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ORIGINAL ARTICLE

Quantitative assessment of minimal effective concentration of erythropoiesis-stimulating agents

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Minimal effective concentration (MEC) was proposed to explain why subcutaneous (SC) administration of erythropoietin (EPO) induces a higher hemoglobin (HGB) increase than intravenous (IV) administration. It has been further used to explain the paradox that erythropoiesis-stimulating agent (ESA) with lower receptor binding affinity may have higher *in vivo* activity. We have developed a pharmacokinetic and pharmacodynamic (PK/PD) model with incorporation of the operational model of agonism to characterize the data from two clinical trials. By using model-based simulations, we demonstrate that SC route is more efficacious than IV route and explain the paradoxical behavior of ESAs. We determined that MEC can be quantified by C_{50}, which represents the concentration of an ESA producing its half-maximal effect of stimulating the proliferation of erythroid precursor cells. The model used may allow joint PK/PD modeling of data from different ESAs, and provide a platform for dosing regimen optimizations and future clinical study designs.
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Erythropoietin (EPO) is a 30.4 kDa, glycoprotein hormone endogenously produced by adult kidney.[1] It increases the red blood cell production by binding to the EPO receptor (EPOR) on the surface of erythroid precursor cells in bone marrow and stimulating their survival, proliferation, and differentiation.[2] The dissociation constant (K_D) of EPO for its receptor in humans is about 0.1 nmol/l, corresponding roughly to 400 mIU/ml.[3,4] In humans, the normal concentration of circulating EPO is between 5 and 30 mIU/ml and is sufficient to maintain the hemoglobin (HGB) concentration within a normal range in healthy subjects.[5] In 1989, the first erythropoiesis-stimulating agent (ESA), recombinant human erythropoietin (rHuEPO, epoetin alfa), received the approval of the Food and Drug Administration for treating anemia associated with chronic renal failure.

The minimal effective concentration (MEC) of rHuEPO was first proposed by Dr. Besarab *et al.* in 1992.[6] They observed that to maintain the same level of hematocrit, the dose of rHuEPO required for subcutaneous (SC) route was half of that required for intravenous (IV) route. However, the peak drug concentration after IV route was more than ten times higher than that after SC route. Based on these observations, it was concluded that the effect of rHuEPO was not dependent on the peak concentration but on the duration the drug concentrations were maintained above a "critical concentration".[6] The "critical concentration" was later renamed as "minimal effective concentration" and utilized to explain the difference of pharmacokinetic and pharmacodynamic (PK/PD) behavior between epoetin and darbepoetin.[7,8] Darbepoetin is a hyper-glycosylation analogue of epoetin.[9] It was created by attaching two extra N-linked carbohydrate side chains to epoetin. Compared with epoetin, darbepoetin has a lower clearance and a longer half-life, which allows less frequent dosing.[9] Further studies demonstrated that the glycosylation reduced both linear and nonlinear clearances of EPO analogue, resulting in an increased half-life.[10] A paradox resulting from EPO analogues developed through glycosylation is that analogues with lower receptor binding affinity may have higher *in vivo* activity.[8] For example, the receptor binding affinity of darbepoetin is 4.3-times lower than that of epoetin, yet it has a higher *in vivo* activity.[9] Another example is the continuous erythropoietin receptor activator (CERA), which was developed by incorporating a 30 kDa methoxy-polyethylene glycol polymer chain to rHuEPO.[11] The receptor binding affinity of CERA is 50–100 times lower than that of epoetin and it has a half-life which is even longer than darbepoetin.[11,12] Given the hypothesis of MEC, it has been argued that due to the longer half-life, the duration of concentration of EPO glycosylation analogue maintained above MEC is increased, leading to an increased *in vivo* activity.[8] Kiss *et al.* extended the MEC concept by suggesting that an ESA with lower receptor binding affinity should have a higher MEC to ensure sufficient receptor binding.[13] For such an ESA, the higher concentration and its prolonged duration above MEC will eventually compensate for the counteracting effect of the lower receptor binding affinity, thereby increasing its *in vivo* activity.[13] They further postulated that if the receptor binding affinity of a given ESA is too low and its MEC is too high, the beneficial effect due to the smaller clearance and longer half-life will be limited.[13]

The MEC hypothesis provides a reasonable explanation for the different efficacy between IV and SC routes for rHuEPO as well as the paradox. However, the exact value of MEC remains undefined. A large amount of clinical trials have been conducted to optimize rHuEPO dosing regimen due to the lack of this information.[7] A quantitative assessment of MEC may be of importance for the selection of optimal dosing regimen for various ESAs. The main aim of this report is to quantitatively decipher MEC by using a PK/PD modeling and simulation approach. We first demonstrated

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the MEC phenomenon through clinical data involving multiple IV and SC dosing regimens. Then, a previously developed PK/PD model based on these data was adopted and expanded to explain why the SC route induces a higher HGB response than the IV route. Afterwards, a series of model-based simulations were conducted to provide further insight for MEC and illustrate the paradox that ESA with lower receptor binding affinity may exhibit higher *in vivo* activity. Our strategy is to find a metrics in the concentration-effect relationship of ESAs that has the similar property as MEC. Given the influence of receptor binding affinity on MEC, we incorporated the formalism of the operational model of agonism in the PK/PD modeling and simulation of ESAs, to dissect the influence of receptor binding affinity on the drug effect, and unravel the intrinsic efficacy of ESAs from clinical data.

