur in virtually any nucleated cell type (including those that lack IL-6Rα expression) because of the ubiquitous expression of membrane gp130.[8]

Previously, Mihara and colleagues showed that tocilizumab bound to sIL-6Rα inhibited IL-6 binding in a dose-dependent manner, and dissociated IL-6 and sIL-6Rα from their preformed complex. Tocilizumab suppressed the IL-6/sIL-6Rα 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.[21] In the present in vitro studies, sarilumab was shown to bind directly to IL-6Rα, but with a binding affinity for both monomeric and dimeric IL-6Rα 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-6Rα complex.

Additional factors can influence binding affinity in vivo, including baseline receptor concentration, receptor turnover, receptor distribution, antibody concentration, and antibody distribution.[22] Therefore, a PK/PD model was developed that incorporated these parameters to profile sIL-6Rα 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.[9,12]

Sarilumab and tocilizumab bind to both the sIL-6Rα and mIL-6Rα forms. It was shown that the TMDD system with 2 targets can be approximated by equations that describe both sarilumab or tocilizumab and sIL-6Rα concentrations, and include 2 target-mediated elimination terms (with different maximum elimination rate [V_{max}] and Michaelis–Menten constant [K_m] parameters). However, mIL-6Rα 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 µg/mL [0.75 nmol/L]) and tocilizumab–mIL-6R binding (0.38–0.43 µg/mL [2.5–2.9 nmol/L]) suggest a similar range of binding affinity for the 2 forms of the target.[14]

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.[23] 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-6Rα 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-6Rα 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.[9,15]

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-6Rα 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.[17] The median percentage reduction of free sIL-6Rα 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.[24] 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,[25] allowing PK/PD comparisons to be made. The IL-6Rα 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-6Rα 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-6Rα 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.[15] 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-6Rα isoform, because mIL-6-Rα 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-6Rα 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-6Rα 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-6Rα and is more potent at inhibiting IL-6-mediated signaling at lower concentrations in serum than tocilizumab.

Conclusions

In summary, sarilumab demonstrated higher sIL-6Rα binding affinity and potency compared with tocilizumab. Higher and more sustained RO with IL-6Rα 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-6Rα 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-6Rα signaling in HEK293/gp130/STAT3-Luc cells exposed to 10 nM hIL-6 and (C) 1 nM hIL-6Rα or (D) 10 nM hIL-6Rα. 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 (± 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.*

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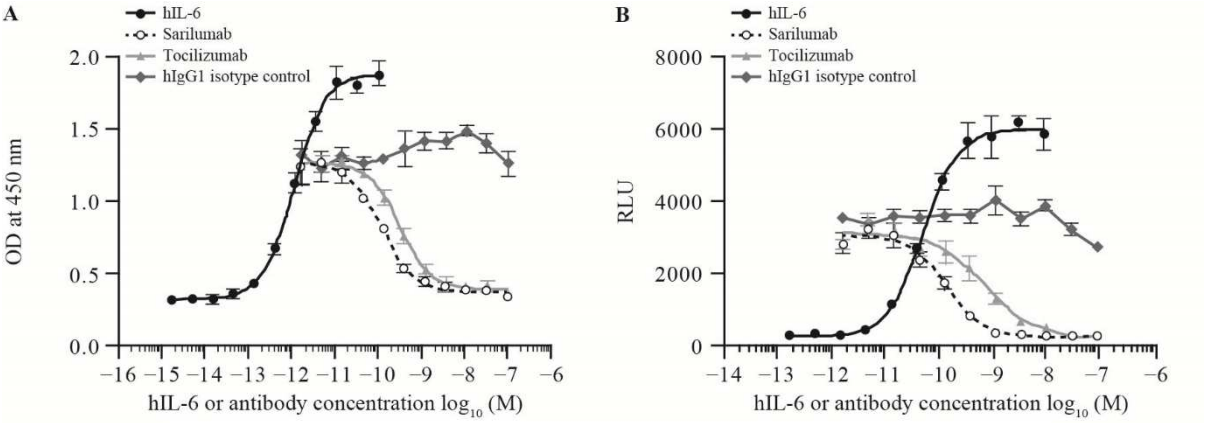
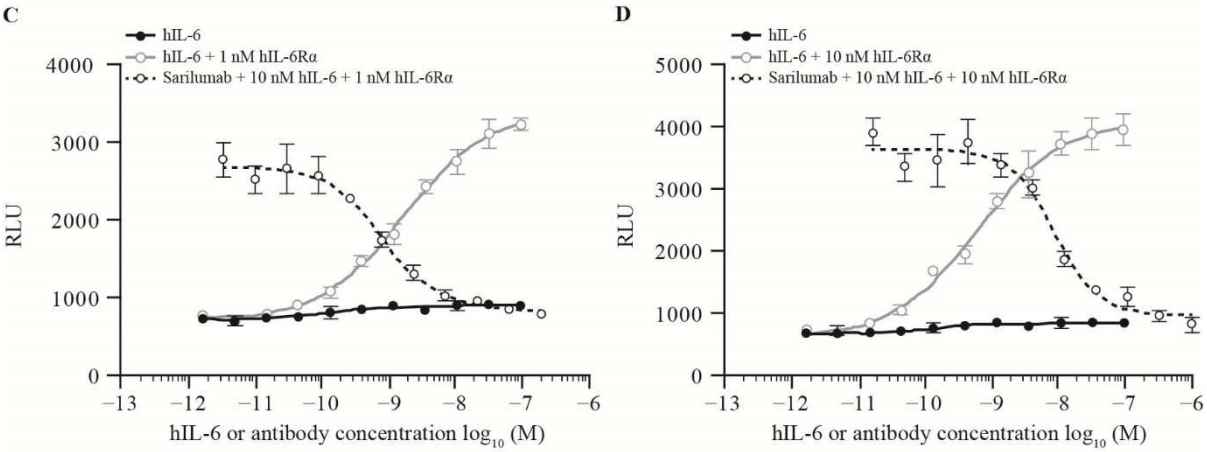


