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Citation for final published version:

Xu, Christine, Rafique, Ashique, Potocky, Terra, Paccaly, Anne, Nolain, Patrick, Lu, Qiang, Iglesias-Rodriguez, Melitza, St John, Gregory, Nivens, Michael C., Kanamaluru, Vanaja, Fairhurst, Jeanette, Ishii, Tomonori, Maldonado, Rafael, Choy, Ernest and Emery, Paul 2021. Differential binding of sarilumab and tocilizumab to IL-6Rα and effects of receptor occupancy on clinical parameters. Journal of Clinical Pharmacology 61 (5) , pp. 714-724. 10.1002/jcph.1795

Publishers page: http://dx.doi.org/10.1002/jcph.1795

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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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Target journal: Rheumatology

Word count: 3425/3500 max (249/250 max for the abstract)

Tables/Figures: 6/6 max

References: 24/50 max

Abstract

Objective. To evaluate IL-6Rα signalling 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.

Methods. We measured IL-6Rα binding and signalling inhibition with sarilumab and tocilizumab *in vitro*, simulated sIL-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.

Results. Sarilumab binds IL-6Rα *in vitro* with 15–22-fold higher affinity than tocilizumab, and inhibits IL-6-mediated classical and *trans* signalling via membrane-bound and soluble IL-6Rα. Sarilumab 200 and 150 mg SC q2w achieved >90% RO after first and second doses, respectively, maintained through 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 reductions in CRP levels were observed with sarilumab (both doses) and tocilizumab 8 mg/kg IV q4w (reductions proportionally smaller with 4 mg/kg IV q4w).

Conclusion. Higher IL-6R 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.

Trial registration. MOBILITY Part A (NCT01061736); ASCERTAIN (NCT01768572); SUMMACTA (NCT01194414); BREVACTA (NCT0123256)

Key words: Rheumatoid arthritis, Pharmacology, Cell receptor-ligand interaction and activation,

Cytokines and inflammatory mediators, Clinical trials and methods

Rheumatology key messages
• Higher IL-6Rα binding with sarilumab translates to higher receptor occupancy *vs* lower approved tocilizumab doses • Higher IL-6R receptor occupancy is associated with greater effects on pharmacodynamic/clinical parameters • The data support sarilumab q2w dosing; both doses resulted in rapid and sustained CRP reduction

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 signalling node in the complex pro-inflammatory cytokine network that underpins RA (1, 2). IL-6 elevations have been noted in serum and synovial fluid in 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-α (IL-6Rα). IL-6 activates classical (*cis*) signalling through membrane-bound IL-6Rα (mIL-6Rα), expressed on the surface of hepatocytes and haematopoietic cells, and *trans* signalling through soluble IL-6Rα (sIL-6Rα), found in serum and synovial fluid. Signalling 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 reactant. For instance, C-reactive protein (CRP), which can 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 signalling 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) at 200 mg once every 2 weeks (q2w), with reduction to 150 mg q2w if required to manage laboratory abnormalities (11, 12). In the US, the recommended tocilizumab dose for intravenous (IV) administration is 4 mg/kg every 4 weeks (q4w) or for SC administration it is 162 mg q2w. Up-titration to 8 mg/kg IV q4w or 162 mg SC weekly (qw) (if clinical response is inadequate) is recommended for IV administration and SC administration, respectively. In the European Union, the higher tocilizumab doses of 8 mg/kg IV q4w and 162 mg SC qw are recommended, down-titrating 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 explore, using a pharmacokinetic (PK)/PD modelling 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 American College of Rheumatology [ACR]20/50/70 responses), observed in a dose-ranging study in patients with RA, were also evaluated.

Methods

In vitro IL-6 binding and signalling

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 Supplementary Appendix. Binding kinetics calculated were association rate constant (ka), dissociation rate constant (kd), and half-life ($t_{1/2}$). The overall equilibrium dissociation constant (K_D) was calculated from the ratio of kd to ka. Further details are included in the Supplementary Appendix.

Blockade of dimeric hIL-6Rα binding to IL-6

hIL-6Rα binding to IL-6 was assessed using an enzyme-linked immunosorbent assay (ELISA) competition assay. Further details are included in the Supplementary Appendix. Values for the inhibitory concentration at 50% activity (IC_{50}) and effective concentration at 50% activity (EC_{50}) were calculated for hIL-6, sarilumab, and tocilizumab. Further details are included in the Supplementary Appendix.

Inhibition of classical IL-6Rα signalling

The activity of sarilumab and tocilizumab in blocking classical IL-6Rα signalling was 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 Supplementary Appendix.

Inhibition of trans-IL-6Rα signalling

The ability of sarilumab to block *trans* signalling stimulated by the IL-6/sIL-6Rα complex was assessed in a functional cell-based luciferase assay using a gp130-overexpressing human embryonic kidney/STAT3/luciferase reporter cell line. Further details are included in the Supplementary Appendix.

PK/PD modelling 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 PK of sarilumab and tocilizumab by a two-compartmental 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-6R was described by the 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 structure model was used to develop the PK/PD model and 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 five sarilumab SC regimens (100 mg q2w, 150 mg q2w, 100 mg qw, 200 mg q2w, 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 Supplementary Fig. 1. The PK/PD model for binding to sIL-6Rα was used to predict the time-course of free sIL-6R 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 the accuracy of parameter estimates. Model verification was performed by comparing observed data with literature-reported data.

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-course of tociliziumab or sarilumab concentrations in serum, and estimate binding to sIL-6R and free sIL-6R concentrations for the approved dosage regimens of sarilumab SC (200 mg and 150 mg q2w), tocilizumab IV (8 mg/kg and 4 mg/kg q4w), and

tocilizumab SC (162 mg qw and q2w). sIL-6Rα occupancy dynamic profiles (% RO over time) were calculated based on unbound sIL-6R concentrations:

$$RO=1-(\text{Free sIL-6R}_{\text{post-treatment}}/\text{Free sIL-6R}_{\text{baseline}})$$

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 SC 200 mg or 150 mg 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 SC 162 mg qw *vs* IV 8 mg/kg q4w (*n*=1262) and tocilizumab SC 162 mg q2w *vs* placebo (*n*=656), respectively (19) (20).

Association between sIL-6Rα receptor occupancy and CRP, DAS28-CRP, and ACR response

The association of median RO calculated from observed concentrations of free sIL-6R 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.

sIL-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 occupancy profiles were compared visually with changes in reported mean CRP levels from SUMMACTA and BREVACTA.