RESULTS

SC route of epoetin induces a higher HGB response than IV route

Figure 1a shows the comparison of HGB response and PK in healthy volunteers receiving thrice-weekly (TIW) IV or SC administration of epoetin for 4 weeks. It can be seen that HGB increased faster after the SC administration. Calculation of the area under the HGB vs. time curve (AUEC) indicates that the AUEC after the SC administrations is significantly higher than that after IV administrations. A comparison of the pharmacokinetic profiles after 11th IV and SC administrations shows that the peak concentration for IV route is over 15 times higher than the peak concentration for the SC route (**Figure 1b**). The PK profile after the SC administration exhibited flip-flop kinetics due to the prolonged absorption of drug. From 16 h post-injection, the drug concentrations after the SC route started to be higher than that after the IV route. These results are consistent with previous observations by Besarab *et al.* and suggest that the erythropoietic response is not dependent on the peak epoetin concentration but on the duration of drug concentrations above the "critical concentration."[6]

The duration of epoetin levels that are maintained above C_{50} for the SC route is longer than that for the IV route

The PK/PD model in **Figure 2a** is capable of capturing the pharmacokinetics and time courses of HGB responses shown in **Figure 1**, based on the diagnostic plots from the previous publication.[14] To illustrate how this model explains the MEC phenomenon demonstrated in **Figure 1**, we introduced the C_{50} of ESAs as shown in equation 13, which is defined as the concentration of EPOR agonist that produces the half-maximal effect of stimulating the proliferation of erythroid precursor cells (see "Methods" section). **Figure 2c** shows the model-based simulation for PK profiles and the time courses of drug–receptor complex for IV and SC dosing regimens. Consistent with the relative HGB responses induced by IV and SC dosing regimens (**Figure 2b**), the duration of rHuEPO concentrations that are maintained above C_{50} for SC administrations is longer than that for IV administrations (**Figure 2ci–ii**). However, the peak concentration for IV administrations is over 15-fold higher than that for SC

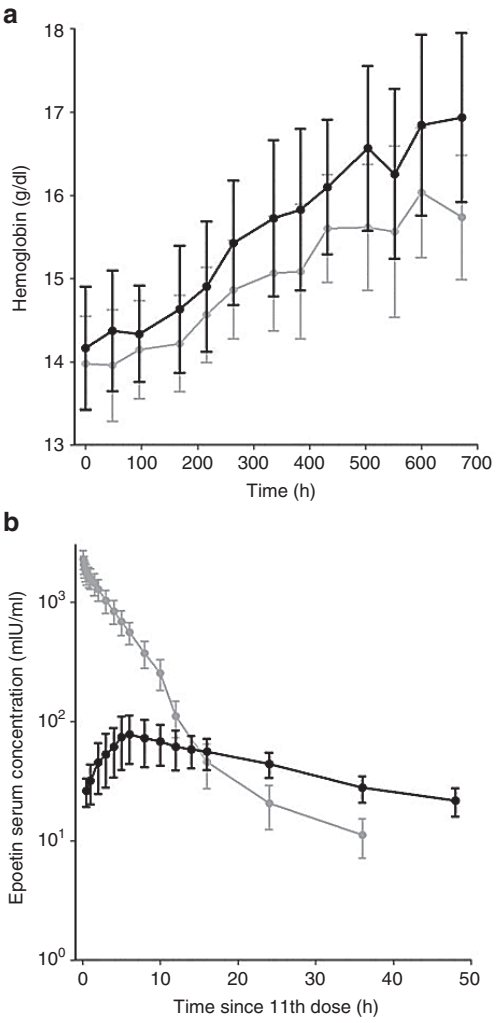

Figure 1 The HGB response after SC administration of epoetin is greater than that after IV administration. (**a**) Mean HGB vs. time profiles after multiple IV (grey) and SC (black) doses with standard deviation (SD) error bars. Statistical analysis (one tailed, *t*-test, n = 38 for IV group, n = 37 for SC group) showed that the area under the HGB curve (AUEC) after IV administration is smaller than that after SC administration ($P < 0.0001$). (**b**) Mean concentration vs. time profiles after the 11th IV (grey) and SC (black) doses with SD error bars. Mean AUC_{0-36h} after the 11th IV dose is 9371.3 mIU/ml·h with SD = 1811.2 mIU/ml·h. Mean AUC_{0-36h} after the 11th SC dose is 1801.9 mIU/ml·h with SD = 451.4 mIU/ml·h. AUC and AUEC were calculated using the NCA module of Phoenix WinNonlin 6.0 (Pharsight Corporation, Cary, NC).

administrations. Similar to the observation for drug concentration, the duration of drug–receptor complex concentrations above SRC_{50}, is also longer for the SC route, even though the peak concentration of drug–receptor complex for IV administrations is around seven-times higher than that for SC administrations (**Figure 2ciii–iv**). SRC_{50} is the concentration of drug–receptor complex that produces the half-maximal effect (see "Methods" section).

C_{50} is dosing regimen dependent

The simulated C_{50} vs. time profiles for IV and SC routes are shown in **Figure 3a**. The C_{50} decreased from

