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COVID-19 acute respiratory distress syndrome: A simulation study of the effects of combination therapy with tocilizumab and siltuximab 📊 🟠

Eileen B. Doyle 🟢 | **Darren Bentley** 🟢 | **Michael G. Dodds** 🟢

Certara USA, Inc, Princeton, NJ, USA

Correspondence
Michael G. Dodds, PhD, Certara USA, Inc.,
100 Overlook Center, Suite 101, Princeton, NJ
08540, USA.
Email: michael.dodds@certara.com

Aims: To assess the potential of interleukin-6 (IL-6) signalling blockade in the lung to treat SARS-CoV-2 infection via model-based simulation by exploring soluble IL-6 receptor (sIL-6R) sequestration by tocilizumab (TCZ) and IL-6 sequestration by siltuximab (SIL).

Methods: Literature values of IL-6, IL-6 antagonist SIL, sIL-6R, IL-6R antagonist TCZ and their respective binding constants were used to develop a model to predict the impact of treatment on IL-6 signalling. Models were used to generate simulated bronchoalveolar lavage fluid concentrations for normal subjects, subjects at risk of developing acute respiratory distress syndrome (ARDS), and subjects with ARDS under 4 conditions: without treatment; treatment with TCZ; treatment with SIL; and treatment with TCZ + SIL.

Results: With TCZ intervention, IL-6 levels are unaffected and sIL-6R is reduced somewhat below the Normal case. IL-6:sIL-6R complex only slightly decreased relative to the no-intervention case. With SIL intervention, sIL-6R levels are unaffected and IL-6 is greatly reduced below the Normal case. IL-6:sIL-6R complex is greatly decreased relative to the no-intervention case. With TCZ + SIL intervention, IL-6 and sIL-6R levels are reduced below the Normal case and achieve suppression equivalent to monotherapy results for their respective targets. IL-6:sIL-6R complex reduction is predicted to be greater than that achieved with monotherapy. This reflects sequestration of both components of the complex and the nonlinear binding equilibrium.

Conclusion: Coadministration of both IL-6 and IL-6R sequestering products such as SIL and TCZ may be necessary to effectively treat COVID-19 patients who have or are at risk of developing ARDS.

KEYWORDS
acute respiratory distress syndrome, COVID-19, IL-6, modelling, siltuximab, simulation, tocilizumab

The authors confirm that there is no Principal Investigator for this paper. Studies were not performed on human subjects.

1 | INTRODUCTION

To date, the most serious symptoms from COVID-19 are pulmonary complications. An overwhelming number of patients with COVID-19 present with viral pneumonia and acute respiratory failure. COVID-19 acute respiratory distress syndrome (ARDS) is diagnosed when someone with confirmed COVID-19 infection meets the Berlin 2012 ARDS diagnostic criteria.[1]

COVID-19 ARDS follows a predictable time course, with a median time to intubation of approximately 8 days after symptom onset,[2] yet cytokine changes are dynamic. The correlation between cytokine response and disease severity is still a point of contention. The worst outcome of COVID-19 ARDS is cytokine release syndrome (CRS), leading to multiorgan failure.

Interleukin-6 (IL-6) plays multiple roles in the immune response, and seems to play a major role in COVID-19 ARDS. A meta-analysis of studies investigating the immunological response of COVID-19 revealed mean IL-6 concentrations were 2.9-fold higher in patients with complicated COVID-19 compared with patients with noncomplicated disease.[3] Results of single-centre studies continue to support this finding. Mortality rates among patients with elevated IL-6 of up to 5% have been reported, with corresponding mortality rates in patients with normal serum IL-6 of the order of 0.16%.[4]

Results from deep-profiling longitudinal studies using whole-blood proteomics identified 23 clusters of cytokines that are differentially expressed in patients with mild disease, severe disease without end organ damage and severe disease with end organ damage. Patients with severe disease had increased IL-6 and other proinflammatory cytokines, with typical IL-6 levels of 30–400 pg-mL-1, consistent with non-COVID-19 ARDS elevation of IL-6.[5]

No studies in COVID-19 ARDS have quantified IL-6's cognate receptors soluble IL-6R (sIL-6R), membrane-bound IL-6R (mIL-6R), or other proteins necessary for IL-6 signalling (glycoprotein 130 [gp130] or soluble gp130 [sgp130]). IL-6 pathway blockade has been discussed, with focus on IL-6 inhibitors, which include the IL-6R antagonists such as sarilumab and tocilizumab (TCZ) as well as the IL-6 antagonist siltuximab (SIL).

This study references a clinical example from non-COVID-19 ARDS, in which the concentrations of serum IL-6, sIL-6R and the IL-6: sIL-6R complex were measured in bronchoalveolar lavage fluid (BALF) of patients with or at risk for non-COVID-19 ARDS, in order to develop a model for COVID-19 ARDS.

2 | BACKGROUND

2.1 | IL-6 signalling pathways

IL-6 signalling is achieved through 2 signalling pathways: classic and trans (Figure 1).

What is already known about this subject

- To date, the most serious symptoms from COVID-19 are pulmonary complications. Patients with respiratory symptoms (COVID-19 pneumonia) may deteriorate and develop COVID-19 acute respiratory distress syndrome. Most of these patients will require intensive care and respiratory support. Respiratory deterioration is concomitant with high levels of inflammatory markers, including but not limited to interleukin-6 (IL-6) and IL-6receptor (IL-6R).

- Treatment with IL-6 inhibitors (such as siltuximab) and IL-6R inhibitors (such as sarilumab and tocilizumab) is being assessed in clinical trials. Sanofi and Regeneron stopped their US study of Kevzara (sarilumab) in mechanically ventilated COVID-19 patients after the drug failed to prevent deaths or get patients off ventilation. Similar results were reported by Genentech for Actemra (tocilizumab) in hospitalized adults with severe COVID-19-associated pneumonia, as the study did not meet its primary endpoint of improved clinical status or key secondary endpoints such as a difference in patient mortality at week 4. Interim data reported with the use of Sylvant (siltuximab) in the first 21 patients of an EUSA-sponsored study showed 33% of patients experienced a clinical improvement with reduced need for oxygen support and 43% of patients achieved stable disease.

What this study adds

- The combination of IL-6 inhibitors and IL-6R inhibitors has not been studied in clinical trials. Results from this simulation study of the combination of tocilizumab and siltuximab suggest that the combination may provide more efficient clearance of IL-6 and, consequently, enhance the effect of IL-6R blockade, as compared to the use of individual agents.