Results

In vitro IL-6 binding and signalling

Sarilumab bound with high affinity to recombinant monomeric and dimeric human IL-6Rα in SPR assays, with K_D values of 61.9 pM and 12.8 pM, respectively (Table 1, Supplementary Fig. 2A). Sarilumab showed 15–22-fold higher affinity than tocilizumab in binding to monomeric and dimeric hIL-6Rα forms (Table 1, Supplementary Fig. 2B).

In the ELISA competition assay, sarilumab directly blocked the binding of hIL-6Rα-Fc to plate-coated hIL-6 with an IC_{50} of 108 pM (achieving complete blockade to baseline levels), whereas the IgG1 isotype control showed no blocking activity under the same conditions (Supplementary Fig. 3B). A constant concentration of 100 pM IL-6Rα-hFc 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 signalling, Fig. 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 signalling via the STAT-3 pathway (Fig. 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* signalling in the presence of 1 nM or 10 nM soluble IL-6Rα-mmH, with EC_{50} values of 1.8 nM and 0.7 nM, respectively; signalling could not be activated by IL-6 alone (Fig. 1C and 1D). Sarilumab blocked *trans* signalling 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 (Fig. 1C and 1D). Again, at the time, only sarilumab was available for evaluation in this assay.

PK/PD modelling of RO and effects on clinical efficacy parameters

Parameter estimates of the sIL-6Rα PK/PD models are presented in Supplementary Table 1. GOF evaluation indicated that the final sIL-6Rα PK/PD model was consistent with the observed data (Supplementary Fig. 4) and the VPC showed that the time-course profiles with the observed concentrations (2.5th, 50th, and 97.5th percentiles) fitted the predicted parameters well (Supplementary Fig. 5).

Simulation of sIL-6Rα occupancy by sarilumab and tocilizumab

Simulated sIL-6Rα occupancy profiles over 24 weeks for sarilumab SC *vs* tocilizumab IV and sarilumab SC *vs* tocilizumab SC are shown in Fig. 2A and 2B. Sarilumab 200 mg SC q2w achieved >90% RO after the first dose, maintained over the dosing interval and through the 24-week simulated treatment period (Fig. 2A). Simulated RO for sarilumab SC 150 mg 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 IV 8 mg/kg q4w and the SC 162 mg qw dose regimens achieved >90% simulated RO from the first dose, maintained over the dosing interval and through the 24-week simulated treatment period. In contrast, with the tocilizumab IV 4 mg/kg q4w and the SC 162 mg q2w dose regimens, trough RO values below the 90% threshold were predicted by the end of each dosing interval over the 24-week period.

At Week 24, steady-state trough IL-6Rα occupancy levels were 98% and 94% for sarilumab SC 200 mg and 150 mg q2w regimens, respectively, 99% and 60% for tocilizumab IV 8 mg/kg q4w and 4 mg/kg q4w regimens, respectively (Fig. 2A), and >99% (99.6%) and 84% for tocilizumab SC 162 mg qw and q2w regimens, respectively (Fig. 2B).

Association between sIL-6Rα occupancy and clinical efficacy parameters

In MOBILITY Part A, the association of sIL-6Rα RO was investigated for the following clinical efficacy parameters: percentage CRP reduction, DAS28-CRP score, and ACR20/50/70. Week 12 RO was calculated for placebo and 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 (Fig. 3A and 3B). There was also an apparent association between higher RO and ACR20/50/70 responses (Fig. 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 (Fig. 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 (Fig. 5), the inverse relationship between sIL-6Rα occupancy 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 to tocilizumab and inhibits IL-6-induced cellular responses (i.e. cell proliferation and STAT signalling) with higher potency and at lower concentrations than tocilizumab. Consistently, PK/PD modelling 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 receptor occupancy was associated with better clinical parameters.

IL-6 activates cells via a signalling mechanism that requires two receptor components, the 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 (*cis*) signalling 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* signalling 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 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-6R complex-induced proliferation of human gp130-transfected cells (BAF-h130) and bound human IL-6R expressing COS-7 cells. It also suppressed growth of the 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- to 22-fold higher than that of tocilizumab. The studies confirm the ability of sarilumab to block both classical- and *trans*-mediated signalling. Sarilumab blocked IL-6-induced growth of the human B cell line DS-1 and inhibited IL-6-induced STAT signalling 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* signalling assay in which cells expressing gp130 were stimulated by the soluble form of 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 occupancy for approved dose regimens of sarilumab SC (200 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 serum 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-6R and mIL-6R forms. It was shown that the TMDD system with two targets can be approximated by equations that describe both sarilumab or tocilizumab and sIL-6R concentrations, and include two target-mediated elimination terms (with different maximum elimination rate [V_{max}] and Michaelis–Menten constant [K_m] parameters). However, mIL-6R was not measured in the clinical studies and insufficient data did not allow for separation of the two 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 two 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 IV 8 mg/kg q4w and SC 162 mg qw regimens are able to achieve and maintain target sIL-6Rα occupancy (>90%) for the entire dosage interval over a 24-week treatment period (for sarilumab 150 mg SC dosing 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 US-recommended tocilizumab starting doses, RO fell below 90% towards the end of each dosing period. 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 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 regimen (9, 15).

Besides being of scientific interest, the importance of the current findings becomes apparent with the associations between RO, PD parameters, and clinical parameters. Concentrations of sIL-6R in serum measured in MOBILITY Part A, 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. 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 (24), allowing PK/PD comparisons to be made. The IL-6Rα occupancy 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 IV 4 mg/kg q4w group. As anticipated based on RO simulations, patients on the 4 mg/kg q4w dose who up-titrated their tocilizumab IV dose to 8 mg/kg 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 a SC tocilizumab dose group as none was 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 two 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 mIL6-Rα was not measured in either the tocilizumab or sarilumab trials.

These findings on the effect of RO on clinical efficacy parameters support selection of dosing regimens of sarilumab (150 mg and 200 mg q2w SC) 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 sarilumab has a higher relative binding affinity for IL-6Rα and is more potent at inhibiting IL-6-mediated signalling at lower concentrations in serum than tocilizumab.

Acknowledgements

The authors would like to thank Ching-Ha (Vicki) Lai for providing support in 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.