Treatment	Proliferation	STAT3 signalling
hIL-6, EC_{50}	0.5 pM	59 pM
Sarilumab, IC_{50}	226 pM	146 pM
Tocilizumab, IC_{50}	812 pM	496 pM

EC_{50} and IC_{50} values are an average of 3 independent experiments

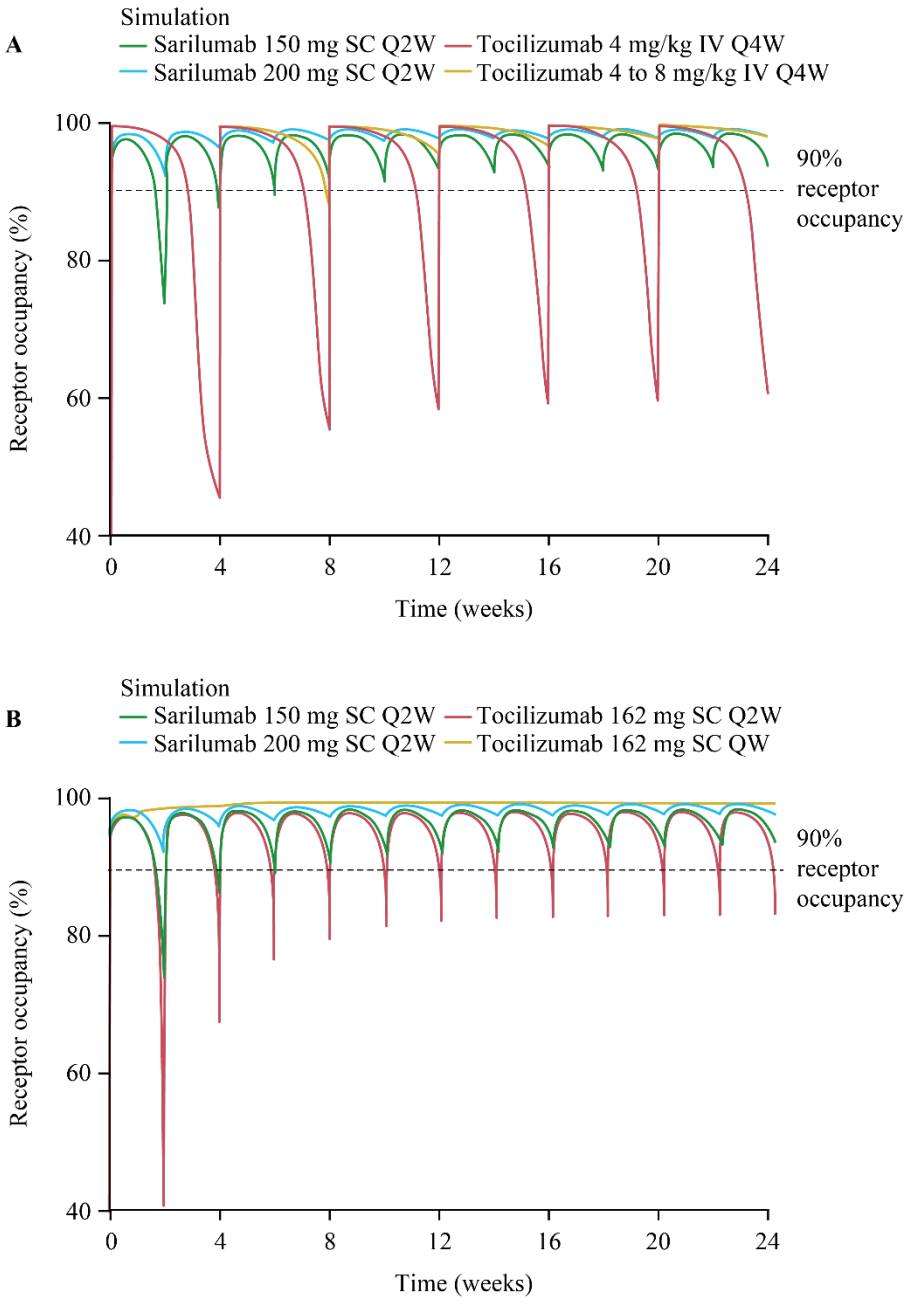


Treatment	1 nM hIL-6Rα	10 nM hIL-6Rα
hIL-6, EC_{50}	1.8 nM	0.7 nM
Sarilumab + 10 nM hIL-6, IC_{50}	0.9 nM	8.9 nM