- This study is an example of the use of modelling and simulation to aid in consideration of drugs to repurpose for the treatment of SARS-CoV-2.

In classic IL-6 signalling, IL-6 binds membrane-bound IL-6R (mIL-6R), then the complex binds with and dimerizes the glycoprotein 130 (gp130) transmembrane protein to elicit signal transduction.[6] mIL-6R is only found on certain cell types (such as macrophages, neutrophils, some T cells, and all hepatocytes). The classic signalling pathway has been associated with anti-inflammatory, or protective, effects of IL-6 signalling.[6]

(A) Classic Pathway
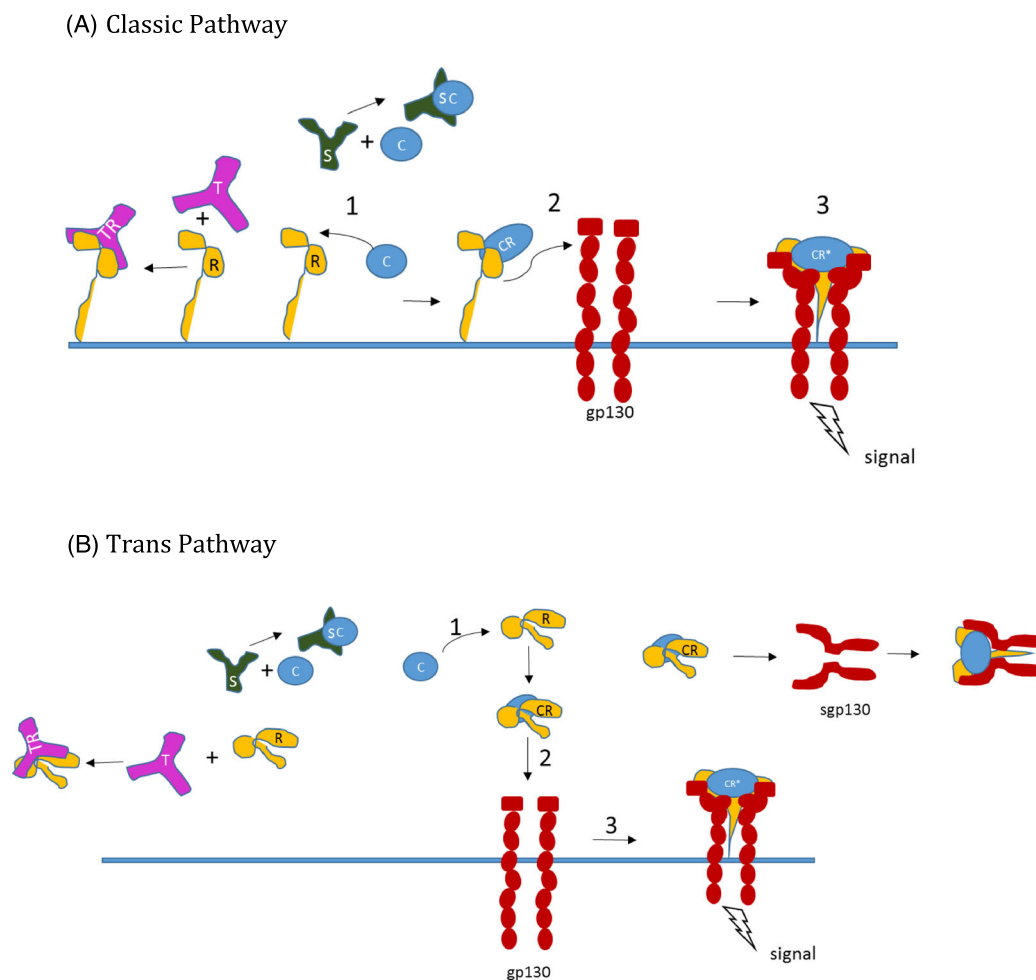
(B) Trans Pathway

FIGURE 1   Interleukin-6 (IL-6) classic and trans signalling pathways. (A) Classic pathway. C: IL-6; S: siltuximab; SC: IL-6:siltuximab complex; R: IL-6R; T: TCZ; TR: IL-6R:TCZ complex; CR: IL-6:IL-6 receptor (IL-6R) presignalling complex; CR*: IL-6:IL-6R complex bound to gp130 (signalling complex). (B) Trans pathway. C: IL-6; S: siltuximab; SC: IL-6:siltuximab complex; R: soluble (s)IL-6R; T: tocilizumab; TR: sIL-6R:TCZ complex; CR: IL-6:sIL-6R presignalling complex; CR*: IL-6:sIL-6R complex bound to gp130 (signalling complex)

Trans signalling relies on soluble IL-6R (sIL-6R), which is constitutively present in all tissue types at ng mL-1 concentrations.[6] IL-6 binds sIL-6R, then the IL-6:sIL-6R complex binds and dimerizes gp130, leading to signalling. Trans signalling is associated with proinflammatory processes, including infiltration of granulocytes and macrophages during the late phase of an acute inflammation (described as the possible cause of tissue damage in the acute lung injury). Activation of gp130 via trans signalling is crucial for lymphocyte trafficking into inflamed areas by controlling chemokine expression[6]; it promotes T-cell proliferation (for example, during colon cancer development) and is involved in regulating adhesion molecule expression on endothelial cells.[6]

One additional player in trans signalling is soluble gp130 (sgp130), which is also constitutively present.[7] sgp130 binds the IL-6:sIL-6R complex with high affinity (~10 pM). Since sgp130 can bind to the IL-6:sIL-6R complex in the circulation, it acts as a specific inhibitor of IL-6-mediated trans signalling. Taken together, sgp130 and sIL-6R serve to buffer levels of circulating IL-6.[8]

2.2 | SIL clinical experience

SIL (Sylvant) is an IL-6 antagonist indicated for the treatment of patients with multicentric Castleman's disease who are human immunodeficiency virus negative and huma herpesvirus-8 negative, approved in 2014 by the Food and Drug Administration (FDA). SIL is administered as an 11 mg/kg intravenous (IV) dose given over 1 hour every 3 weeks. SIL is not approved for the treatment of CRS, but has been administered as a single IV dose of 11 mg/kg IV for this indication. Trials for use in COVID-19 are ongoing.