Disclosure statement: 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. 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, Phytoplant, 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, Eli Lilly, Ferring Pharmaceuticals, GlaxoSmithKline, Hospira, ISIS, Janssen, Jazz Pharmaceuticals, MedImmune, Merck Sharp & Dohme, Merrimack Pharmaceuticals, Napp, Novartis, Novimmune, ObsEva, Pfizer, Regeneron Pharmaceuticals Inc., Roche, R-Pharm, Sanofi-Aventis, Sanofi Genzyme, SynAct Pharma, Tonix, and UCB; P. Emery has received grant/research support from AbbVie, Merck, Pfizer and Roche, and is a consultant for AbbVie, Bristol-Myers Squibb, Lilly, Merck, Novartis, Pfizer, Roche, and Samsung Bioepis.

Institutional review board approval and ethics: For each of the clinical studies described in this manuscript, institutional review board and ethics committee approval of the protocols were obtained prior to study initiation, and all patients provided written informed consent.

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 dataset 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

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FIGURES AND TABLES

FIG 1 Blockade of classical IL-6Rα signalling by sarilumab and tocilizumab in (A) proliferation assay in DS-1 cells, and (B) STAT3 signalling in HepG2/STAT3-Luc cells, and sarilumab blockade of *trans* IL-6Rα signalling in HEK293/gp130/STAT3-Luc cells exposed to 10 nM hIL-6 and (C) 1 nM hIL-6Rα or (D) 10 nM hIL-6Rα

FIG 2 Simulated receptor occupancy (RO) profile through Week 24 for (A) sarilumab SC *vs* tocilizumab IV and (B) sarilumab SC *vs* tocilizumab SC

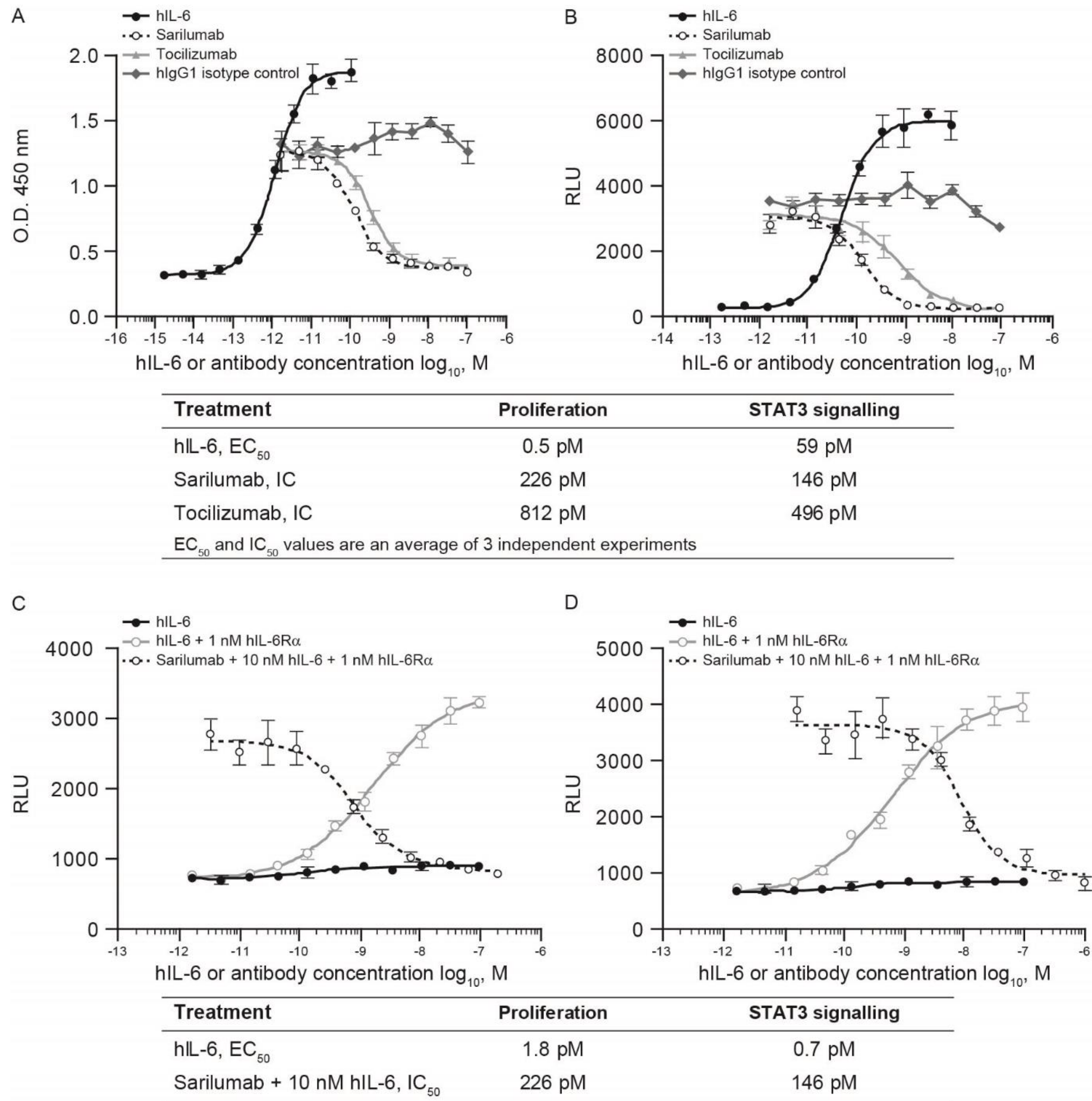
FIG 3 Relationship between receptor occupancy (RO) and (A) CRP reduction, (B) DAS28-CRP reduction, and (C) ACR20/50/70 responses with placebo and sarilumab (MOBILITY Part A)

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

FIG 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 Fig. 3 by *The Journal of Clinical Pharamcology*

TABLE 1 Pharmacokinetic and binding parameters for sarilumab and tocilizumab to monomeric (mmH-tagged) and dimeric (mFc-tagged) human IL-6Rα proteins

FIG. 1 Blockade of classical IL-6Rα signalling by sarilumab and tocilizumab in (A) proliferation assay in DS-1 cells, and (B) STAT3 signalling in HepG2/STAT3-Luc cells, and sarilumab blockade of *trans* signalling in HEK293/gp130/STAT3-Luc cells exposed to 10 nM hIL-6 and (C) 1 nM hIL-6Rα or (D) 10 nM hIL-6Rα