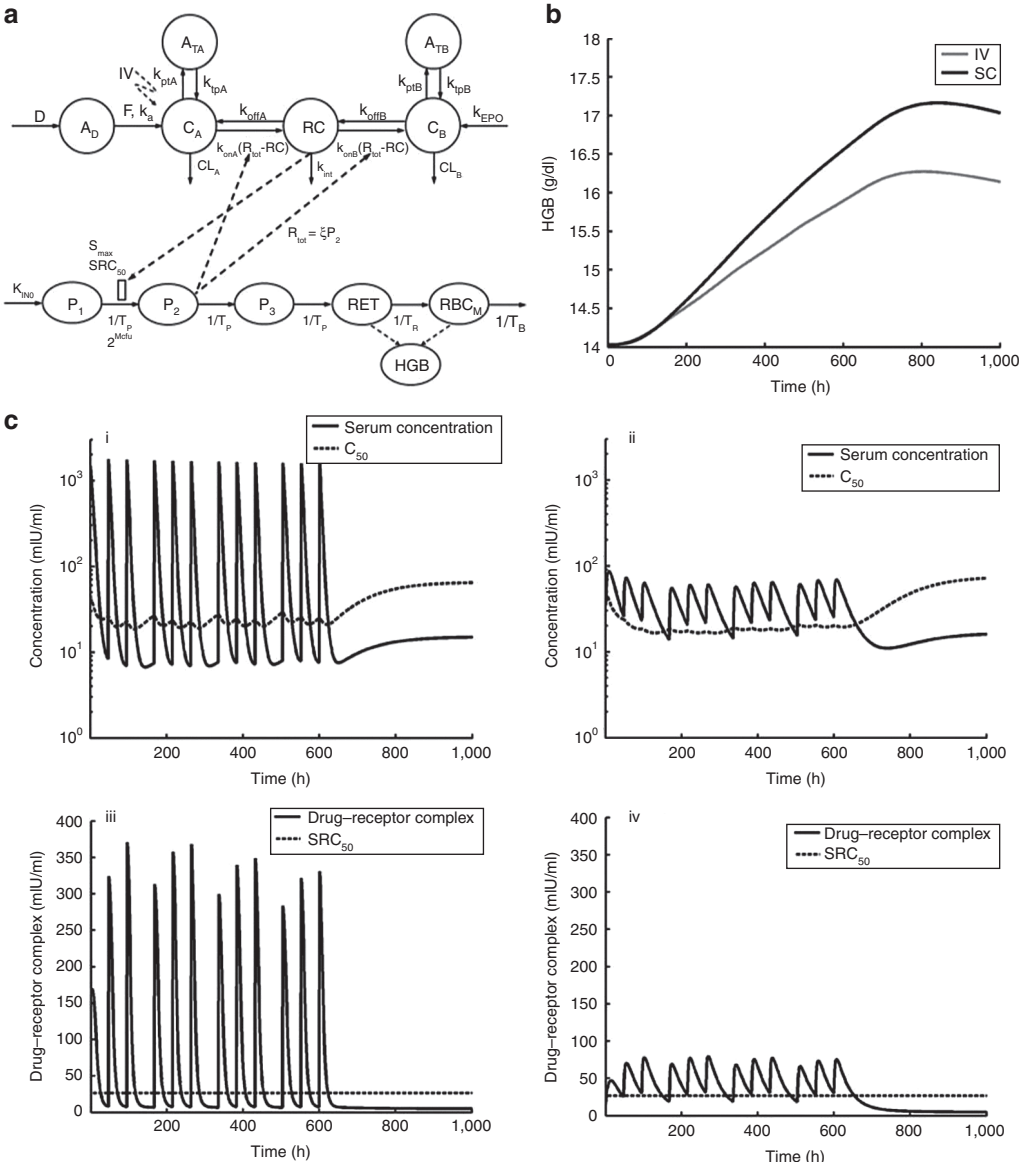

Figure 2 Model-based simulation shows that duration of epoetin concentration maintained above C_{50} for the SC route is longer than that for the IV route. (**a**) PK/PD model for ESAs with incorporation of the competitive interaction between the endogenous and exogenous EPO. Model compartments are defined as follows: C_A and C_B, free exogenous and endogenous EPO; RC, drug–receptor complex; A_D, absorption compartment for the SC route; A_{TA} and A_{TB}, tissue compartments; P_1, P_2 and P_3, erythroid precursor cell compartments; RET, reticulocytes; RBC_M, mature red blood cell; HGB, hemoglobin. Symbols for parameters are defined as follows: D, duration of the zero-order input for the SC route; F, bioavailability; k_a, first-order absorption rate constant; k_{onA} and k_{onB}, second-order rate constants for forming the EPO-EPOR complex; k_{offA} and k_{offB} first-order dissociation rate constants; k_{int}, first-order internalization and degradation rate constant; CL_A and CL_B first-order elimination processes; k_{tpA}, k_{tpB}, k_{ptA} and k_{ptB}, tissue distribution rate constants; K_{EPO}, production process for endogenous EPO; K_{IN0}, production for P_1 cells; T_P, T_R, T_B, mean residence times for precursor compartments, RET and RBC_M; S_{max}, the maximal effect of EPO-EPOR complex on the proliferation of precursor cells; SRC_{50}, concentration of EPO-EPOR complex inducing half of S_{max}; ξ, factor of proportionality. (**b**) Simulated HGB vs. time profiles for IV and SC dosing regimens. (**c**) Panels i–ii show simulated rHuEPO concentration vs. time profiles overlaid with the C_{50} for thrice-weekly IV (i) and SC (ii) dosing regimens. Panels iii-iv show simulated drug–receptor complex vs. time profile for IV (iii) and SC (iv) dosing regimens overlaid with SRC_{50}.

60 to 20 mIU/ml after rHuEPO administration and oscillated during the dosing period. For most part of the profiles, the C_{50} for the SC route is smaller than that after the IV route, which is due to its relative bigger $\tau_{in\ vivo}$ (**Figure 3b**). $\tau_{in\ vivo}$ is known as the efficacy parameter in the operational model of agonism (see "Methods" section) and this parameter is estimated based on the clinical data. The efficacy parameter started to increase from 7.0 and peaked at about 20 for the IV route and about 24 for the SC route after the third dose (**Figure 3b**). Afterwards, a slow and oscillatory decline was observed. For most of the dosing period, the $\tau_{in\ vivo}$ for the SC route is higher than that for the IV route. The dynamic change of $\tau_{in\ vivo}$ is due to the change of total receptor concentration (R_{tot}), which is dependent on the amount of precursor cells