2.3 | TCZ clinical experience

TCZ (ACTEMRA) is an IL-6 receptor inhibitor with several indications (rheumatoid arthritis, giant cell arteritis, polyarticular juvenile idiopathic arthritis, systemic juvenile idiopathic arthritis, cytokine release syndrome), approved in 2018 by the FDA. For the management of

CRS, the recommended dose is 8 mg/kg IV over 1 hour for patients at or above 30 kg and 12 mg/kg for patients <30 kg in weight. Doses exceeding 800 mg per infusion are not recommended in CRS patients. Trials for use in COVID-19 are ongoing.

2.4 | Hypothesis

A significant anti-inflammatory response early in ARDS may provide a key mechanism for limiting the net inflammatory response in the lungs.[9]

Therefore, preferential IL-6 trans pathway blockade may be superior to global (classic and trans) pathway blockade in the treatment of COVID-19 ARDS.

The complexity of IL-6 signalling gives insight into why blockade of IL-6 (via, for example, SIL) or IL-6R (via, for example, TCZ) alone may not be sufficient to decrease IL-6-mediated signal transduction to a clinically relevant extent in COVID-19-related ARDS.

3 | METHODS

3.1 | Nomenclature

Drug and molecular target nomenclature conforms to the IUPHAR/BPS Guide to Pharmacology nomenclature classification.[10]

3.2 | Clinical study conduct

Methods from Park et al., and the literature-cited clinical study providing estimates, are reported here.[9]

Patients with sepsis or trauma were identified by prospective screening of patients admitted to the medical and surgical intensive care units of Harborview Medical Center (Seattle, WA, USA) between 14 February 1994 and 13 July 1997 and categorized as either having ARDS or as at risk for developing ARDS. Criteria for sepsis and trauma risks and for ARDS were described in prior studies from the Seattle ARDS research programme. Patients with ARDS met the American European Consensus Conference definition of ARDS.[9]

Sixty-nine patients were enrolled in the study. Forty-six patients with ARDS were identified and categorized based on their Day post-ARDS diagnosis. Patients were followed for 21 days post-ARDS diagnosis. Twenty-three patients were categorized as at risk for ARDS on Day 1 based on their sepsis and trauma exams. Of these, 7 developed ARDS and were included in the ARDS population. The rest of the at-risk patients were followed daily in the clinical course of their care. The BALF from 6 normal volunteers was included for comparison.

Of the 23 at risk patients, BALF samples were obtained from of 20 patients on Day 1 of the study and 14 patients on Day 3 of the study. Of the 52 patients who developed ARDS, BALF samples

were obtained from 36 on Day 1 of ARDS, 41 on Day 3 of ARDS, 30 on Day 7 of ARDS, 16 on Day 14 of ARDS, and 11 on Day 21 of ARDS. The variation in the number of patients on different study days reflects changes in clinical status, including successful extubation or death, as well as the enrolment of some patients on Day 3 of ARDS.

3.3 | Modeling and simulation study conduct

Literature values of IL-6, the IL-6 antagonist SIL, sIL-6R, the IL-6R antagonist TCZ and their respective binding constants were used to develop a model to predict the impact of treatment on IL-6 signalling. Models were used to generate simulated BALF concentrations for normal subjects, subjects at risk of developing ARDS, and subjects with ARDS were simulated under 4 conditions: without treatment, treatment with TCZ, treatment with SIL, and treatment with TCZ + SIL.

3.4 | IL-6 and sIL-6R concentration in the lung: Non-COVID-19 ARDS

Measurements of IL-6 and sIL-6R concentrations in normal subjects, patients at-risk for non-COVID-19 ARDS, and subjects experiencing non-COVID-19 ARDS were obtained from the literature.[9] These patients would correspond to patients on the World Health Organization Ordinal Scale for Clinical Improvement of 3 (hospitalized with mild disease, no oxygen requirement) to 7 (hospitalized with severe disease, ventilated plus additional organ support).[11] Patients were not stratified by mild, moderate or severe ARDS, and the oxygen requirements of individual patients were not reported.

For the purpose of the present analysis, with respect to IL-6 and sIL-6R concentrations, normal levels, levels in at-risk subjects on Day 1, and levels in ARDS subjects on Day 1 were extracted (median, 25th, 75th percentiles) and analysed as log-normal distributions. In total, 300 subjects were simulated from these lognormal distributions. The degree of variability reported in Park et al. exceed 100% for both IL-6 and sIL-6R in the at-risk and ARDS population, so between-patient variability is an important consideration. Table 1 reports summaries of virtual subject values, as well as the calculated IL-6: sIL-6R complex using the formula [IL-6:sIL6R] = [IL-6]*[sIL-6R] Kd-1 and assuming the reported values in Park et al. are free IL-6 and free sIL-6R.

Notably, the induction level of IL-6 and sIL-6R in ARDS relative to normal is 370-fold and 4.88-fold, respectively. Given a reported Kd value for sIL-6R to IL-6 of 5500 pM, binding is not favoured and the free moiety forms predominate over the complex IL-6:sIL-6R (CR). The induced IL-6:sIL-6R complex level in ARDS relative to normal is 1810-fold. While out-of-scope for this analysis, affinity for the IL-6: sIL-6R complex for gp130 is in the range of 10 pM, so the predicted increase in available IL-6:sIL-6R complex should be understood in that context. This is a placeholder, suggesting the location of Table 2.

TABLE 1   IL-6, sIL-6R and complex concentrations in normal subjects, patients at risk for ARDS and patients with ARDS

Subjects	IL-6 pM (%BSV)	sIL-6R pM (%BSV)	IL-6:sIL-6R pM (%BSV)
Normal	0.138 (55.7%)	2.13 (81.3%)	5.35e-05 (101%)
At risk	7.03 (278%)	5.64 (170%)	0.00721 (318%)
ARDS	51 (196%)	10.4 (100%)	0.0968 (220%)

ARDS: acute respiratory distress syndrome; BSV: between-subject variability; IL-6: interleukin-6; sIL-6R; soluble IL-6 receptor.

TABLE 2   Published binding constants (Kd) for sIL:IL-6 and TCZ:sIL-6R

Source	A	B	Kd, pM
Van Zaanen 1996 [38]	SIL	IL-6	6.25
Deisseroth 2015 [39]	SIL	IL-6	34
Wang 2014 [40]	SIL	IL-6	15
Gibiansky 2012 [26]	TCZ	sIL-6R	759; 1241[a]
Mihara 2005 [41]	TCZ	sIL-6R	2540

IL-6: interleukin-6; sIL-6R; soluble IL-6 receptor; SIL: siltuximab; TCZ: tocilizumab.
[a]model-estimated.