EC_{50}, effective concentration at 50% activity; gp, glycoprotein; hIgG1, human immunoglobulin G1; hIL-6, human interleukin-6; hIL-6Rα, human interleukin-6 receptor alpha subunit; IC, inhibitory concentration; IC_{50}, inhibitory concentration at 50% activity;

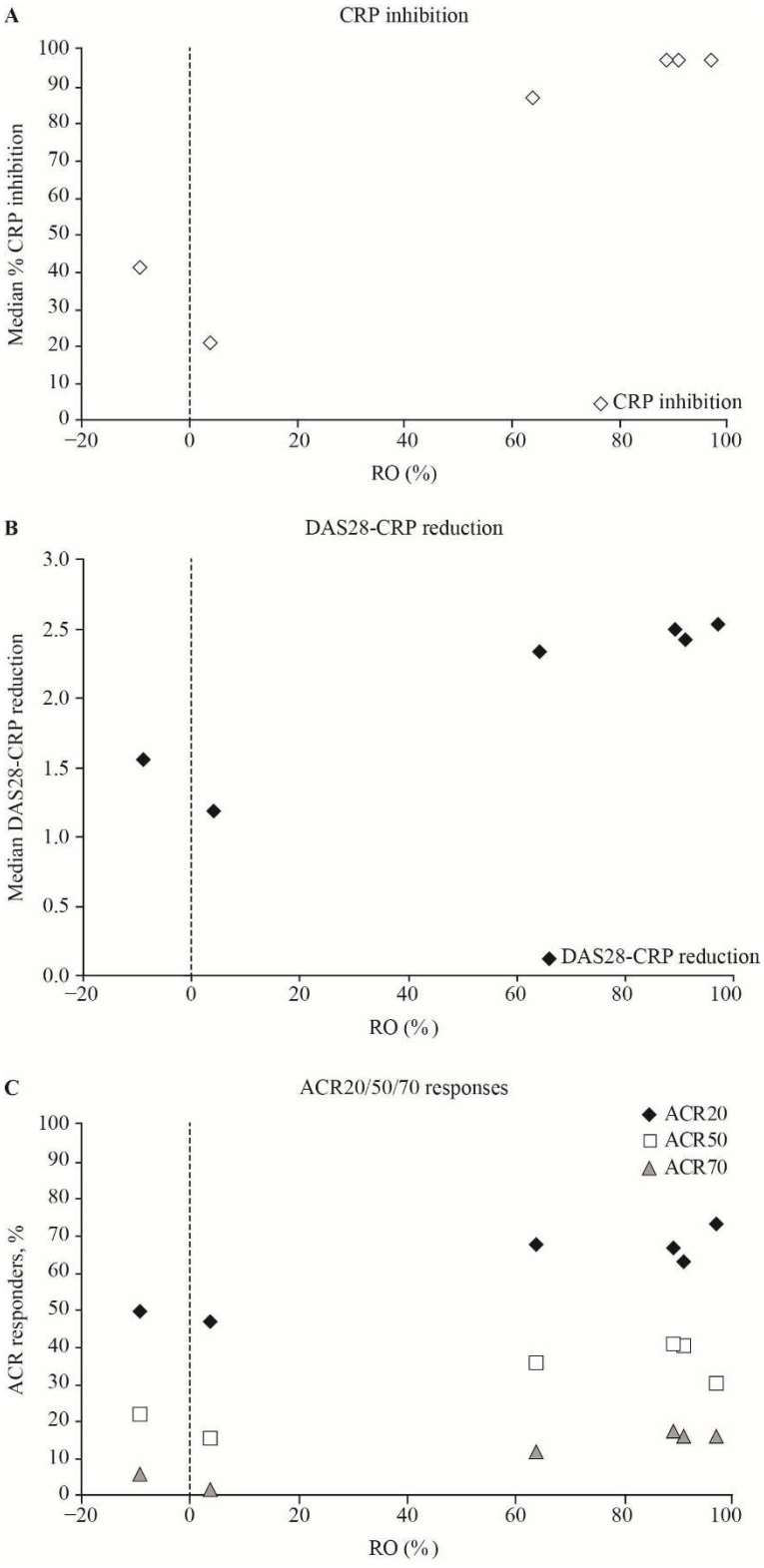
IL-6Rα, interleukin-6 receptor alpha subunit; Luc, luciferase; OD, optical density; RLU, relative luminescence unit; STAT3, signal transducer and activator of transcription 3.

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.