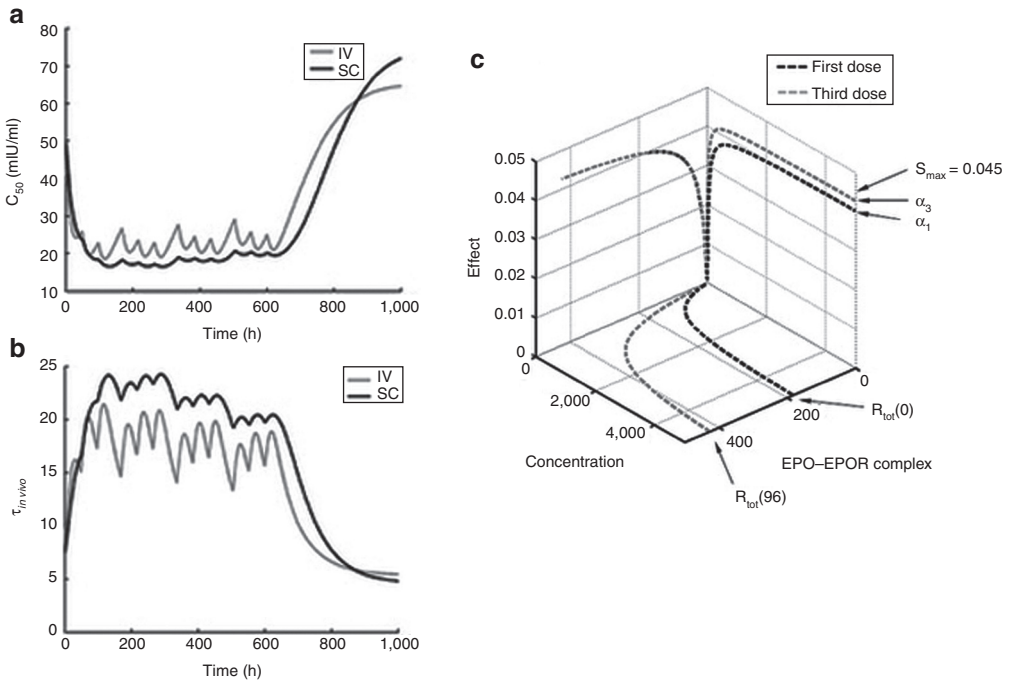

Figure 3 C_{50} is dosing regimen dependent. (**a**) C_{50} vs. time profiles after thrice-weekly IV and SC dosing regimens. (**b**) $\tau_{in\ vivo}$ vs. time profiles after thrice-weekly IV and SC dosing regimens for 4 weeks. (**c**) A schematic diagram shows three relationships in the operational model of agonism including: relationships between rHuEPO concentration and effect, between rHuEPO concentration and drug–receptor complex, and between drug–receptor complex concentration and effect, at the predose time point of the first and third IV dose. α_1 and α_3 represent the α value at the predose time point of these two doses.

(P_2) as shown in equation 6. Because the precursor compartment (P_2) is a response compartment and influenced by dosing regimens, C_{50} is dosing regimen dependent. A "stronger" dosing regimen will lead to a larger expansion of the P_2 cells and lower C_{50} value, creating a positive feedback mechanism. The existence of the positive feedback loop has been supported by experimental results.[27–29] Accordingly, the change of the total receptor concentration dynamically changes the concentration-effect relationship of rHuEPO. **Figure 3c** shows the schematic diagram of various relationships in the operational model of agonism at the predose time point of the first and third IV dose. Compared with the baseline condition (prior to the first dose), at the beginning of the third dose, total receptor increased over twofold. The increase of the total receptor resulted in an increase of the drug efficacy, which is reflected by the change of the concentration-effect relationship as shown in the right-hand side of **Figure 3c**.

The difference of *in vitro* activity between epoetin and darbepoetin is due to their different receptor binding affinity (K_D)

In the framework of the operational model of agonism, different ESAs may have different values for the efficacy parameter (τ), which contributes to their different stimulatory effect on the erythroid precursor cells. To evaluate this hypothesis, the operational model of agonism was fitted to the *in vitro* data produced from colony forming cell assays for epoetin and darbepoetin.[8] The parameter estimates are listed in **Table 1**. Statistical analysis indicates that there is no significant difference for all the estimated parameters between epoetin and darbepoetin. The model fittings are shown in **Figure 4a**. **Figure 4b** shows the simulated stimulus-effect described by equation 15, overlaid with observations. The proximity between two curves in **Figure 4b** suggests that the stimulus-effect relationship is same for epoetin and darbepoetin, implying that these two drugs induce the same conformational change of EPOR after binding. Therefore, the difference in the *in vitro* activity between these two drugs is due to their different receptor binding affinity.

Compared with epoetin, darbepoetin has a higher C_{50}, a longer duration that the drug concentrations are maintained above C_{50} and a higher *in vivo* activity

Model-based simulations were employed to demonstrate and explain the paradoxical behavior that ESAs with lower receptor binding affinity may have higher *in vivo* activity. Epoetin and darbepoetin were used as examples of ESAs. The parameter values are listed in **Supplementary Table S1** online. SRC_{50} was assumed to be same for epoetin and darbepoetin based on the *in vitro* data analysis. The model in **Figure 2a** also assumes that the receptor-mediated internalization and degradation is not affected by different species of ESAs, which is supported by published experimental results.[15] **Figure 5** shows the simulated temporal profiles of free drug concentration, drug–receptor complex concentration and HGB response for TIW IV dosing regimens for 4 weeks with equal amount of epoetin and darbepoetin. Darbepoetin exhibits a longer half-life compared with epoetin and the C_{50} of darbepoetin is higher than that of epoetin (**Figure 5a**). The duration

of drug concentrations above C_{50} is longer for darbepoetin, that is, $T_2 > T_1$. The peak concentration of drug–receptor complex for epoetin is higher than that for darbepoetin, due to the stronger receptor binding affinity of epoetin (**Figure 5b**). However, the drug–receptor complex of epoetin also declines faster because of the faster clearance of the drug, which is demonstrated in its PK profile in **Figure 5a**. Consequently, the duration of drug–receptor complex above SRC_{50} is longer for darbepoetin. Accordingly, darbepoetin has a higher *in vivo* activity than epoetin, demonstrated by the HGB response (**Figure 5c**).

Table 1 Parameter estimates for the operational model of agonism fitted to *in vitro* data

Parameter	Estimate	Standard error	CV%	95% CI
E_{max}	56.7	3.23	5.69	49.7, 63.4
$\tau_{in\ vitro\_EPO}$	6.63	1.30	19.6	3.84, 9.42
$\tau_{in\ vitro\_DA}$	5.32	1.06	19.9	3.05, 7.60
K_{D_EPO} (ng/ml)	3.04[a]	N/A	N/A	N/A
K_{D_DA} (ng/ml)	13.072[a]	N/A	N/A	N/A

CI, confidence interval; DA, darbepoetin; EPO, epoetin; N/A, not available.
[a]Fixed parameter based on publication by Egrie and Browne.[9]