3.5 | Monoclonal antibody systemic concentration

The mean peak serum concentration of SIL following the first dose of SIL 11 mg/kg IV in patients with multicentric Castleman's disease was derived from the mean steady-state maximum concentration (Cmax) of 322 μg/mL divided by the accumulation ratio (1.7), or 195 μg/mL.[12] A dissociation equilibrium binding constant (Kd) for SIL has been reported as 15 pM.[13]

The mean peak serum concentration of TCZ following the first dose of TCZ 8 mg/kg in patients with CRS during chimeric antigen receptor T-cell treatment is 99.5 μg/mL.[14] A Kd for TCZ has been reported as 1240 pM.[15]

3.6 | Monoclonal antibody concentration in the lung

Biodistribution mechanisms and data for therapeutic monoclonal antibodies were taken from published literature.[16] Specifically, distribution to the lung has been studied in the context of respiratory syncytial virus (RSV), where anti-RSV antibodies have been developed. BALF concentrations have been quantified in studies in cynomolgus monkeys relative to serum concentrations, and suggest extremely low BALF:Serum concentration ratios of 0.1–0.2%.

For the purpose of these calculations, an optimistic BALF:Serum concentration ratio of 0.2% was used. Applying this ratio to peak SIL and TCZ serum concentrations, peak BALF concentrations are then 2690 and 1370 pM, respectively.

3.7 | Binding constant selection

A review of the literature provided estimates for binding constants for SIL:IL-6 complex and TCZ:IL-6R complex. These values represented data across clinical and nonclinical studies, and modelling from clinical and nonclinical species. Binding constants for use in our model were set at the median value encountered, or 15 pM for SIL:IL-6 (Kd_SC in Figure 2) and 1241 pM for TCZ:sIL-6R (Kd_TR in Figure 2).

3.8 | Binding models

CR is the complex that (presumably) signals through the ubiquitously expressed gp130 receptor.

In stoichiometric form:

- S + C < − Kd_SC –> SC *where* Kd_SC = S * C / SC
- T + R < − Kd_TR –> TR *where* Kd_TR = T * R / TR
- C + R < − Kd_CR –> CR *where* Kd_CR = C * R / CR

where: S and T are the free concentrations of SIL and TCZ in BALF, respectively; C and R are the free concentrations of IL-6 cytokine and sIL-6R, respectively; SC is the concentration of SIL:IL-6 complex; TR is the concentration of TCZ:sIL-6R; CR is the concentration of sIL-6R bound to IL-6. Kd_SC, Kd_TR and Kd_CR are the equilibrium dissociation binding constants for SIL:IL-6, TCZ:sIL-6R and IL-6:sIL-6R, respectively.

The binding reactions are assumed reversible following receptor theory. The concentrations of free and bound form of each species, after equilibration time, can be calculated from their initial concentrations and the strength of the binding interaction given by the dissociation equilibrium constant, Kd. Antibody drugs, such as SIL and TCZ interact with their targets in this manner.

These reactions were implemented as a system of ordinary differential equations. Initial conditions of IL-6 (C) and sIL-6R (R) were given from the BALF concentration data in normal, pre-ARDS and ARDS subjects from the above Table. IL-6:sIL-6R (CR) was calculated, as above. Binding constants are given for SIL:IL-6 (SC) of 15 pM,
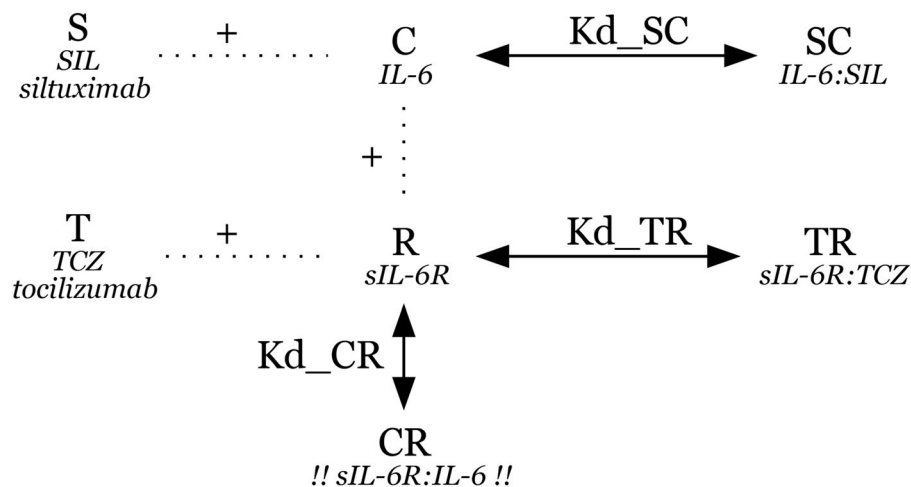
FIGURE 2 Binding model. C: IL-6; S: siltuximab; SC: IL-6:siltuximab complex; R: sIL-6R; T: tocilizumab; TR: sIL- 6R: tocilizumab complex; CR: IL-6:sIL-6R pre-signalling complex; Kd: binding constant

IL-6:sIL-6R (CR) of 5500 and TCZ:sIL-6R (TR) of 1241. Initial conditions for SIL and TCZ concentrations were taken as the peak BALF concentrations of 2690 pM and 1370 pM, respectively, as calculated above.

Solving the resulting equilibrium equations may be possible, but these authors opted instead to simply simulate from the (dynamic) ordinary-differential equations out to steady-state. Off-rates were set to 0.1 s^{-1} for each reaction, so on-rates were derived kon = koff Kd-1. Although the off-rate is much more rapid than is typical for antibodies, the simulations were run out to steady-state so this assumption plays no role in the simulation results. Simulations at off-rate values of 0.01s^{-1} and 0.001 s^{-1} were performed to confirm similar results at equilibrium (time > > koff-1.) Should an analytical solution to the equilibrium equations be derived, the results would be expected to match these.

The model solutions for C (IL-6), R (sIL-6R) and CR (IL-6:sIL-6R complex) at binding equilibrium were produced for each synthetic subject.

Supplemental Materials provides an R-script that performs these simulations and fully reproduces these methods and the following results. Critical assumptions include: no additional effect of gp130 binding, instantaneous and constant lung concentration for SIL and TCZ, constant concentration of total IL-6 and sIL-R in the lung, and equilibrium binding, The latter assumption is likely reasonable, as binding events are fast relative to antibody kinetics. The former 2 assumptions generate *optimistic* predictions for the interventions. Antibody concentrations would wane over time, and new IL-6 and sIL-6R would be synthesized over time, leading to waning antagonism of IL-6 and sIL-6R with time. However, there is no information on the dynamics of IL-6 and sIL-6R in the lung, so the approximation/ assumptions taken here match the available data.