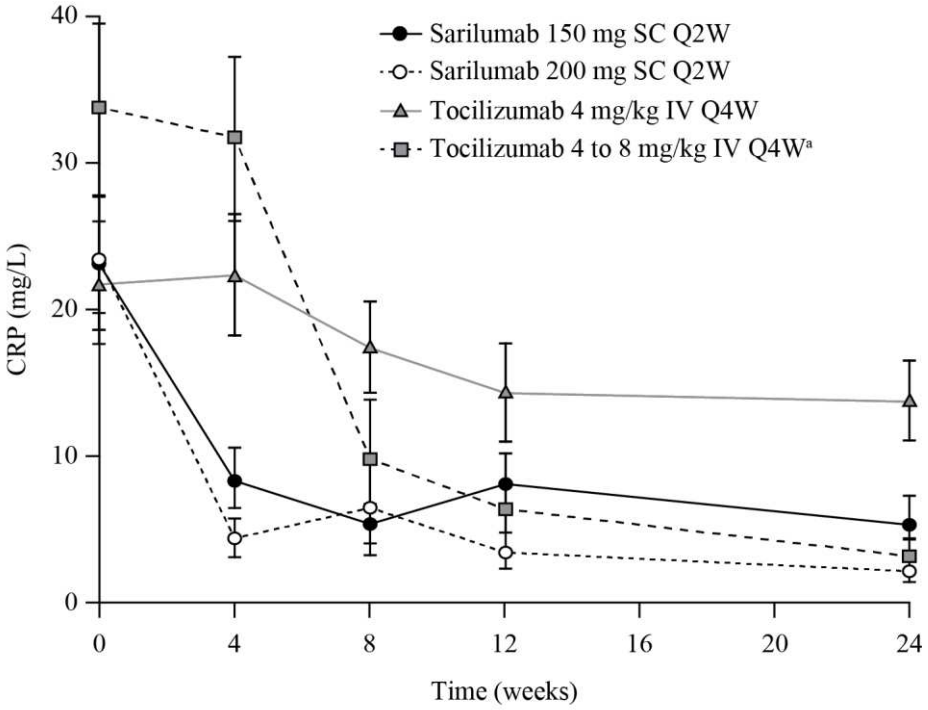
Figure 3. Relationship between RO and (A) CRP reduction, (B) DAS28-CRP reduction, and (C) ACR20/50/70 responses with placebo and sarilumab (MOBILITY Part A [NCT01061736]).



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-6Rα levels were used to calculate receptor occupancy and associations were assessed against clinical parameters. Receptor occupancy and pharmacodynamic endpoints were at 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-6Rα, soluble interleukin-6 receptor alpha subunit.

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

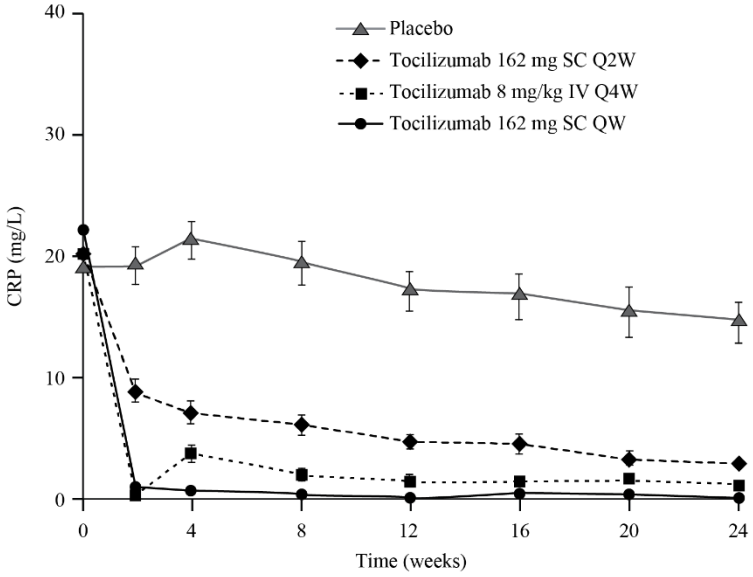


Number of patients					
Sarilumab 150 mg	49	47	45	44	39
Sarilumab 200 mg	51	47	51	43	39
Tocilizumab 4 mg/kg	40	37	37	37	35
Tocilizumab 4 to 8 mg/kg	39	39	39	39	39

[a]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-6Rα proteins.

Antigen	Antibody	K_{on} (1/Ms)	K_{off} (1/s)	K_D (M)	$t_{1/2}$ (h)
Human IL-6Rα 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-6Rα 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-6Rα, 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.

Supplemental Appendix

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

Supplemental Methodology

Supplemental Figure S1. Sarilumab and tocilizumab sIL-6Rα 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-6Rα-mmH 10, 5, 2.5, 1.25, and 0.625 nM, and (B) tocilizumab binding to monomer human IL-6Rα-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-6Rα binding to IL-6 (ELISA competition assay). (A) Dose–response of hIL-6Rα-hFc binding to hIL-6 and (B) concentration-dependent blockade of hIL-6Rα-hFc binding to hIL-6 by sarilumab.