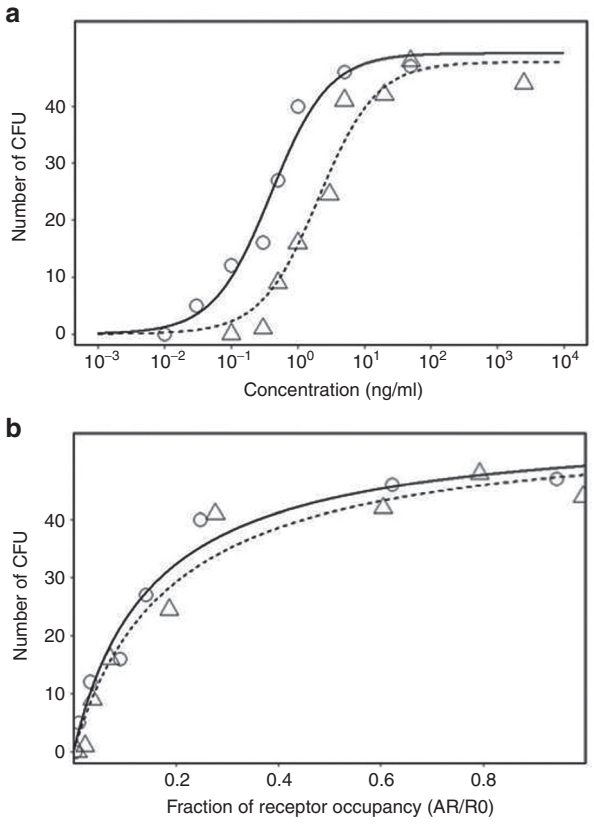

Figure 4 *In vitro* data fitted by the operational model of agonism. (**a**) Open circles and triangles represent data for epoetin and darbepoetin. Solid and dashed lines represent model fittings. (**b**) Open circles and triangles represent observations for epoetin and darbepoetin. Observations for the fractional receptor occupancy were calculated based on the receptor binding affinity for epoetin and darbepoetin listed in **Table 1**. Solid and dashed lines represent model predictions for epoetin and darbepoetin.

The effect of receptor binding affinity (K_{DA}) and linear clearance (CL_A) of ESAs on the HGB response

The development of ESAs usually involves modifying the molecule of rHuEPO through glycosylation or PEGylation.[16,19] These ESAs exhibit higher *in vivo* activity than rHuEPO, due to the smaller clearance and longer half-life. However,

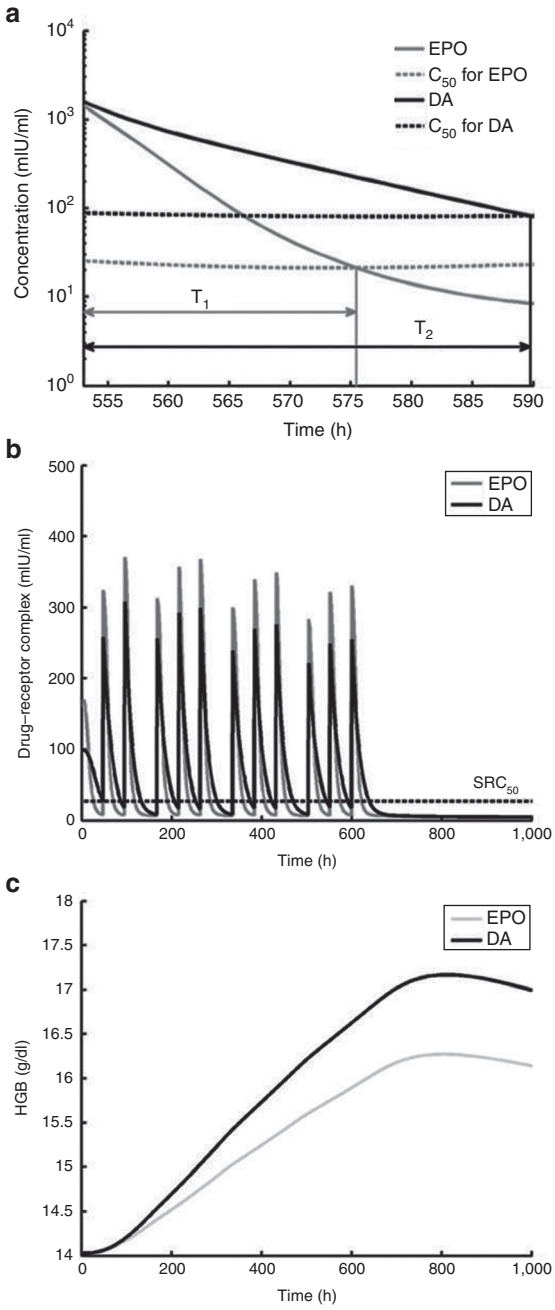

Figure 5 Compared with epoetin, darbepoetin has a higher C_{50}, a longer duration that the drug concentrations are maintained above C_{50} and a higher *in vivo* activity. (**a**) Simulated serum concentration vs. time profiles for epoetin (EPO) and darbepoetin (DA) in thrice-weekly IV dosing regimens for 4 weeks. Their profiles after the 11th IV dose were shown. T_1 and T_2 represent the duration of epoetin and darbepoetin levels above their C_{50}. (**b**) Simulated drug–receptor complex vs. time profiles for epoetin and darbepoetin. (**c**) HGB response for epoetin and darbepoetin.

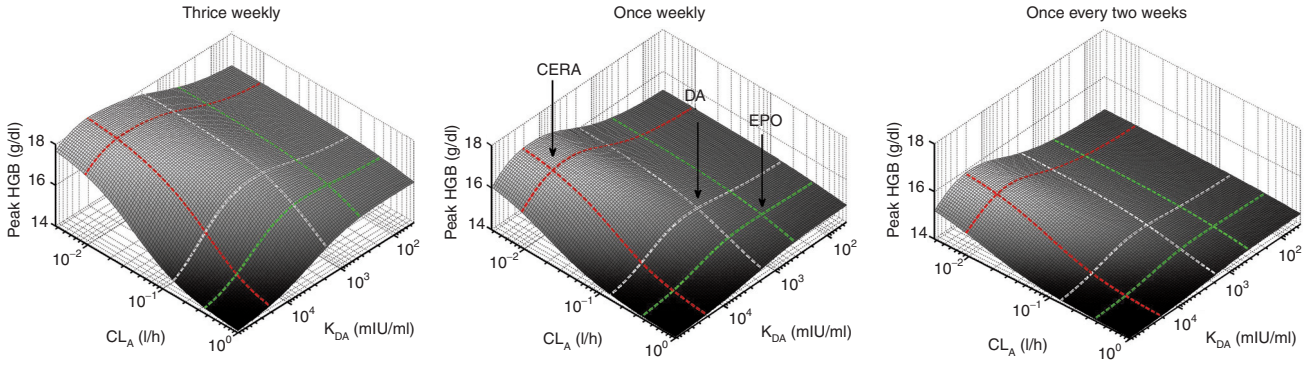

Figure 6 The effect of receptor binding affinity (K_{DA}) and linear clearance (CL_A) of ESAs on the HGB response. Simulated peak HGB values with varying CL_A (from 3.73×10^{-3} to 1 h/l) and K_{DA} values (from 41.8 to 83,600 mIU/ml) after IV administrations of ESAs for thrice-weekly, once-weekly and once every 2 weeks dosing regimens for 4 weeks. The linear CL_A and K_{DA} value for epoetin (green), darbepoetin (white) and CERA (red) were highlighted to find their expected peak HGB response on these surfaces.