4 | RESULTS

The binding models were used to generate simulated BALF concentrations of IL-6, sIL-6R and the IL-6:sIL-6R complex for normal subjects,

subjects at risk of developing ARDS, and subjects with ARDS. Each model was used to simulate concentrations from 300 virtual subjects. Complex concentrations were simulated under 4 conditions: without treatment, treatment with TCZ, treatment with SIL, and treatment with TCZ + SIL.

Results are displayed in Figure 3 and provided in numerical form in Table 3.

Concentrations simulated from the virtual group of subjects without ARDS and not at risk for ARDS are represented as Normal subjects. Dashed lines capture 90% of the simulated Normal Subject with Treatment: None cases. Observed concentrations of IL-6 and sIL-6R[9] are summarized and plotted as Treatment: None.

Simulation of concentrations in at-risk subjects and subjects with ARDS following no treatment (black circles), treatment with TCZ (orange circles), SIL (blue circles) and TCZ + SIL (green circles) are summarized and plotted (point: median, interval: 5th–95th percentile for n = 300 simulated subjects.)

IL-6 and IL-6:sIL-6R are greatly elevated in both simulated populations, while sIL-6R elevations are more modest.

With TCZ intervention, IL-6 levels are unaffected and sIL-6R is reduced somewhat below the Normal case. IL-6:sIL-6R complex only slightly decreased relative to the no-intervention case. While this is somewhat counterintuitive, IL-6 competes with TCZ for sIL-6R and IL-6 is greatly induced in at-risk (corresponding to a World Health Organization [WHO] Score of 3–4[11]) and ARDS populations (corresponding to a WHO Score of 5–7[11]). This idea is consistent with the findings of Swaroopa et al.,[17] where APACHE II score, together with Day 1 serum IL-6 and serum-IL-8 concentrations predicted survival in ARDS patients.

With SIL intervention, sIL-6R levels are unaffected and IL-6 is greatly reduced below the Normal case. IL-6:sIL-6R complex is greatly decreased relative to the no-intervention case. Here, sIL-6R competes with SIL for IL-6 and sIL-6R is only modestly induced in at-risk and ARDS populations.

With TCZ + SIL intervention, IL-6 and sIL-6R levels are reduced below the Normal case and achieve suppression equivalent to

FIGURE 3 Model-derived simulations of concentrations of IL-6, sIL-6R, and IL-6:sIL-6R Complex in Virtual Subjects with Varying ARDS Status. ARDS: acute respiratory distress syndrome; BALF: bronchoalveolar lavage fluid; IL-6: interleukin-6; sIL-6R; soluble IL-6 receptor; SIL: siltuximab; TCZ: tocilizumab
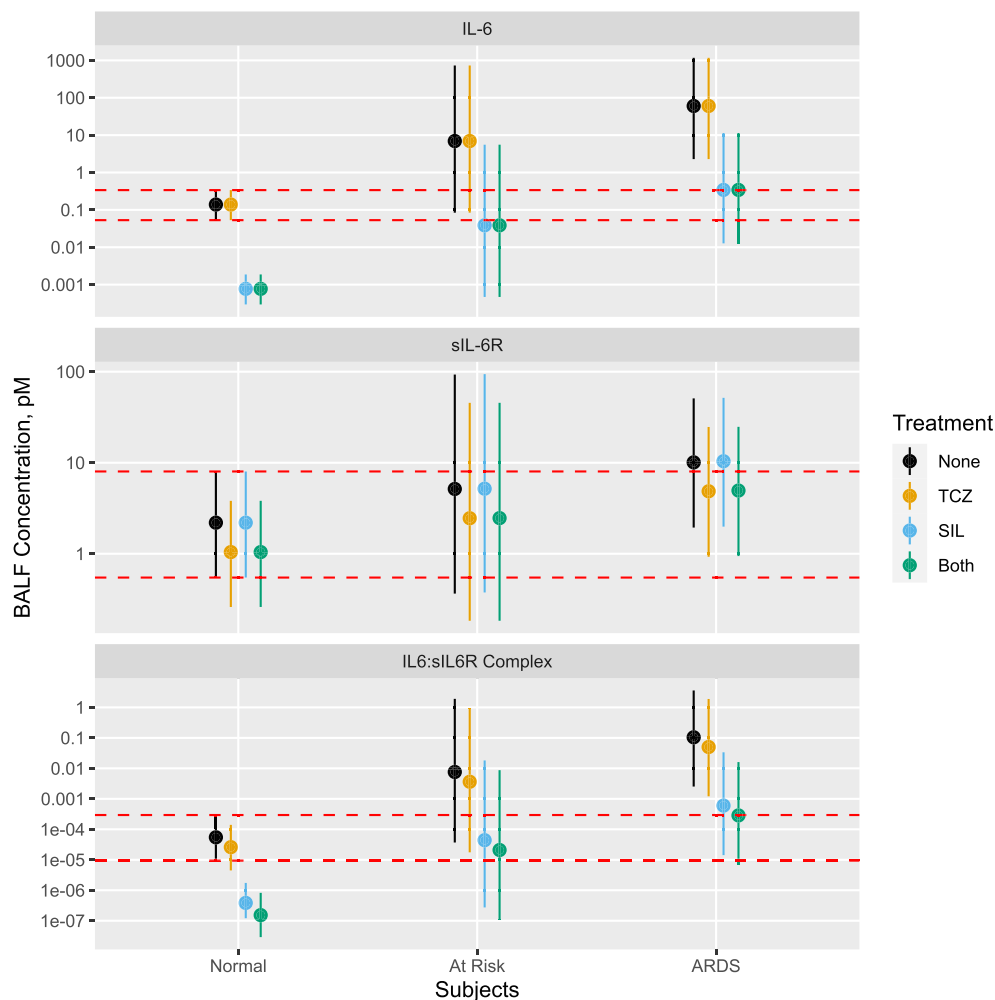
monotherapy results for their respective targets. Interestingly, IL-6:sIL-6R complex reduction is predicted to be greater than monotherapy. This reflects sequestration of both components of the complex and the nonlinear binding equilibrium.