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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-α (IL-6Rα) 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-6Rα (hIL-6Rα) 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-6Rα 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-6Rα with a C-terminal human IgG1 fragment crystallizable (Fc) tag (IL-6Rα-hFc), after which the mixtures were transferred to 96-well microtiter plates onto which human IL-6 (hIL-6) (2 μg/mL) had been immobilized. Bound IL-6Rα-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.

Inhibition of Classical IL-6Rα 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-6Rα 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-6Rα and glycoprotein (gp)130
- HepG2: a hepatocytic cell line endogenously expressing IL-6Rα 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 (37ºC, 5% CO_2 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 (37ºC, 5% CO_2 for 6 hours) and visualized using Steady-Glo or One-Glo luciferase substrate.

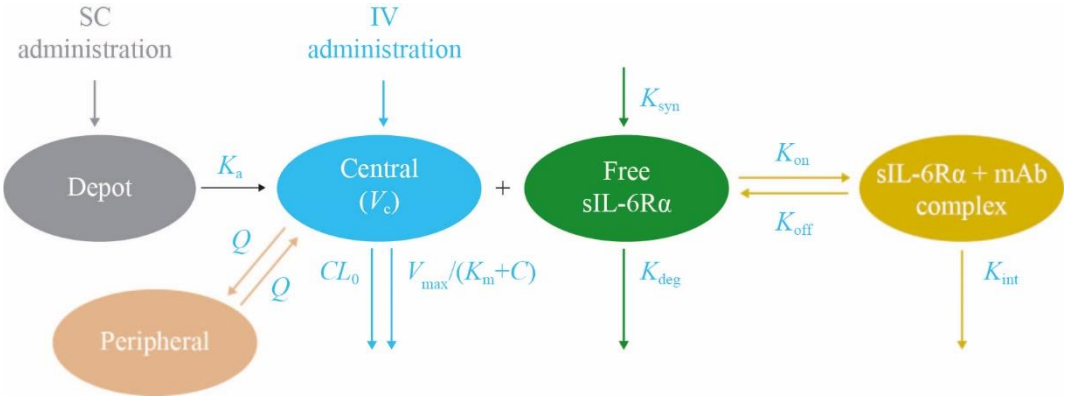
In both assays, plates were read on a Victor X5 multilabel counter, and EC_{50} and IC_{50} values calculated as described above.

Inhibition of Trans IL-6Rα Signaling (Luciferase Assay)

The ability of sarilumab to block *trans* signaling stimulated by a soluble complex of IL-6 and IL-6Rα 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-6Rα with a C-terminal myc-myc-hexahistidine tag (IL-6Rα-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-6Rα-mmH (1 nM). Following incubation (37ºC, 5% CO_2 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-6Rα 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-6Rα concentrations:

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

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

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

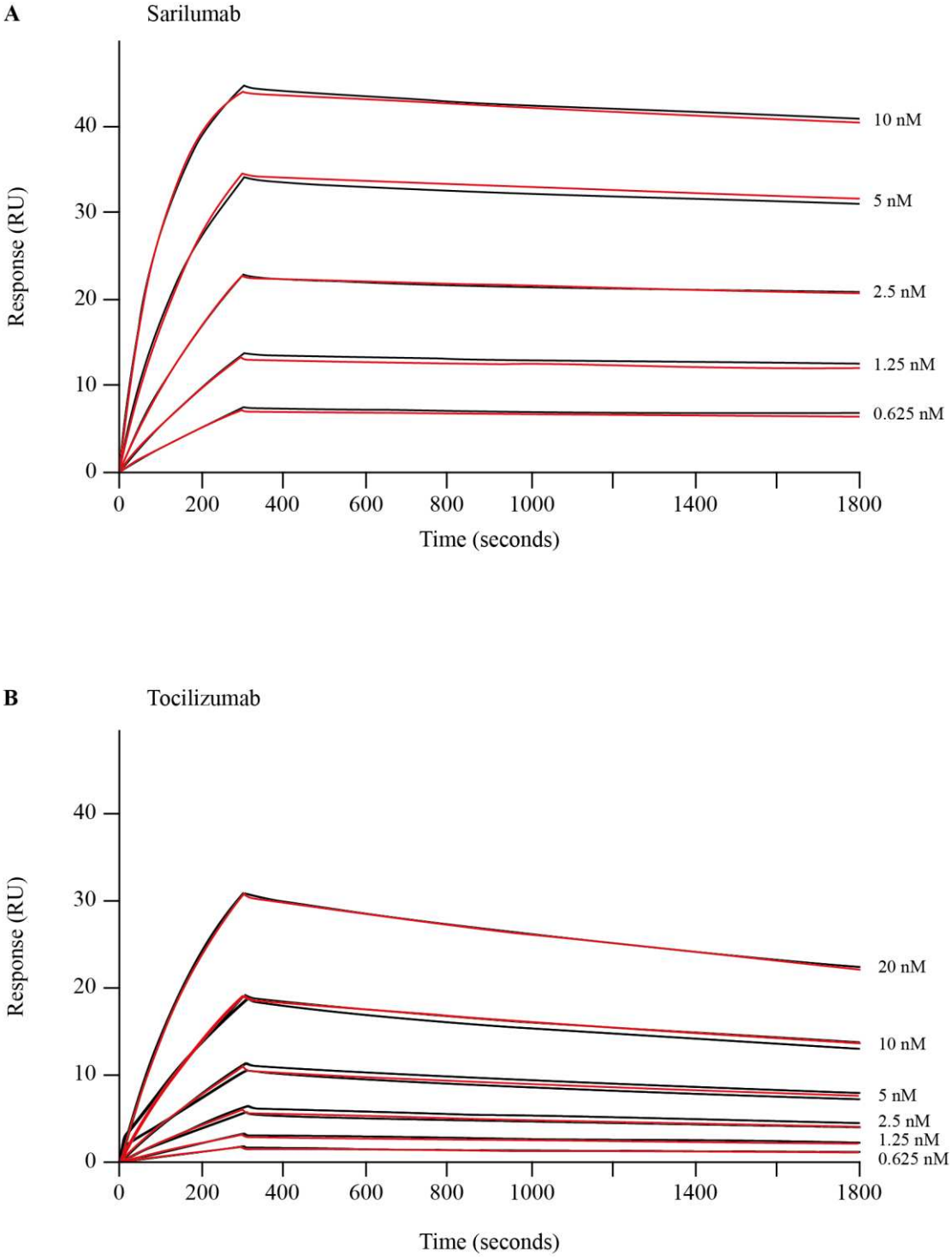
The unbound sIL-6Rα concentration and concentration of sIL-6Rα bound to tocilizumab:

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

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

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

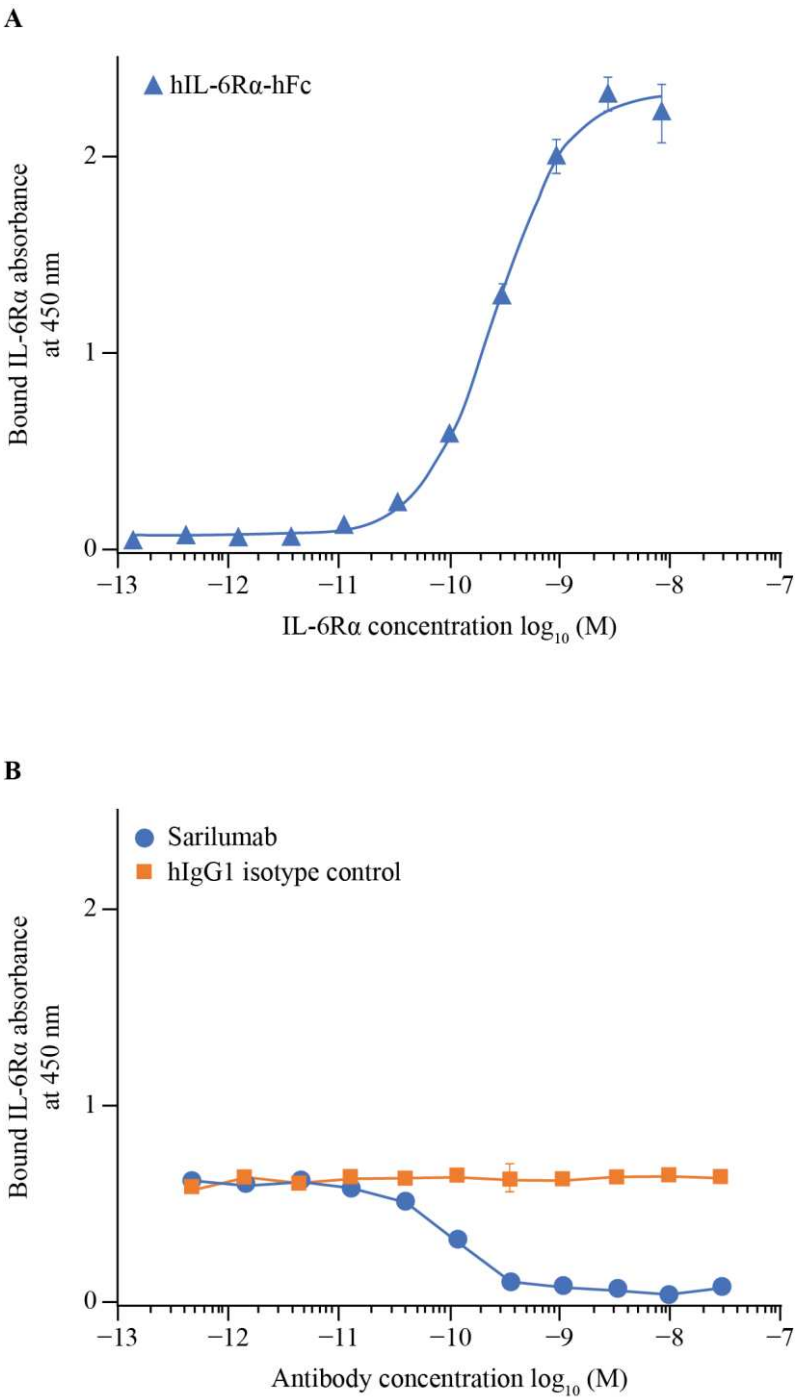
Supplemental Figure S2. Ligand-binding properties of sarilumab (107 RU) and tocilizumab (108 RU). Representative sensograms of (A) sarilumab binding to monomer human IL-6Rα-mmH 10, 5, 2.5, 1.25, and 0.625 nM, and (B) tocilizumab binding to monomer human IL-6Rα-mmH 20, 10, 5, 2.5, 1.25, and 0.625 nM are 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-6Rα proteins; sarilumab (107 RU) was captured on an anti-human Fc region-coupled chip surface. Human IL-6Rα proteins were tested in duplicate in a 2-fold dilution series: the association phase of human IL-6Rα was monitored at 50 µl/minute for 5 minutes over each of the captured surfaces.