these molecules may exhibit lower receptor binding affinity, which decreases the *in vivo* activity and limit the benefit of longer half-life.[13] The following simulations were conducted to comprehensively evaluate the influence of the receptor binding affinity and linear clearance of ESA molecules on the *in vivo* activity. Peak HGB concentration after multiple IV administrations was used as a marker. A 3D surface was generated by simulation to illustrate the effect of different combinations of K_{DA} (from 41.8 to 83,600 mIU/ml) and CL_A (from 3.73×10^{-3} to 1 h/l) on the peak HGB concentrations (**Figure 6**). TIW, once-weekly, and once every 2 weeks (Q2W) dosing regimens for 4 weeks were used. These dosing frequencies were selected to mimic the manufacturer suggested dosing regimens for epoetin, darbepoetin, and CERA.[17–19] For CERA, it has been recommended that Q2W is used for initial period of treatment, and afterwards Q2W or Q4W is employed in maintaining the target HGB level.[19] Therefore, Q2W was used in simulation, since the model was developed to capture the PK/PD profiles during initial period of rHuEPO treatment.[14] The dose level of 100 IU/kg corresponds to 0.5 μg/kg for darbepoetin and CERA, which is also very close to the recommended dose for darbepoetin (0.45 μg/kg) and CERA (0.4 μg/kg).[18–20]

The expected HGB responses of these ESA molecules on the surface were located based on their receptor binding affinity and linear clearance. For all the three dosing regimens, **Figure 6** shows the *in vivo* activities are higher for ESAa with CL and K_D similar to CERA, than darbepoetin, and finally epoetin. For highlighted lines which are parallel to the axis for K_{DA}, "bell" shaped profiles for HGB peak concentrations can be observed when the receptor binding affinity decreases (K_{DA} value increases). This "bell" shape behavior of the peak HGB concentration demonstrates that if the receptor binding affinity is too low, the benefit of longer half-life due to smaller clearance might be limited and the *in vivo* activity can decrease.

DISCUSSION

The MEC was proposed to explain why the SC route of rHuEPO was more effective than the IV route. Based on the MEC, the underlying reason is that the duration of rHuEPO concentrations above MEC for the SC route is longer than that for the IV route. **Figures 1–2** demonstrate this phenomenon and further show that the duration of rHuEPO level above C_{50} for the SC route is longer than that for the IV route. According to the operational model of agonism, $\tau_{in\ vivo}$ serves as the marker of drug efficacy. The greater $\tau_{in\ vivo}$ associated with the SC route indicates that the SC administration is more efficacious (**Figure 3b**). It should be noted that it is the higher total receptor induced by the SC administration that results in the greater $\tau_{in\ vivo}$. MEC has also been used to explain the paradox that ESAs with lower receptor binding affinity may have higher *in vivo* activity.[7,8,13] By using epoetin and darbepoetin as examples, we demonstrate that the duration of darbepoetin level above its C_{50} is longer than that of epoetin, and consequently darbepoetin induces a greater HGB response (**Figure 5**). A higher C_{50} for darbepoetin than for epoetin agrees with the property of MEC that ESAs with lower receptor binding affinity have higher MEC.[13] In **Figure 6**, we further show that if the receptor binding affinity is too low and C_{50} is too high, the response of such an ESA can decrease. This finding is also consistent with the postulation from Kiss *et al.*[13] Consequently, we suggest that the MEC of an ESA can be quantified by its C_{50}.

It is worth mentioning that the $\tau_{in\ vitro}$ value (**Table 1**), which is the efficacy parameter estimated based on *in vitro* data (see Methods section), is similar to the $\tau_{in\ vivo}$ at $t = 0$ (**Figure 3b**) estimated from *in vivo* data, indicating that there is an agreement between the *in vivo* and *in vitro* efficacy of the drug. Consequently, C_{50} = 54.8 (mIU/ml) for rHuEPO (K_D = 418 mIU/ml) estimated from *in vitro* data also agrees with *in vivo* C_{50} at $t = 0$ (**Figure 3a**). These observations suggest that the efficacy (or receptor binding affinity) parameter estimated from *in vitro* data could be used to predict the *in vivo* PD of ESAs. The correlation between *in vitro* and *in vivo* estimates of operational model parameters is not unexpected and has been previously observed for adenosine A_1 receptors agonists.[20] It should be noted that ideally, to make the above conclusion, the analysis should involve the *in vitro* and *in vivo* data from a series of EPOR agonists, including both partial and full agonists, with a range of receptor binding affinity and efficacy. Then, the efficacy parameter of partial agonists can be obtained by comparison with the full agonists, in the so called "comparative method."[21] However, such data for ESAs is not available.

This also contributes to fixing the receptor binding affinities of rHuEPO and darbepoetin, whereas the knowledge of receptor binding affinities of these two ESAs is well documented in the literature.[9] Nevertheless, the estimates of *in vivo* and *in vitro* efficacy for rHuEPO and darbepoetin are justifiable. The large τ values also suggest that both drugs are full EPOR agonists.

The MEC actually exists for many drugs and has been previously illustrated through the indirect response model.[22] The origin of MEC for EPO can be traced back to the 1960s when it was found that a fixed amount of EPO when divided and administered via several small fractions will have a greater effect than a single dose.[23,24] This phenomenon that divided doses have a greater therapeutic effect than a single large dose, is a characteristic for drugs that act through the indirect response mechanism.[23,25] It has been demonstrated that targeting drug concentration above IC_{50} or SC_{50} will induce an optimal effect per unit of dose, where IC_{50} and SC_{50} is the concentration inducing half-maximal effect in the indirect response model.[22] The operational model of agonism separates the receptor binding and transduction process. Incorporation of the operational model into the PK/PD modeling of ESAs allowed us to dissect the influence of receptor binding affinity on the drug effect, and to quantify the intrinsic efficacy of the erythropoietic system from *in vivo* data. Equation 13 for C_{50} consists of these components and reflects their influence on the MEC.