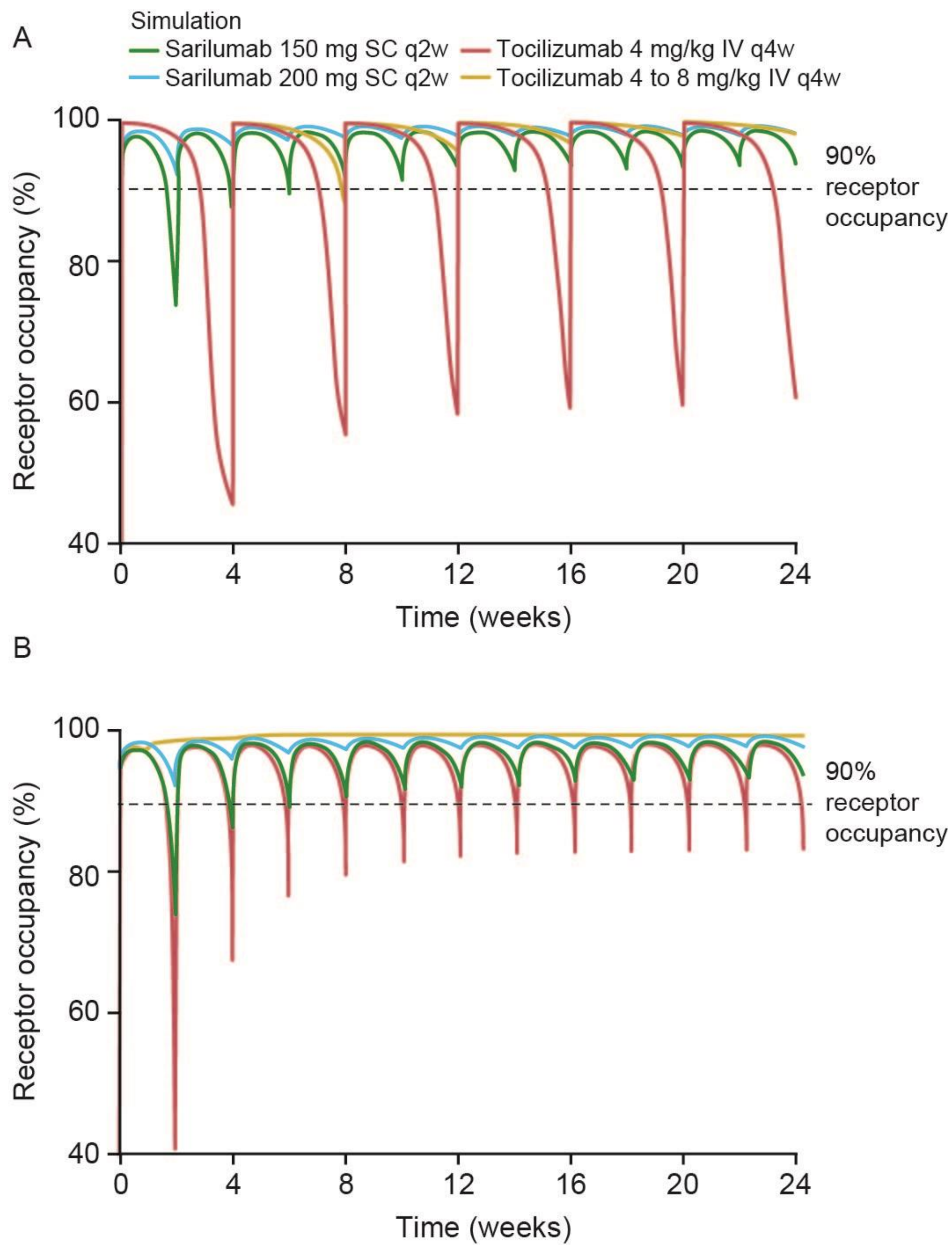
Treatment	Proliferation	STAT3 signalling
hIL-6, EC_{50}	0.5 pM	59 pM
Sarilumab, IC	226 pM	146 pM
Tocilizumab, IC	812 pM	496 pM

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

Treatment	Proliferation	STAT3 signalling
hIL-6, EC_{50}	1.8 pM	0.7 pM
Sarilumab + 10 nM hIL-6, IC_{50}	226 pM	146 pM

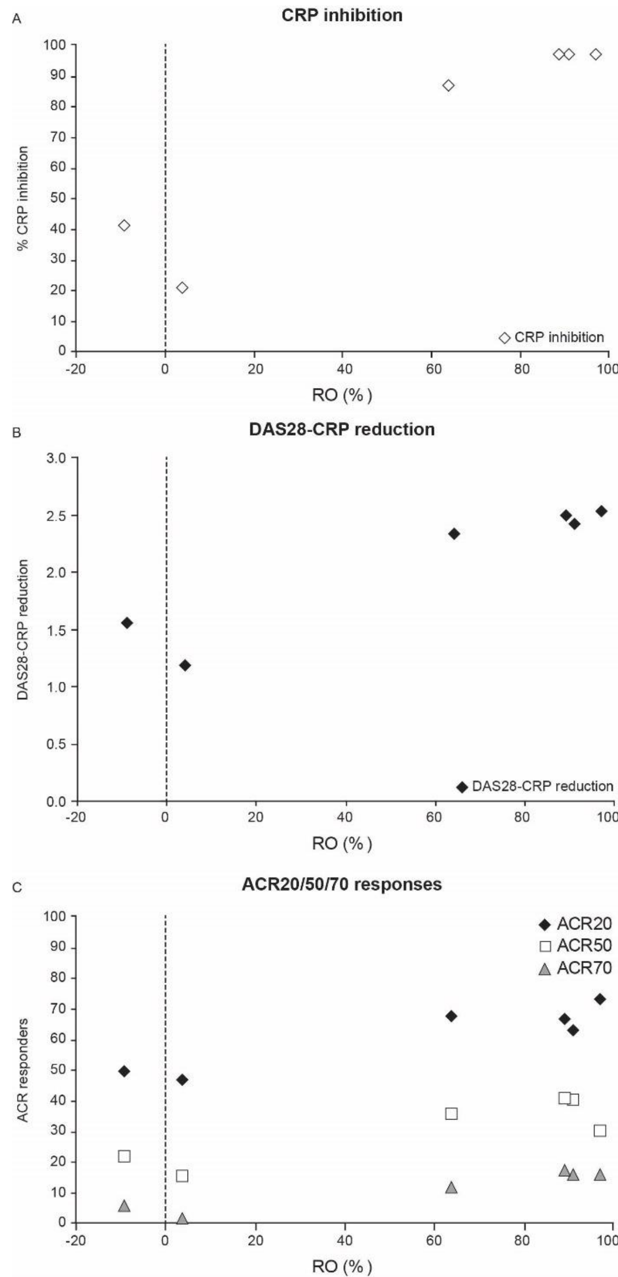
EC_{50}, effective concentration at 50% activity; HepG2, human hepatocellular carcinoma cell line HepG2; hIgG1, human immunoglobin G 1; hIL-6, human interleukin-6; IC_{50} inhibitory concentration at 50% activity; IL-6Rα, interleukin-6 receptor alpha subunit; O.D., optical density; RLU, relative luminescence units; STAT3, signal transducer and activator of transcription 3.