Fc, fragment crystallizable; IL-6Rα, interleukin-6 receptor alpha subunit; RU, resonance unit.

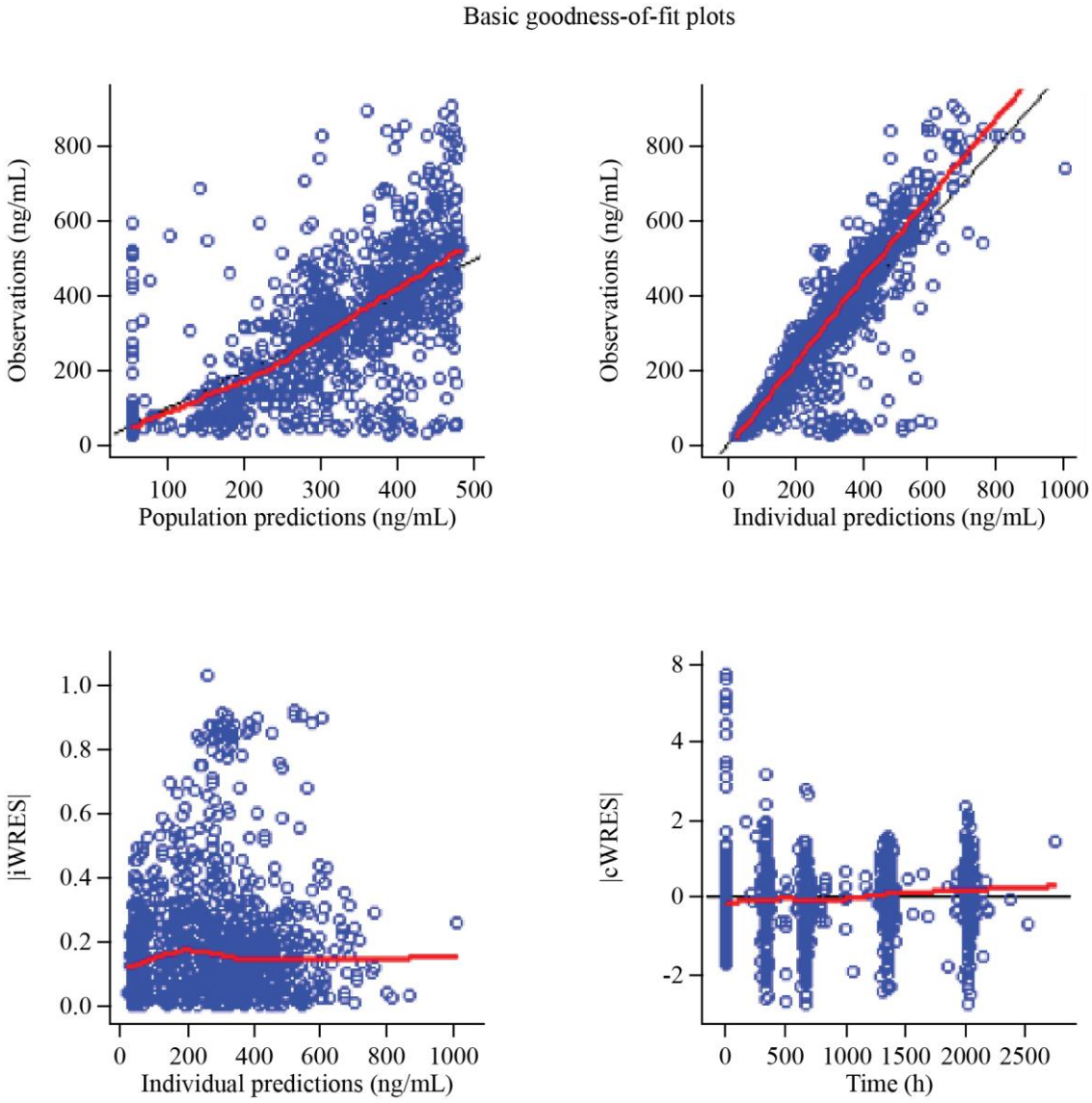
Supplemental Figure S3. Blockade of dimeric hIL-6Rα binding to IL-6 (ELISA competition assay). (A) Dose–response of hIL-6Rα-hFc binding to hIL-6 and (B) concentration-dependent blockade of hIL-6Rα-hFc binding to hIL-6 by sarilumab.