The hyperbolic relationship between stimulus (drug–receptor complex) and effect is present for many receptor agonists.[26] This illustrates a general phenomenon called "receptor reserve," that the maximal effect can be achieved with the submaximal receptor occupancy.[26] For EPO, it has been suggested only 20–30% receptor occupancy is required to stimulate erythropoiesis.[7] *In vitro* data analysis suggested only 6.8% of receptors were occupied to achieve the half-maximal effect.[27] The model-based simulation also suggested that the receptor occupancy for half-maximal effect was between 3 and 12%.[18] Consistently, the C_{50} for IV and SC administrations of rHuEPO is between 20 and 30 mIU/ml (**Figure 3a**). All of these pieces of evidence suggest that high concentration is not required to stimulate erythropoiesis. It should be pointed out that the C_{50} of rHuEPO in this report is for healthy volunteers. The C_{50} of rHuEPO for anemic patients is expected to be higher. Due to their bone marrow malfunction, anemic patients will have a smaller R_{tot}, resulting in a smaller $\tau_{in\ vivo}$ value, and consequently a higher C_{50} compared with healthy volunteers based on equation 13.

A tremendous effort has been devoted to optimize dosing regimens for epoetin in various patient populations through clinical trials.[7] The observation of MEC phenomenon has served as one of the rationales for these efforts.[7] However, because MEC is not the quantitatively defined, many clinical trials have been repeated after darbepoetin and CERA were developed.[7,28] By using a PK/PD modeling and simulation approach, we have demonstrated the MEC phenomenon and provided a quantitative definition for MEC. Our findings suggest that the observation of MEC for ESAs is due to the nonlinear binding between ESA and EPOR, and the nonlinear stimulus-effect relationship. We further show that MEC can be affected by the receptor binding affinity of ESAs and dosing regimens. This may contribute to the different outcomes of many clinical trials that have been conducted for various ESAs.[7,28] Furthermore, the results in this report indicate that the model we proposed may allow the joint PK/PD modeling of data from different ESAs. A model-based meta-analysis may be of great help to design clinical trials for new ESAs as well as to find the desired dosing regimen for existing ESAs. A potential application of our model emerges in designing dosing regimens for clinical trials addressing personalized treatment with ESAs of chronic renal failure and cancer patients. Reported adverse effects to ESA therapy can be linked to excessively high doses. Administration of large doses of EPO contributes to rapid changes in HGB as well as HGB oscillations which have been identified as potential factors in cardiac toxicity.[29] It has been hypothesized that at very high EPO concentrations EPORs expressed at low levels on non-erythroid cells (e.g., tumor or endothelial) can be activated and increase cell survival.[30] These examples implicate a shift to personalized ESA administration with more frequent, but lesser doses. According to the MEC hypothesis, such regimens will be equally effective and avoid unnecessary patient exposure to high levels of ESA.

METHODS

Data source. The mean pharmacokinetic profile and HGB response were obtained from two clinical trials, both of which were open, randomized, parallel group studies.[31,32] Two groups of 40 healthy volunteers received TIW IV or SC administrations of 100 IU/kg epoetin for 4 weeks. The demographic characteristics and complete PK/PD data have been published elsewhere.[31,32]

Model structure. The model structure for PK/PD simulation is presented in **Figure 2a**. This model stems from a previously published model developed based on the PK/PD data for epoetin and model fitting was performed using NONMEM.[14] Different from the previous model, our current model separates the endogenous and exogenous EPO, which competitively bind to the EPOR. This modification enables us to simulate PK/PD profiles of ESA having different clearance and receptor binding affinity from epoetin. If the exogenous EPO (e.g., epoetin) shares the same clearance and receptor binding affinity with endogenous EPO, this model can be mathematically reduced to the previous model. The target-mediated drug disposition (TMDD) model was applied to describe the disposition of both endogenous and exogenous EPO.[33]

The rapid binding approximation of the TMDD model for two drugs competing for the same receptor was used for simulations.[34] For the rapid binding approximation, the microconstants for the receptor binding process were replaced by the following dissociation constants:

$$\frac{k_{offA}}{k_{onB}} = \frac{R \cdot C_A}{RC_A} = K_{DA}$$

and

$$\frac{k_{offB}}{k_{onB}} = \frac{R \cdot C_B}{RC_B} = K_{DB} \qquad (1)$$

where K_{DA} and K_{DB} are dissociation equilibrium constants for exogenous and endogenous EPO. Upon introducing the total drug plasma concentrations:

$$C_{Atot} = C_A + RC_A$$

and

$$C_{Btot} = C_B + RC_B \tag{2}$$

and total receptor plasma concentration:

$$R_{tot} = R + RC_A + RC_B \tag{3}$$

the EPO-EPOR complex concentrations for exogenous and endogenous EPO RC_A and RC_B can be calculated from equation 1:

$$RC_A = \frac{R_{tot}C_A / K_{DA}}{1 + C_A / K_{DA} + C_B / K_{DB}}$$

and

$$RC_B = \frac{R_{tot}C_B / K_{DB}}{1 + C_A / K_{DA} + C_B / K_{DB}} \tag{4}$$

Above equations are known in pharmacology as the Gaddum equations.[26] The stimulatory function is given by:

$$S(RC) = \frac{S_{max}RC}{SRC_{50} + RC} \tag{5}$$

where S_{max} is the maximal effect of EPO-EPOR complex on the proliferation of precursor cells, and SRC_{50} is the concentration of EPO-EPOR complex inducing 50% of S_{max}. RC is the sum of RC_A and RC_B. The stimulus-effect relationship described by equation 5 was introduced by Black and Leff in the operational model of agonism.[35] The total receptor number (R_{tot}) is assumed to be proportional to the number of the EPOR expressing precursor cells (P_2):

$$R_{tot} = \xi P_2 \tag{6}$$

where ξ is a factor of proportionality. The model equations for PK and PD are provided in the **Supplementary Material** online.