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



IV, intravenous; q2w, every two weeks; q4w, every four weeks; SC, subcutaneous.

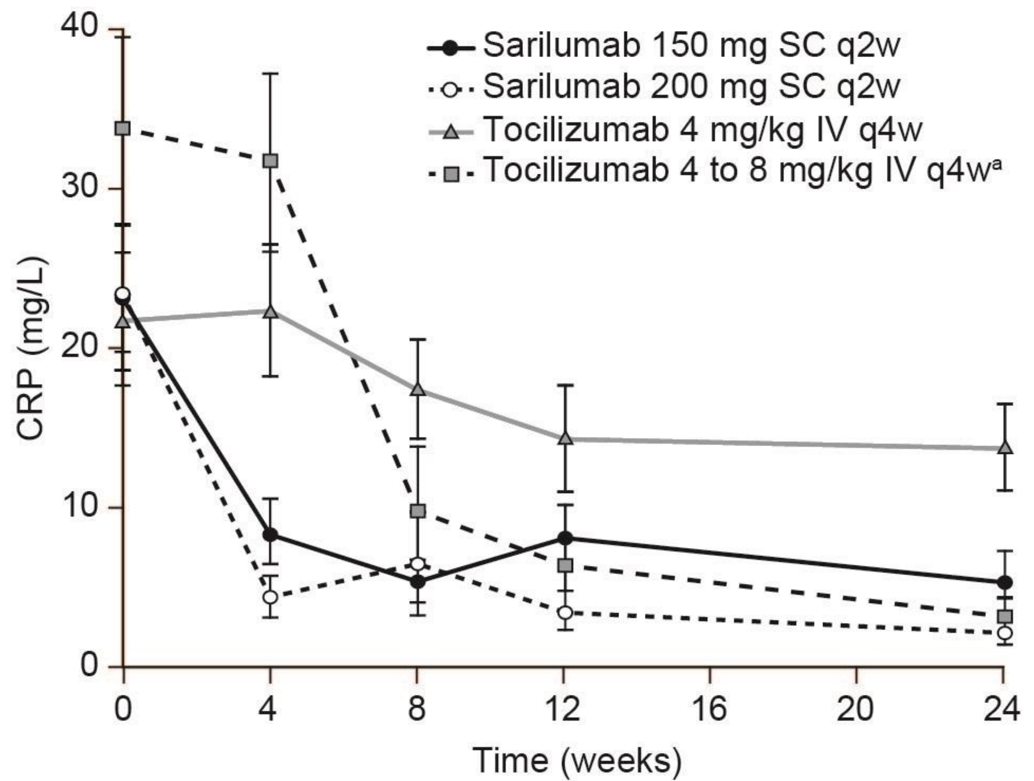
FIG. 3 Relationship between receptor occupancy (RO) and (A) CRP reduction, (B) DAS28-CRP reduction, and (C) ACR20/50/70 responses with placebo and sarilumab (MOBILITY Part A)



Patients received placebo or sarilumab 100 mg q2w, 150 mg q2w, 200 mg q2w, 100 mg qw, or 150 mg q2w in MOBILITY Part A. Changes in free sIL-6R levels were used to calculate receptor occupancy, and associations were assessed against clinical parameters.

ACR, American College of Rheumatology; ACR20/50/70, 20%/50%/70% improvement in American College of Rheumatology criteria; CRP, C-reactive protein; DAS28-CRP, DAS28-CRP, Disease Activity Score of 28 joints using C-reactive protein; RO, receptor occupancy.

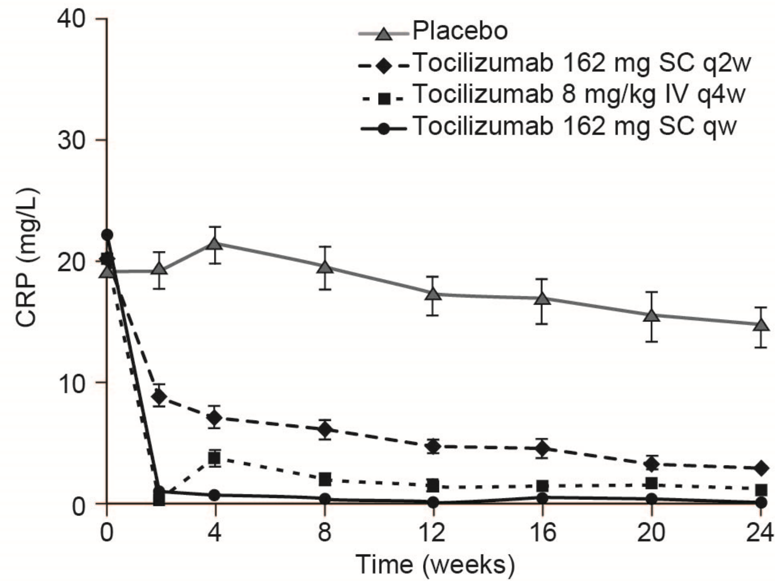
FIG. 4 Observed mean CRP levels (± SE) in patients with RA treated with sarilumab SC or tocilizumab IV (ASCERTAIN)



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

[a]Patients started at 4 mg/kg followed by an increase to 8 mg/kg, based on clinical response; ASCERTAIN (NCT01768572); CRP, C-reactive protein; IV, intravenous; q2w, every two weeks; q4w, every four weeks; RA, rheumatoid arthritis; SC, subcutaneous; SE, standard error.

FIG. 5 Observed mean CRP levels (± SE) in patients with RA treated with tocilizumab SC and IV (SUMMACTA, BREVACTA) (Abdallah H, et al. 2017), is reprinted under CC BY-NC-ND 4.0 from Fig. 3 by *The Journal of Clinical Pharmacology*



Number of patients

		0	2	4	8	12	16	20	24
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 Fig. 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; RA, rheumatoid arthritis; SC, subcutaneous; SE, standard error.