5 | DISCUSSION

In 1 study,[9] individual cytokines increased in patients before and after the onset of non-COVID-19 ARDS, yet greater increases occurred in cognate receptors and/or antagonists, so that the molar ratios of agonists to antagonists declined dramatically at the onset of non-COVID-19 ARDS and remained low for at least 7 days. In that study,[9] IL-6 increased an average 400-fold (mean peak 1230 pg/mL) over normal in 53 patients on the first day of non-COVID-19 ARDS, while sIL-6R increased only 2–3 fold. IL-6 steadily declined in these patients from Day 1–21 of non-COVID-19 ARDS, but remained elevated compared with normal levels. Importantly, the molar ratios of IL-6 and its cognate receptor sIL-6R (a specific agonist) increased >10-fold in patients at risk for non-COVID-19 ARDS and approximately 100-fold in patients with non-COVID-19 ARDS.[9]

Using the results of this study to build a model of IL-6, sIL-6R and IL-6:sIL-6R complex in bronchoalveolar lavage fluid, and using binding constants to describe the kinetics 1 might encounter, we simulated the concentrations of players in the IL-6 signalling pathway and simulated how those concentrations might change upon treatment with SIL, TCZ, or a combination of SIL and TCZ. This modelling exercise gives us *proof of hope* that a combination treatment is worth exploring in clinical studies of COVID-19.

Cytokine signalling through the IL-6 pathway is complex and depends on multiple factors, including cell type and agonist or antagonist concentration in the tissue environment.

Though IL-6R is necessary for IL-6 signalling, and is a reasonable target for IL-6 signalling inhibition, the increase in IL-6 in non-COVID-19 and COVID-19 ARDS is at least 10–50 times greater than the increase in IL-6R, and up to 1000-fold above normal. Near-complete receptor occupancy of IL-6 by TCZ in the lung could be necessary to achieve a robust decrease in signal transduction. An ability to achieve this depends on 3 factors: the relative concentrations of IL-6 and sIL-6; the concentrations of antibodies such as SIL and TCZ; and the strength of the binding in each of their respective complexes (SIL:IL-6, TCZ:sIL-6R and sIL-6R:IL-6).

TABLE 3 Simulated IL-6, sIL-6R and IL-6:sIL-6R BALF Concentrations

Subjects	Treatment	IL-6, pM pM [5th–95th PI]	sIL-6R pM [5th–95th PI]	IL6:sIL6R complex pM [5th–95th PI]
Normal	None	0.139 [0.0531–0.338]	2.19 [0.548–8.02]	5.47×10^{-5} [9.39×10^{-6}–0.000294]
Normal	TCZ	0.139 [0.0531–0.338]	1.04 [0.26–3.82]	2.6×10^{-5} [4.47×10^{-6}–0.00014]
Normal	SIL	0.000771 [0.000294–0.00188]	2.2 [0.548–8.02]	3.85×10^{-7} [1.21×10^{-7}–1.74×10^{-6}]
Normal	Both	0.000771 [0.000294–0.00188]	1.04 [0.26–3.82]	1.53×10^{-7} [2.9×10^{-8}–8.24×10^{-7}]
At risk	None	6.94 [0.084–731]	5.15 [0.365–93.1]	0.0076 [3.68×10^{-5}–1.91]
At risk	TCZ	6.95 [0.0842–732]	2.46 [0.183–45.5]	0.00364 [1.75×10^{-5}–0.952]
At risk	SIL	0.0387 [0.000468–5.56]	5.18 [0.375–94.1]	4.4×10^{-5} [2.73×10^{-7}–0.0182]
At risk	Both	0.0387 [0.000468–5.56]	2.46 [0.184–45.5]	2.09×10^{-5} [1.07×10^{-7}–0.00872]
ARDS	None	60.3 [2.28–1170]	10.1 [1.94–50.9]	0.105 [0.00253–3.6]
ARDS	TCZ	60.3 [2.28–1170]	4.86 [0.926–24.7]	0.0502 [0.00121–1.89]
ARDS	SIL	0.342 [0.0127–11.4]	10.4 [1.98–51.7]	6×10^{-4} [1.42×10^{-5}–0.0337]
ARDS	Both	0.343 [0.0127–11.4]	4.95 [0.943–24.8]	0.000286 [6.78×10^{-6}–0.0161]

ARDS: acute respiratory distress syndrome; IL-6: interleukin-6; sIL-6R; soluble IL-6 receptor; SIL: siltuximab; TCZ: tocilizumab; PI: prediction interval.

Recently, the effect of TCZ *vs.* standard care on clinical worsening in patients hospitalized with COVID-19 pneumonia was evaluated in a randomized clinical trial.[18] The trial evaluated early administration of TCZ in 60 patients (standard care in 63 patients) between March and June 2020. The median time from symptom onset to randomization was 8 days, and the median time from hospital admission to randomization was 2 days. Treatment was initiated within 8 hours of randomization to the study. No benefit on disease progression was observed compared with standard care. Interim results of a study of SIL in patients with COVID-19 respiratory failure (SISCO) demonstrated a significantly lower (54% reduction in risk) all-cause 30-day mortality rate in the SIL-treated *vs.* the matched-control cohort patients.[19] Risk factors were not taken into account, so the results could be confounded. A phase 3 study to confirm efficacy of SIL as a COVID-19 therapy is underway (SILVAR).

The clinical results are consistent with the simulation findings. With TCZ intervention alone, IL-6 levels are unaffected, sIL-6R is normalized and the IL-6:sIL-6R complex largely unchanged. In contrast, with SIL intervention alone, sIL-6R levels are unaffected, IL-6 is greatly reduced and the IL-6:sIL-6R complex is greatly reduced. If the IL-6:sIL-6R complex signalling is responsible for clinical manifestations, that would suggest SIL is superior to TCZ, which appears to be bearing out in contemporary clinical trials. While both SIL and TCZ block IL-6:sIL-6R signalling, they do so through binding different components (Figure 2) so the assumption that these treatments are interchangeable may not be valid. Further, these simulations suggest that the combination of SIL + TCZ would yield further reductions in IL-6:sIL-6R complex responsible for trans-signalling. The complexity of IL-6 signalling gives insight into why blockade of IL-6 (via, for example, SIL) or IL-6R (via, for example, TCZ) alone may not be sufficient to decrease IL-6-mediated signal transduction to a clinically relevant extent in COVID-19-related ARDS.