ELISA, enzyme-linked immunosorbent assay; Fc, fragment crystallizable; hIgG1, human immunoglobulin G1; hIL-6, human interleukin-6; hIL-6Rα, human interleukin-6 receptor

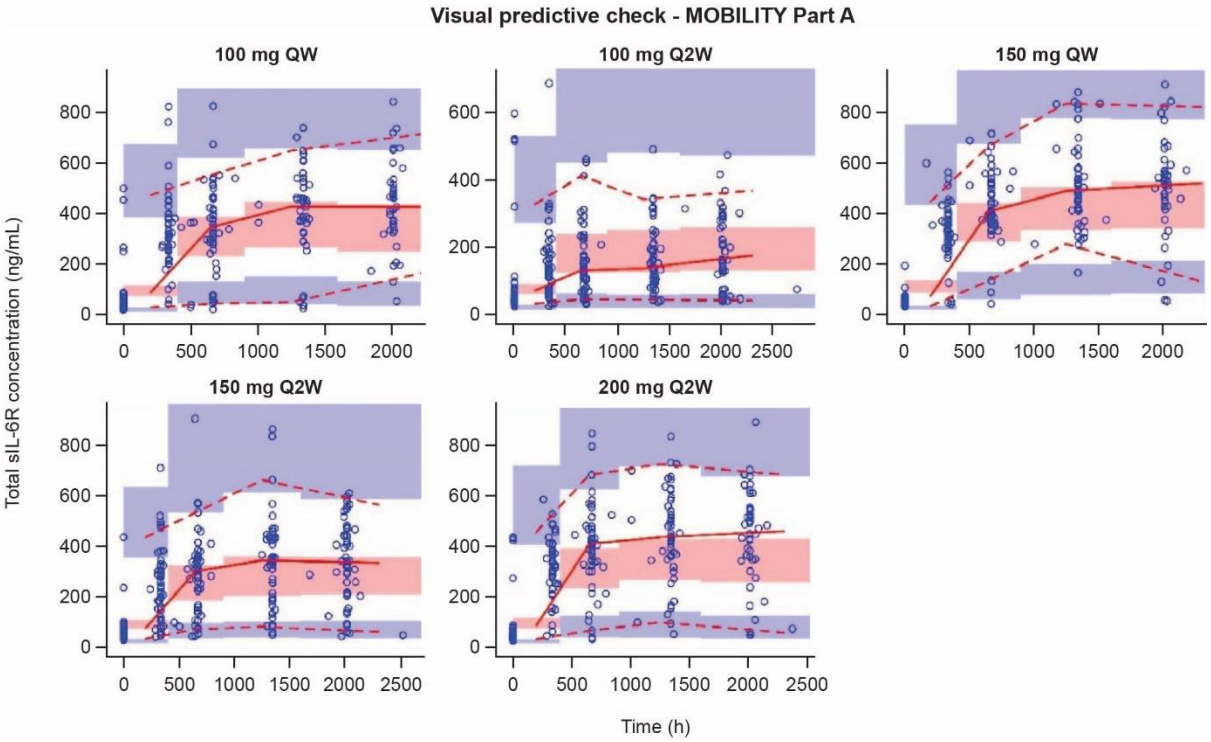
alpha subunit; hIL-6Rα-hFc, recombinant extracellular domain of human IL-6Rα generated with an N-terminal amino acid linker sequence comprising the Fc of human IgG1; IgG1, immunoglobulin G1; IL-6, interleukin-6; IL-6Rα, interleukin-6 receptor alpha subunit.

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, 2.5th and 97.5th 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^{-1})	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%)			
σ^{2c}	0.395	0.811	0.366–0.421

[a]Interindividual variability is expressed as % coefficient of variation (CV%).

[b]Estimate of covariance between two variances is expressed as correlation coefficient.

[c]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 × 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_{syn} (ng/mL/day)	40.1	7.8	33.8–46.3
K_{deg} (1/h)	0.734	9.2	0.600–0.871
I_{max}	0.891	0.7	0.879–0.902
K_{ss} (µg/mL)	0.264	22.4	0.146–0.381
Interindividual variability (CV%)			
K_{syn}	47.8	19.3	37.4–56.2
K_{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_{max}, maximum intensity; K_{deg}, degradation rate constant; K_{ss}, steady-state constant; K_{syn}, synthesis rate constant; mAb, monoclonal antibody; PD, pharmacodynamic; PK, pharmacokinetic; RSE, relative standard error; sIL-6R, soluble interleukin-6 receptor.