TABLE 1 Pharmacokinetic and binding parameters for sarilumab and tocilizumab to monomeric (mmH-tagged) and dimeric (mFc-tagged) human IL-6Rα proteins, determined by SPR-Biacore

Antigen	**Antibody**	**ka (1/Ms)**	**kd (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

Supplementary appendix

Christine Xu et al. Differential binding of sarilumab and tocilizumab to IL-6Rα and effects of receptor occupancy on clinical parameters

Supplementary methodology

Supplementary FIG S1. Sarilumab and tocilizumab sIL-6Rα receptor occupancy models

Supplementary FIG S2. Ligand-binding properties of sarilumab and tocilizumab. 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 fit 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

Supplementary FIG 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

Supplementary FIG S4. Basic goodness-of-fit plots with locally weighted scatterplot smoothing (LOWESS) (red lines) for the final model. |*iWRES*| individual weighted residuals

Supplementary FIG 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

Supplementary Table S1. Parameter estimates of the sIL-6Rα population PK/PD model

Supplementary methodology

Kinetic binding analysis

The binding kinetics of sarilumab and tocilizumab to IL-6Rα were measured using Surface Plasmon Resonance (SPR; Biacore™ T200). 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 nM to 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 association rate constant (ka), dissociation rate constant (kd), and half-life ($t_{1/2}$). The overall equilibrium dissociation constant (K_D) was calculated from the ratio of kd to ka.

Blockade of dimeric hIL-6Rα binding to IL-6 (ELISA competition assay)

A 3-fold dilution series of sarilumab or an isotype control IgG1 antibody (30 nM to 0.5 pM) were pre-incubated for 1 hour with 100 pM of dimeric hIL-6Rα with a C-terminal human IgG1 Fc tag (IL-6Rα-hFc), after which the mixtures were transferred to 96-well microtitre plates onto which 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). IC_{50} and EC_{50} values, the concentration 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 signalling (cell proliferation and STAT3 response element activation assays)

The activity of sarilumab and tocilizumab in blocking classical IL-6Rα signalling was 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

Serial dilutions of sarilumab (100 nM to 1.7 pM) and tocilizumab (100 nM to 1.7 pM) were added to DS-1 cells (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).

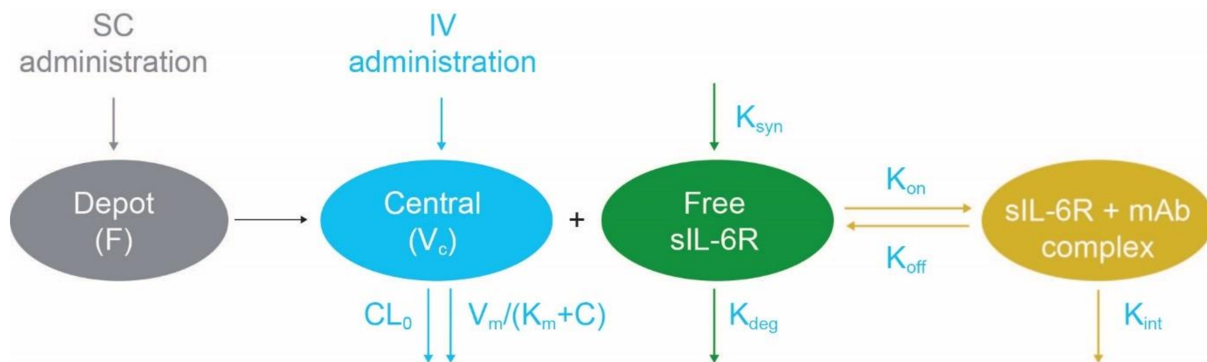
In the second assay, HepG2 (ATCC, HB-8065) was transiently transfected with a STAT3-luciferase reporter plasmid. Dilution series of sarilumab (100 nM to 1.7 pM) and tocilizumab (100 nM to 1.7 pM) were added to the transfected cells seeded in 96-well plates, followed by hIL-6 (50 pM). Plates were incubated (6 hours at 37ºC, 5% CO_2) 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 signalling (luciferase assay)

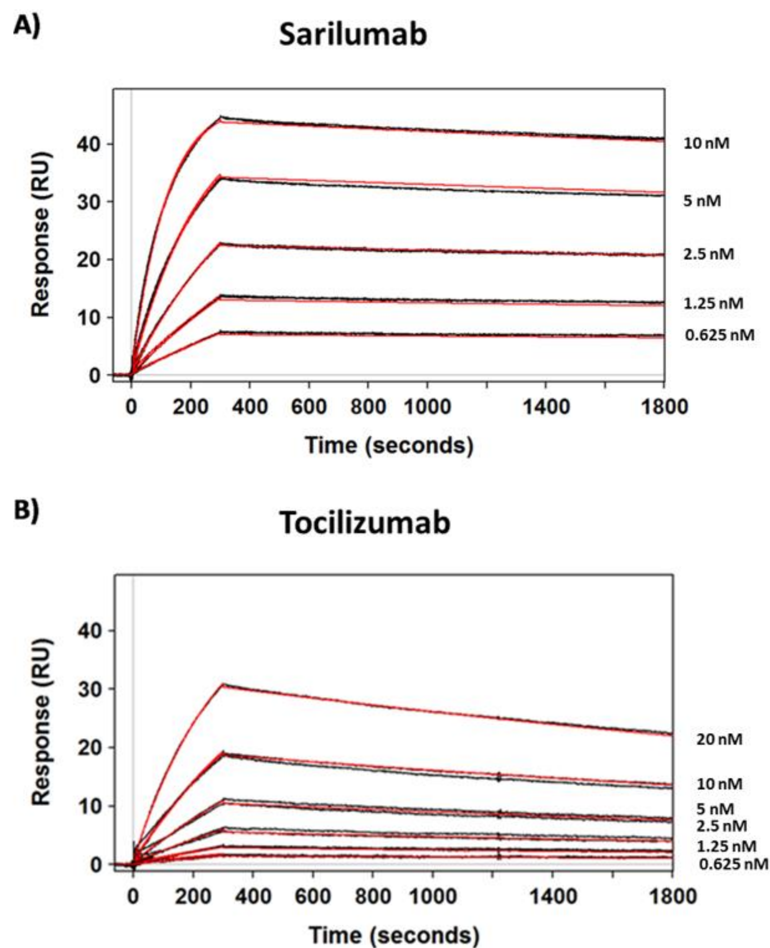
The ability of sarilumab to block *trans* signalling stimulated by a soluble complex of IL-6 and IL-6Rα was assessed in a functional cell-based luciferase assay using a gp130-overexpressing human embryonic kidney/STAT3/luciferase reporter cell line. Serial dilutions of sarilumab (200 nM–3.4 pM) were pre-incubated with 1 nM of monomeric sIL-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 (5 hours at 37ºC, 5% CO_2), response was measured using One-Glo luciferase substrate (Promega) and read on the Victor X5 multilabel counter.