Maximal achievable serum SIL concentration exceeds TCZ concentration by approximately 2-fold, based on approved posology. The dissociation equilibrium concentration for SIL is 2–3 orders of magnitude lower than TCZ, meaning that SIL is better at sequestering IL-6 than TCZ is at sequestering sIL-6R. Taking these 3 factors into account, results of the simulation greatly favour the use of SIL over TCZ to inhibit IL-6 signalling. However, these results are dependent on several key assumptions:

The value for antibody penetration (BALF:serum ratio) of SIL or TCZ has not been reported. The value used in the simulation (0.2%) represented the upper end of reported values for unrelated monoclonal antibodies. Clearly, having experimentally obtained penetration values for these compounds would be ideal, but difficult to source for repurposed drugs where the lung has not been a studied as a site of action.

The binding model introduced here is complex, yet only captures a portion of the interactions involved in IL-6 signalling. Specifically, IL-6 binds to both sIL-6R (via the trans pathway in all cells) and membrane-bound IL-6R (mIL-6R; via the classic pathway on certain cells such as some immune cells). mIL-6R, and therefore classic signalling, are not included in the model. Similarly, sgp130 (an important IL-6 modulator in the trans pathway) was not included in the model. Signalling complex formation (IL-6:sIL-6R:gp130 and IL-6:mIL-6R:gp130) is not accounted for directly in the model.

Still, suppression of free IL-6 reduces IL-6:mIL-6R and IL-6:sIL-6R, and IL-6:sIL-6R is tracked in the model. While signalling complex formation is not tracked in the model (for classic or trans signalling), gp130 is constitutively expressed and is therefore assumed not to be a limiting factor in IL-6 signalling. Similarly, gp130 transmembrane protein binding is not included in the model. However, gp130 affinity for IL-6:sIL-6R is higher than IL-6 for sIL-6R, suggesting that the limiting step is the binding of IL-6 to sIL-6R.

Finally, a review of the literature revealed a range of binding constants reported for SIL:IL-6 and TCZ:sIL-6R (Table 2). The selection of Kd for use in the model was the median reported value (Table 2, Figure 2). The impact of the true Kds being lower or higher is not accounted for in this work.

5.1 | Considerations for place in therapy

A rapid, coordinated innate immune response is the initial line of defence against viral infections. Hyperinflammatory responses, however, can cause immunopathology. Low pathogenic coronaviruses typically infect the upper airways, while highly pathogenic coronaviruses infect the lower respiratory tract and can cause severe pneumonia, sometimes leading to acute lung injury and ARDS. Disease severity of the highly pathogenic coronaviruses SARS and MERS was influenced by factors such as initial viral titres in the airways, age and comorbid conditions.[20]

The clinical course of SARS progressed in 3 stages. Robust viral replication dominated the first phase, which lasted a few days. The second phase was associated with high fever, hypoxemia, and progression to pneumonia despite a decrease in viral load. The third phase is characterized by strong inflammatory response, in which ~20% of patients progressed to ARDS and often death.[20] MERS progresses more rapidly, and has a higher fatality rate than SARS. Common clinical manifestations of MERS resemble those of SARS-CoV-2 and include rapid progression to pneumonia.[20] Like SARS-CoV-2, the majority of MERS patients with shortness of breath progressed to severe pneumonia and required admission to the intensive care unit. Analyses of lungs from patients who died from SARS-CoV showed infection of both the airway and alveolar epithelial cells, vascular endothelial cells, macrophages, monocytes and lymphocytes. Neutrophils and macrophages extensively infiltrated cells.[20] The only tissue samples available for MERS is the analysis of lung tissue from 1 patient, which were consistent with what was seen in SARS.[20]

Virus-induced cytopathic effects and viral evasion of host immune responses are thought to play major roles in disease severity. This argues for antiviral therapy as early as possible in treatment, with adjunctive immunotherapy during the time when patients are at risk for ARDS and in early ARDS. This paradigm would be similar to therapeutic interventions aimed at MERS viral load reduction, which were somewhat beneficial when administered early (but not later in) MERS-CoV infection.[20]

IL-6 concentrations skyrocket at the onset of ARDS (i.e. WHO Score ≥ 5[11]). Immediate treatment with an antibody such as SIL upon hospital admission for patients with low oxygenation needs (i.e. 200 mmHg < PaO$_2$ to FIO$_2$ ≤ 300 mmHg with positive end-expiratory pressure or continuous positive airway pressure ≥5 cmH$_2$O, delivered invasively or noninvasively; corresponding to WHO Score of 4[11]) could drive the immune response out of hyperreactivity. SIL treatment in early ARDS (i.e. Days 1–3 following ARDS diagnosis) would be crucial to preventing the IL-6 onslaught that leads to lung damage.

5.2 | Limitations

As is the case in all novel pandemics, this approach is limited by a lack of time and a lack of information. The novel nature of the disease limits the information we have on the virus and the trajectory of disease progression. The severity of disease limits the time we have to research the disease and attack drug development in the typical paradigm. For these reasons, we are constrained to make many assumptions, some of which are highlighted below.

5.3 | Antibody penetration

Antibody penetration into the lung has been studied for anti-RSV antibodies. There, BALF:serum concentration ratios are reported in the range 0.1–0.2%. While it is tempting to make dose selection evaluations based on systemic exposure alone, solely relying on this information would greatly overestimate target binding in lung BALF. Binding equilibrium is related to concentrations of each species and binding constants, so understanding the concentration of each species at the site of action is crucial. Lower or higher BALF:serum concentration ratios would decrease or increase the effect of each drug. Particularly, TCZ would benefit from increased BALF exposure as the IL-6 levels are extremely increased in COVID-19 and non-COVID-19 ARDS patients. The benefit to SIL would be modest, but a lower BALF exposure would certainly hurt efficacy. The Supplemental Materials contains an R-script that would allow interested readers to study the effect of this change.

As we have only 2 *snapshots* of cytokine levels in non-COVID-19 *pre-ARDS* and *ARDS* subjects, the current simulations cannot accurately predict time-dependent antagonism of IL-6 and sIL-6R. As noted in several large studies of COVID-19 ARDS, peak IL-6 may be missed, as cytokine profiling is not a standard of COVID-19 treatment. The antibody concentration in the lungs is assumed to be the maximum (peak) observed in the serum after applying a BALF:serum ratio of 0.2%. The binding reactions were simulated out to steady state given the initial concentration of each species and their binding affinities. As such, these simulations provide *optimistic* predictions for IL-6 and sIL-6R antagonism. In reality, antibody concentrations would wane over time, and new Il-6 and sIL-6R would be created over time, reducing the antibody effectiveness.

