# Pharmacodynamic Modeling of Recombinant Human Erythropoietin Effect on Reticulocyte production Rate and Age Distribution in Healthy Subjects


**Wojciech Krzyzanski[1], Juan Jose Perez Ruixo[2], and Jeremy Hing[2]**

[1]Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, Buffalo, NY.
[2]Clinical Pharmacology, Johnson & Johnson Pharmaceutical Research & Development, Beerse, Belgium (JJPR), and Raritan, NJ (JH).


## Objective

To infer a major mechanism of the rHuEPO action on reticulocyte age distribution and production rate from data obtained in Phase I studies

## Introduction

Erythropoietin (EPO) is a 30.4 kD glycoprotein that is the major growth factor regulating the production of circulating erythrocytes. EPO acts by binding to receptors on the surface of the erythroid progenitor cells in the bone marrow and other hematopoietic tissues, leading to an increase in survival, proliferation, and differentiation of erythroid progenitor cells, which ultimately results in increase in reticulocytes (RET), red blood cell (RBC) and hemoglobin concentrations. Recombinant human EPO (rHuEPO) has been approved for the treatment of anemia associated with renal failure, cancer chemotherapy, and the treatment of acquired immunodeficiency syndrome. The total maturation time of erythroblasts and reticulocytes is of the order of 4 days, of which 3 days are spent in bone marrow and 1 day in the peripheral circulation. A single intravenous bolus administration of rHuEPO into healthy subjects results in an immediate release to the circulation of the immature reticulocytes, followed by a 1.5 day delayed release of reticulocytes arising from the stimulation of erythropoiesis by rHuEPO [1]. This indicates that in addition to an increase in the number of reticulocyte precursors, rHuEPO shortens their transit time from early erythrobrasts to reticulocytes in the bone marrow. Another consequence of rHuEPO is a transient change in the age distribution of circulating reticulocytes. The maturation time of about 1 day for homeostatic reticulocytes in the circulation extends to about 3 days for stress reticulocytes [1]. This phenomenon has been confirmed by other investigators for phlebotomy-induced stress erythropoiesis [2]. Recently, it has been demonstrated in healthy subjects that the maturation time in the circulation for stress reticulocytes depends on rHuEPO dose [3].

## Methods

***Study Design***: Data from three open-label, randomized, placebo-controlled parallel-group Phase I studies performed by Johnson & Johnson Pharmaceutical Research & Development, LLC were used. In these studies, the pharmacokinetics and pharmacodynamics of a single subcutaneous dose of rHuEPO was investigated in 88 adult male healthy volunteers. The subjects were randomized to receive rHuEPO subcutaneously at doses ranging 300 -1800 IU/kg and 20-160 kIU. ***Pharmacokinetic Analysis***: A Bayesian estimation of individual pharmacokinetic parameters of rHuEPO was implemented in the NONMEM software, using the POSTHOC option. The results of a previous population pharmacokinetic analysis of rHuEPO using data from 16 clinical studies were used to describe the time course of rHuEPO after intravenous and subcutaneous administration [4]. The model structure used to characterize the rHuEPO disposition is displayed in Fig. 1.

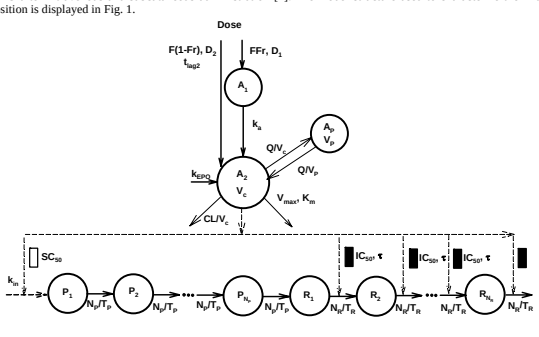


***Fig. 1.*** *Schematic diagram of the PK/PD model describing rHuEPO effect on reticulocyte production and age distribution.*

***Pharmacodynamic Analysis***. The development of the PD model was performed using a sequential process. Individual model parameters obtained from the PK analysis were used to predict the individual time course of rHuEPO serum concentration, which was used as input function into the PD model. To investigate possible mechanisms by which The concept of maturation-structured cytokinetic model introduced previously by Harker et al., [5] was applied to describe rHuEPO increases production of reticulocytes and affects their age distribution. The backbone structure of such a model is a series of compartments linked in a catenary fashion by first-order cell transfer rates (Fig. 1). Each compartment represents a pool of cells of the increased mean age by $1/k$, where $k$ denotes the first-order rate constant between the aging compartments. A cascade of $N_P = 10$ age compartments with the transfer rate constants equal $N_P/T_P$ were selected to account for development and maturation of reticulocyte precursor cells in bone marrow, where $T_P$ is the mean lifespan of the precursor cell. Similarly, a cascade of $N_R = 10$ age compartments represented the circulating reticulocytes of the mean lifespan $T_R$. The precursor cells in the first age compartment $P_1$ were assumed to be produced at the zero-order rate $k_{in}$. The stimulation of production of progenitor cells in bone marrow and the increase of maturation times of the circulating reticulocytes was included in the PD model.