Supplementary FIG S1 Sarilumab and tocilizumab sIL-6Rα receptor occupancy models



C, concentration; CL_0, apparent clearance; F, bioavailability; 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; sIL-6R, soluble form of the interleukin-6 receptor; V_c, apparent volume of central compartment; V_m, maximum elimination rate.

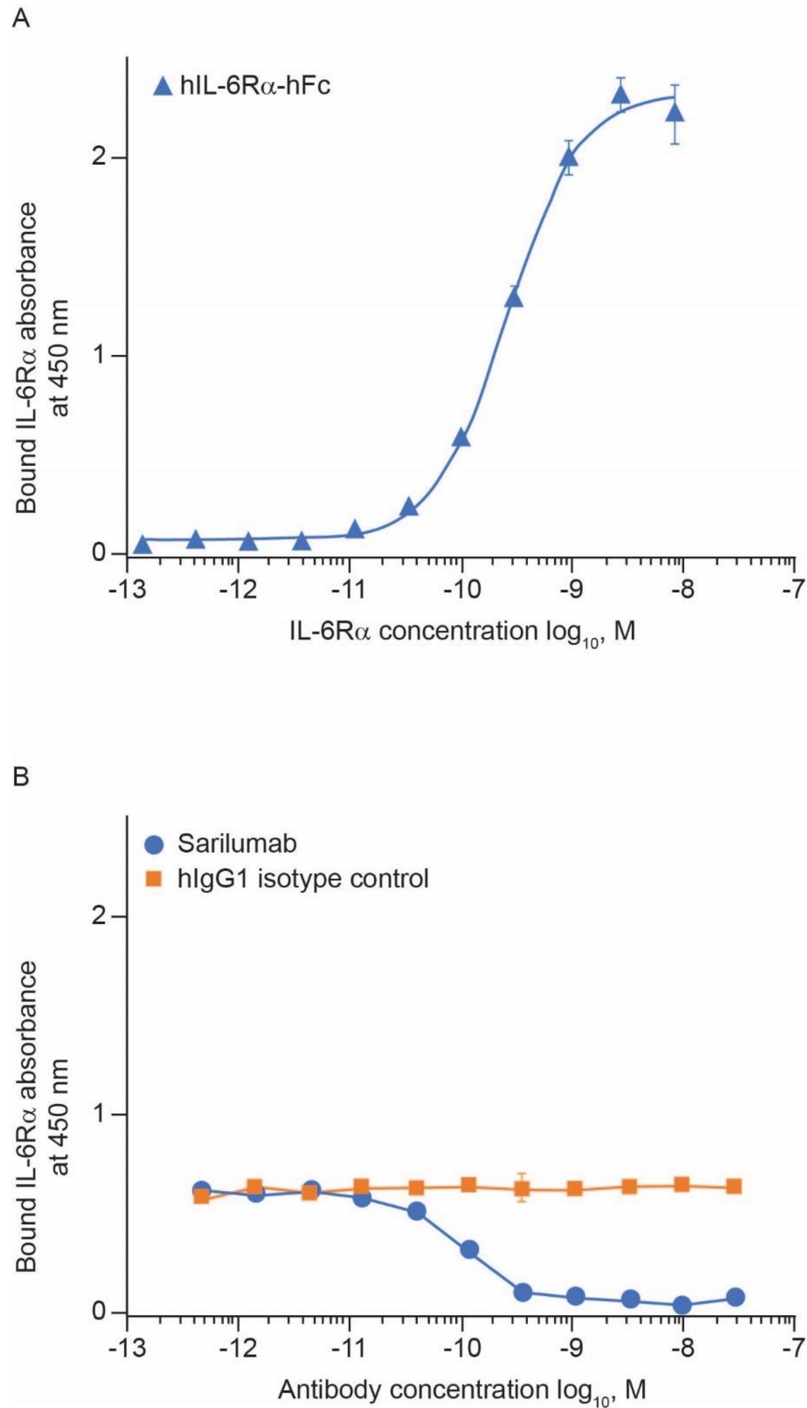
Supplementary FIG S2 Ligand-binding properties of sarilumab and tocilizumab. Representative sensograms of (A) sarilumab binding to monomer human IL-6Rα-mmH 10, 5, 2.5, 1.25, and 0.625 nM, and (B) tocilizumab binding to monomer human IL-6Rα-mmH 20, 10, 5, 2.5, 1.25, and 0.625 nM, are shown as black lines. The data were globally fit 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; (107 RU) sarilumab was captured on an anti-human Fc-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/min for 5 minutes over each of the captured surfaces.