5.4 | Binding and target kinetics

An important caveat of these simulations is to understand that they are made: (i) based on the assumption that the maximal drug concentration instantaneously arrives in the BALF at a predefined ratio; and (ii) equilibrium binding occurs instantaneously and total target concentrations do not change over time. We would expect that unbound TCZ or SIL would constantly cycle into the BALF allowing for more sIL-6R or IL-6, respectively, to be bound. However, TCZ or SIL concentrations would also be falling relative to the maximal concentrations used here. Moreover, these simulations do not account for the synthesis and turnover rates of sIL-6R and IL-6. If IL-6 is formed and turned over more rapidly, that would tilt the results towards TCZ's favour. Far more complex, dynamic calculations would be required to understand the push–pull of these effects on binding outcomes. Additionally, variability in TCZ and SIL pharmacokinetics should be considered in more complex simulations. That variability would be expressed in wider prediction intervals (Figure 3 whiskers) for cytokine concentrations.

5.5 | Translatability of non-COVID-19 ARDS to COVID-19 ARDS

We are unaware of specific BALF data from COVID-19 subjects and are relying on the translatability of non-COVID-19 ARDS cases to the COVID-19 context. Clearly, more specific data from this patient type would be of greater utility and specificity. Moreover, the BALF concentration pattern changes over time, suggesting longitudinal data would provide insight on the timing of treatment. We posit that reducing the serum concentration of IL-6 to normal or near-normal levels before severe lung damage occurs will have a similar effect in COVID-19 ARDS patients as it does in non-COVID-19 ARDS patients.

The hypothesis for the use of combination therapy to modulate the IL-6 pathway comes from work on cytokine balance in the lungs of patients with non-COVID-19 ARDS.[9] To date, clinical studies have not assessed the relative IL-6 and IL-6R concentrations in COVID-19 pneumonia (in serum or BALF), nor correlated a reduction in IL-6 with positive clinical outcome (reduced mortality). Our work provides a pharmacological rationale for the use of the combination IL-6 pathway blockade. We hope that this work will lead to consideration of IL-6-lowering strategies, including the consideration of IL-6 and IL-6R separately.

6 | CONCLUSIONS

Drug repurposing provides an avenue for prioritizing compounds and moving them quickly into clinic. Modelling and simulation can aid in the selection of drugs and drug combinations to take to clinic. In the absence of clinical drug combination studies, in vitro binding studies and simulations based on those scenarios can be a starting point for suggesting rational combinations and prioritizing clinical combination studies.

6.1 | Clinical relevance of the TCZ/SIL interaction

Use of agents to simultaneously bind IL-6 and the IL-6R represent a 2-pronged approach in decreasing IL-6 signalling. Decreasing signalling through both mechanisms might be necessary to achieve a clinical response (i.e. quickly or sustainably drop IL-6 concentrations), as clinically achievable concentrations of either drug alone may not be sufficient to provide adequate down-regulation of IL-6. The question of whether high serum cytokine levels is a cause of or an effect of COVID-19 ARDS still exists. However, evidence that IL-6 drives immune dysregulation and respiratory failure in COVID-cytokine storm syndrome is rapidly accumulating.

Dose-ranging for the optimal combination of SIL and TCZ would need to be run in initial clinical trials. The initial suggestion would be to use both drugs according to their approved posology. However, there is no information on the use of these 2 drugs together. Ultimately, the lowest efficacious doses of each should be sought.

6.2 | Implications of variable IL-6 levels in the target patient population

IL-6 concentrations in COVID-19 patients experiencing severe disease, including ARDS, are variable. Modest serum IL-6 elevations (IL-6 7–45 pg/mL) were reported in early COVID-19 studies.[21,22] In some studies, 1000-fold increases in IL-6 above normal have been reported.[18] It is unclear how targeting normal (<7 pg/mL) serum IL-6 levels for patients with such large variation in serum IL-6 concentration will be beneficial. It is unclear whether IL-6 represents a biomarker or a central pathogenetic element of severe COVID-19 that should be used as a parameter for therapeutic intervention. It is also unclear if the SIL or TCZ dose will need to be adjusted based on serum IL-6 concentration. It is suggested that initial dosing follow approved labelling, and that a dose-ranging study is planned in early clinical development of the combination for COVID-19 ARDS.

6.3 | Timing and duration of treatment relative to the onset of clinical symptoms

Because this simulation does not address the time-dependent aspect of IL-6 pathway blockade, the results cannot be used to justify timing of treatment relative to the onset of clinical symptoms. However, clinical data describing the increase in circulating IL-6, coupled with the differences in magnitude of IL-6 increase in patients with ARDS and those that have not yet developed ARDS suggests early treatment with SIL, bolstered by treatment with TCZ, could be beneficial.

Care should be taken to not over-interpret these simulations to suggest that SIL should be preferred over TCZ. To the contrary, these simulations suggest that the combined effect of these drugs on reducing IL6:sIL6R complex, which is responsible for signalling via the *trans* pathway is better than the monotherapy results. In the context of so much uncertainty around target concentrations, target dynamics and relative importance of each target's contributions, combination therapy should be considered. Finally, it should be noted that anti-IL-6 therapy, anti-IL-6R therapy and combination anti-IL-6:IL-6R therapy could have differing efficacies. The nuances of IL-6 signalling must be taken into account when discussing antibodies that target the IL-6 signalling pathway, and we must not treat IL-6 and IL-6R targets or blockers interchangeably.

COMPETING INTERESTS
All authors are employees of Certara, Inc.

AUTHOR CONTRIBUTION
Eileen Doyle and Darren Bentley contributed to the clinical and clinical pharmacology aspects of this article. Michael G. Dodds developed

and executed the pharmacometric model. All authors contributed to writing and reviewing the manuscript. All authors give final approval of the version to be published.

DATA AVAILABILITY STATEMENT

Data for model development to support the findings of this study were derived from public domain resources:

Data	Reference	
TCZ:sIL-6R Kd	**PMID** 22101760	**DOI** 10.1007/ s10928-011-9227-z
SIL:IL-6 Kd	**PMID** 15239138	**DOI** 10.1002/ijc.20270
TCZ Cmax	**PMID** 29622697	**DOI** 10.1634/theoncologist. 2018-0028
SIL Cmax	Sylvant (SILTUXIMAB)	BLA #125496 drugs@FDA

OPEN RESEARCH BADGES

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at [http://qed.econ.queensu.ca/bcp/datasets/dufays001/].

ORCID

Eileen B. Doyle https://orcid.org/0000-0002-6963-8796
Darren Bentley https://orcid.org/0000-0003-2136-9264
Michael G. Dodds https://orcid.org/0000-0002-0135-7840

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.