C_{50} for EPOR agonists. The stimulatory function in equation 5 can be expressed as a function of free EPO concentrations:

$$E(C_A, C_B) = \frac{S_{max}\left(\frac{R_{tot}C_A / K_{DA}}{1 + C_A / K_{DA} + C_B / K_{DB}} + \frac{R_{tot}C_B / K_{DB}}{1 + C_A / K_{DA} + C_B / K_{DB}}\right)}{SRC_{50} + \frac{R_{tot}C_A / K_{DA}}{1 + C_A / K_{DA} + C_B / K_{DB}} + \frac{R_{tot}C_B / K_{DB}}{1 + C_A / K_{DA} + C_B / K_{DB}}} \tag{7}$$

Let

$$\tau_{in\ vivo} = \frac{R_{tot}}{SRC_{50}} \tag{8}$$

where $\tau_{in\ vivo}$ is known as the transducer constant in the operational model of agonism and serves as a marker of efficacy.[33] Equation 7 can be rewritten as:

$$E(C_A, C_B) = \frac{S_{max}\tau_{in\ vivo}\left(\frac{C_A / K_{DA}}{1 + C_A / K_{DA} + C_B / K_{DB}} + \frac{C_B / K_{DB}}{1 + C_A / K_{DA} + C_B / K_{DB}}\right)}{1 + \tau_{in\ vivo}\left(\frac{C_A / K_{DA}}{1 + C_A / K_{DA} + C_B / K_{DB}} + \frac{C_B / K_{DB}}{1 + C_A / K_{DA} + C_B / K_{DB}}\right)} \tag{9}$$

In the case when endogenous EPO level is far less than that for exogenous EPO ($C_B/K_{DB} << C_A/K_{DA}$), equation 9 can be simplified to:

$$E(C_A) = \frac{S_{max}\tau_{in\ vivo}\left(\frac{C_A}{C_A + K_{DA}}\right)}{1 + \tau_{in\ vivo}\left(\frac{C_A}{C_A + K_{DA}}\right)} \tag{10}$$

As $C_A \to \infty$, the maximal effect (α) to a given agonist can be defined as:

$$\alpha = \frac{S_{max}\tau_{in\ vivo}}{\tau_{in\ vivo} + 1} \tag{11}$$

C_{50}, which represents the concentration of agonist that produces the half-maximal effect, can be calculated from:

$$\frac{S_{max}\tau_{in\ vivo}\frac{C_{50}}{C_{50} + K_{DA}}}{1 + \tau_{in\ vivo}\frac{C_{50}}{C_{50} + K_{DA}}} = \frac{1}{2}\frac{S_{max}\tau_{in\ vivo}}{\tau_{in\ vivo} + 1} \tag{12}$$

Solving the above equation leads to:

$$C_{50} = \frac{K_{DA}}{\tau_{in\ vivo} + 1} \tag{13}$$

In vitro data analysis. Previously published *in vitro* data was used for analysis.[8] In the study, colony forming cell assays were conducted to determine the effect of epoetin and darbepoetin on the proliferation of erythroid progenitor cells (**Figure 4**). The data were fitted to the following operational model of agonism:

$$\mathrm{FRO} = \frac{C}{K_D + C} \tag{14}$$

$$E = \frac{E_{max}\mathrm{FRO}}{K_E + \mathrm{FRO}} \tag{15}$$

where C is ESA concentration, FRO denotes the fractional receptor occupancy, E_{max} represents the maximal effect, and K_E represents the value of percent receptor occupancy that induces the half-maximal effect. The *in vitro* efficacy parameter can be calculated as:

$$\tau_{in\ vitro} = \frac{1}{K_E} \tag{16}$$

Combining equations 14 and 15 gives:

$$E = \frac{\tau_{in\ vitro} E_{max} C}{K_D + C(\tau_{in\ vitro} + 1)} \tag{17}$$

Equation 17 was fitted to the *in vitro* data for epoetin and darbepoetin. Similarly, C_{50} can also be obtained from the *in vitro* data:

$$C_{50} = \frac{K_D}{\tau_{in\ vitro} + 1} \tag{18}$$

K_D for epoetin and darbepoetin was fixed to the literature values.[9] Model fittings were conducted using Phoenix WinNonlin 6.0 (Pharsight Corporation, Cary, NC).

Model-based simulations. For model-based simulations, the dose amount of 7,800 IU was chosen based on the mean weight (78 kg).[14] Given that both darbepoietin and CERA were administered based on the mass of the peptide core and 1 µg of darbepoietin corresponds to 200 IU,[18–20] the same conversion factor can be assumed for CERA. The parameter values are listed in **Supplementary Table S1** online. To assess the influence of receptor binding affinity and the linear clearance of exogenous rHuEPO analogue on the pharmacodynamic effect, HGB response vs. time profiles were simulated based upon the model in **Figure 2a**, but with a combination of K_{DA} values ranging from 41.8 to 83,600 mIU/ml, and CL_A values ranging from 3.73×10^{-3} to 1 l/l. The peak HGB concentrations were extracted and plotted against K_{DA} and CL_A. These simulations were conducted using MATLAB 7.8 (MathWorks, Natick, MA). The MATLAB code for the simulation is provided in the **Supplementary Material** online.

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Conflict of Interest. The authors declared no conflict of interest.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✓ It has been suggested that the pharmacological effect of rHuEPO is not dependent on the peak concentration, but on the duration of rHuEPO concentration above the MEC. An ESA with a lower receptor binding affinity should have a higher MEC. However, the value of MEC remains unknown.

WHAT QUESTION THIS STUDY ADDRESSED?

✓ Our study sought to quantitatively decipher MEC using a PK/PD modeling and simulation approach.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

✓ The MEC of an ESA can be quantified by its C_{50}, which is defined as the concentration of ESA that induces half-maximal effect of stimulating the proliferation of erythroid precursors in bone marrow. C_{50} of an ESA is a function of its receptor binding affinity and *in vivo* efficacy.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

✓ The mode-based simulations predicted PK/PD behavior of three different ESAs. The model used in this report may allow joint PK/PD modeling of data from different ESAs, and provide a platform for dosing regimen optimizations and future clinical study designs.

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Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (http://www.nature.com/psp)