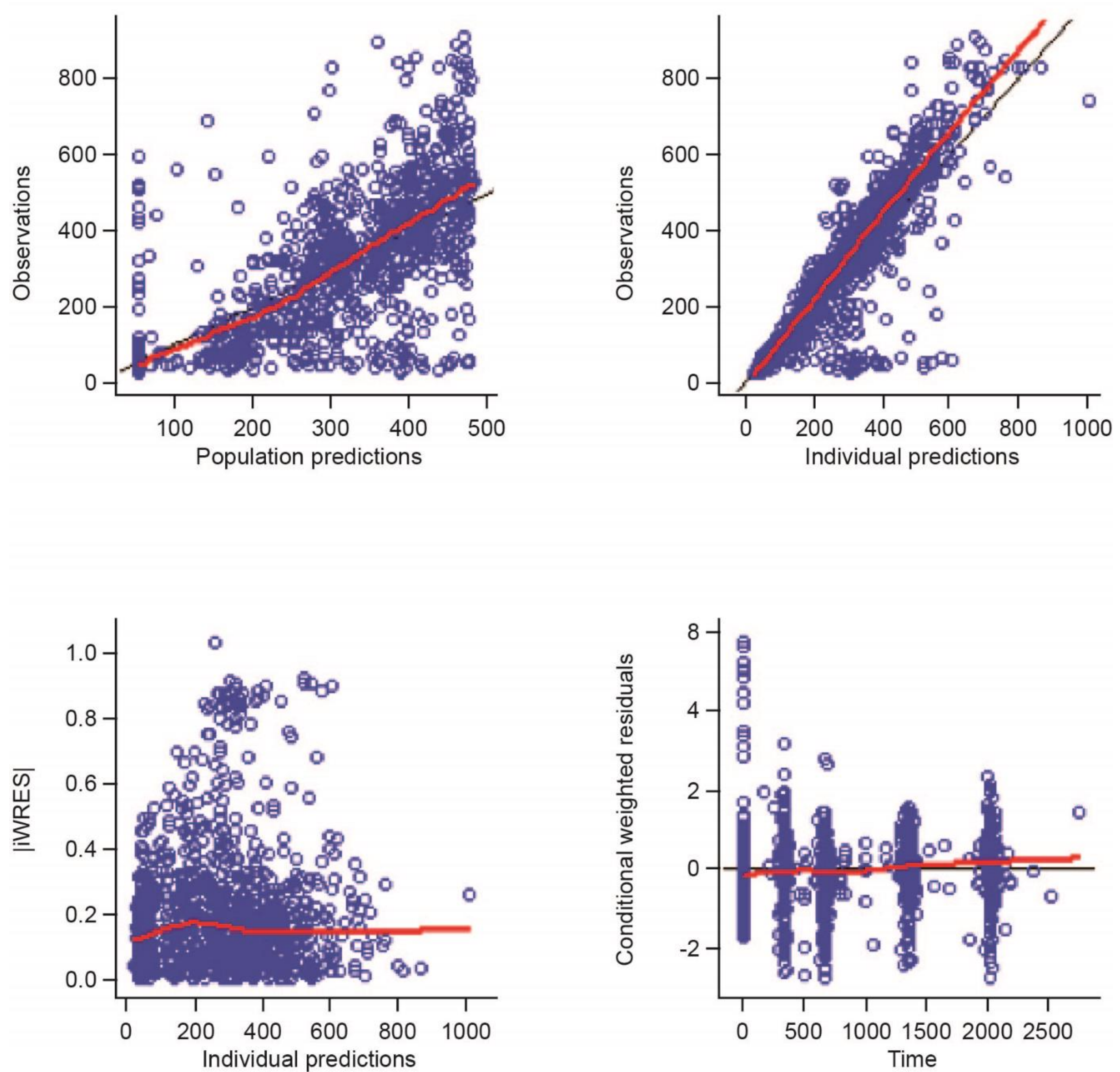
IL-6R, interleukin-6 receptor; RU, resonance units; SPR, surface plasmon resonance.

Supplementary FIG 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



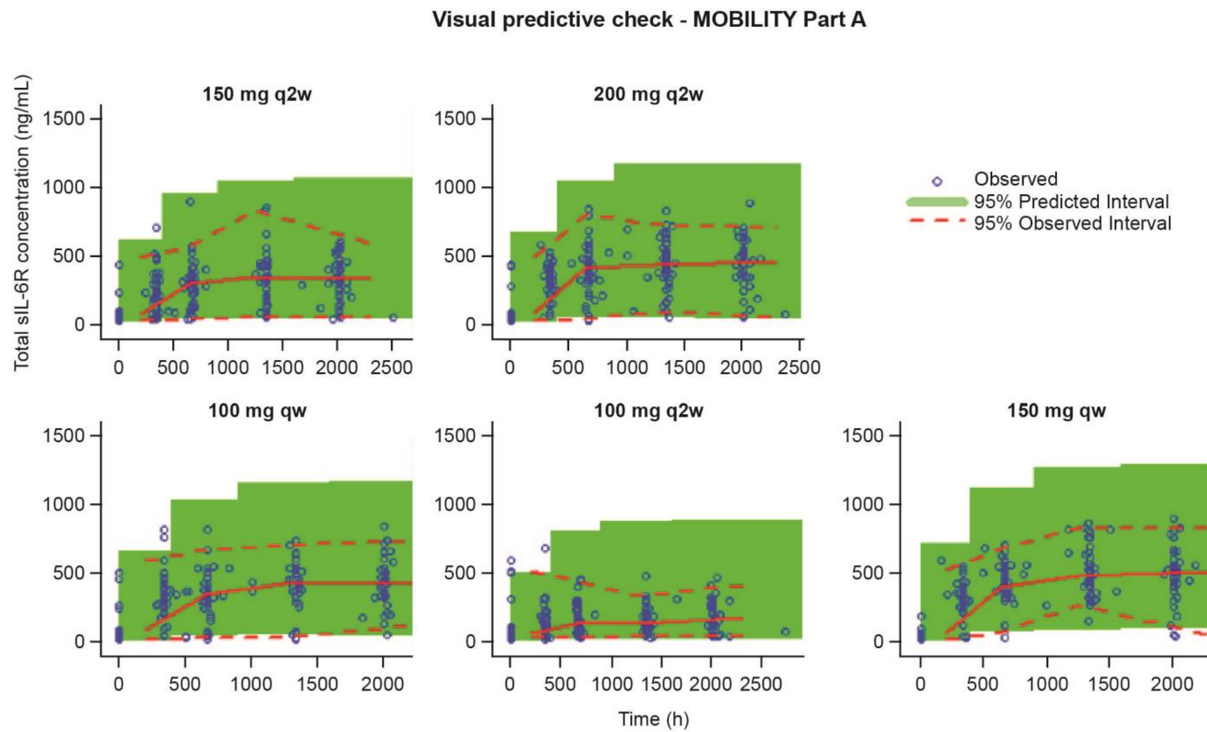
hIgG1, human immunoglobin G1; hIL-6, human interleukin-6; hIL-6Rα-hFc, recombinant extracellular domain of human IL-6Rα generated with an N-terminal amino acid linker sequence comprising the Fc region of human immunoglobin G 1 (IgG1); IL-6Rα, interleukin-6 receptor alpha subunit.

Supplementary FIG S4 Basic goodness-of-fit plots with locally weighted scatterplot smoothing (LOWESS) (red lines) for the final model. */iWRES/* individual weighted residuals



Supplementary FIG 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



In these figures, solid and dashed red lines connect the median and bounds (i.e., 2.5th and 97.5th percentiles) of observed concentrations at each time point. Red and blue rectangles represent the median and bounds, respectively, of predicted concentrations at each time point.

Supplementary TABLE S1 Parameter estimates of the sIL-6R population PK/PD model

Parameter	Estimate	RSE%	95% CI
κ_{syn} (ng/mL/day)	40.1	7.79	33.8–46.3
κ_{dge} (1/h)	0.734	9.16	0.600–0.871
I_{max}	0.891	0.650	0.879–0.902
κ_{ss} (µg/mL)	0.264	22.4	0.146–0.381
Inter-individual variability (CV%)			
κ_{syn}	47.8	19.3	37.4–56.2
κ_{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; RSE, relative standard error; sIL-6R, soluble form of the interleukin-6 receptor.