$$\frac{dP_1}{dt} = \frac{k_{in}\cdot C}{SC_{50}+C} - \frac{N_P}{T_P}\cdot P_1 \qquad \frac{dP_i}{dt} = \frac{N_P}{T_P}\cdot\left(P_{i-1} - P_i\right) \qquad i = 2,\ldots, N_P$$

$$\frac{dR_1}{dt} = \frac{N_P}{T_P}P_{N_P} - \frac{N_R}{T_R}\left(\frac{S_0}{S_M}\right)R_1 \qquad \frac{dR_j}{dt} = \frac{N_R}{T_R}\left(\frac{S_0}{S_M}\right)\left(R_{j-1} - R_j\right) \qquad j = 2,\ldots, N_R$$

$$RET = R_1 + \ldots + R_{N_R}$$

The signal transduction was described by the transduction model with M = 5 transit compartments

$$\frac{dS_1}{dt} = \frac{1}{\tau}\left(\frac{C}{EC_{50}+C} - S_1\right) \qquad \frac{dS_k}{dt} = \frac{1}{\tau}\left(S_{k-1} - S_k\right) \qquad k = 2,\ldots, M$$

***Reticulocyte residence time distribution.*** The jth reticulocyte compartment Rj can be considered as a subpopulation of reticulocytes of the mean residence time in the circulation aj calculated as the sum of the mean residence times for compartments $R_1, \ldots, R_j$: aj(t) = j·MRT(t) , where both aj and MRT (k = 1,…,j) are time dependent, since the first-order transfer rates between the reticulocyte pools were dependent on the transduction signal. For this model, and for time t = $t_0$, MRT($t_0$) were calculated as follows

$$MRT(t_0) = \int_{t_0}^{\infty} A(t,t_0)dt \qquad \frac{dA(t,t_0)}{dt} = -\frac{N_R}{T_R}\left(\frac{S_0}{S_M}\right)A(t,t_0) \qquad A(t_0,t_0) = 1$$

At a time t = $t_0$, the probability density function (p.d.f.) of the reticulocyte age distribution and the mean reticulocyte residence time were determined as follows:

$$pdf.(a,t_0) = \frac{R_1(t_0)}{RET(t_0)}\delta(a-a_1) + \ldots + \frac{R_{NR}(t_0)}{RET(t_0)}\delta(a-a_{NR}) \qquad MRT_{RET}(t_0) = \frac{MRT(t_0)}{RET(t_0)}\sum_{j=1}^{N_R} j\cdot R_j(t_0)$$

***Software.*** Nonlinear mixed effects modeling was performed using NONMEM® V level 1.1 (GloboMax, Hanover, MD, USA). Non-parametric bootstrap analysis was performed using the package Wings for NONMEM (N. Holford, Version 4.04, June 2003, Auckland, New Zealand).

## Results

| Model Parameters | Original dataset | Non-Parametric Bootstrap | | |
|---|---|---|---|---|
| | Estimate | Median (RSE, %) | 95%CI Lower limit | 95%CI Upper limit |
| *Structural Model Parameters* | | | | |
| $RET_0$ | 1.24 | 1.23 (3.23) | 1.15 | 1.30 |
| $T_R$, h | 62.2 | 62.2 (8.39) | 44.1 | 78.6 |
| $T_P$, h | 118 | 117 (13.9) | 86 | 144 |
| $SC_{50}$, IU/L | 7.61 | 7.91 (35.9) | 2.81 | 14.25 |
| $EC_{50}$, IU/L | 56.3 | 71.1 (49.5) | 34.4 | 166.5 |
| $\tau$, h | 4.89 | 4.83 (60.7) | 0.24 | 10.8 |
| *Interindividual Variability (%)* | | | | |
| $\omega RET_0$ | 19.8 | 19.7 (10.5) | 15.6 | 23.8 |
| $\omega T_R$ | 36.2 | 35.8 (11.5) | 27.9 | 44.2 |
| $\omega T_P$ | 27.4 | 25.1 (23.8) | 14.2 | 36.5 |
| $\omega EC_{50}$ | 107 | 108 (18.9) | 74.1 | 154 |
| *Residual variability (%)* | | | | |
| $\sigma$ | 63.1 | 62.9 (4.62) | 57.5 | 68.3 |

***Table I.*** *Descriptive statistics of the rHuEPO individual Bayesian estimates of pharmacokinetic parameters from the population model.*

| Parameter | Prior Mean (Variability, %) | Posterior Mean (Variability, %) | Range |
|---|---|---|---|
| Baseline (IU/L)* | 13.9 (30) | 11.4 (34) | 6.2 – 26.6 |
| CL (L/h) | 0.358 (33) | 0.325 (15) | 0.226 – 0.468 |
| Vmax (IU/h) | 211 (29) | 239 (44) | 83 – 634 |
| $V_c$ (L) | 3.89 (31) | 4.17 (25) | 1.81 – 7.10 |
| F (%) | 62 (35) | 47 (42) | 15 – 100 |
| fr (%) | 60 (45) | 78 (46) | 51 – 93 |
| Ka (/h) | 0.034 (36) | 0.039 (47) | 0.016 – 0.155 |
| $D_1$ (h) | 0.725 (125) | 1.678 (102) | 0.128 – 12.718 |
| lag$_2$ (h) | 2.72 (53) | 2.75 (31) | 1.00 – 5.47 |

Km was fixed to 394 IU/L, Q was fixed to 0.044 L/h, $V_P$ was fixed to 1.64 L, and D2 was fixed to 37.8 h.

***Table II.*** *Estimates of the population parameters of the PD model*

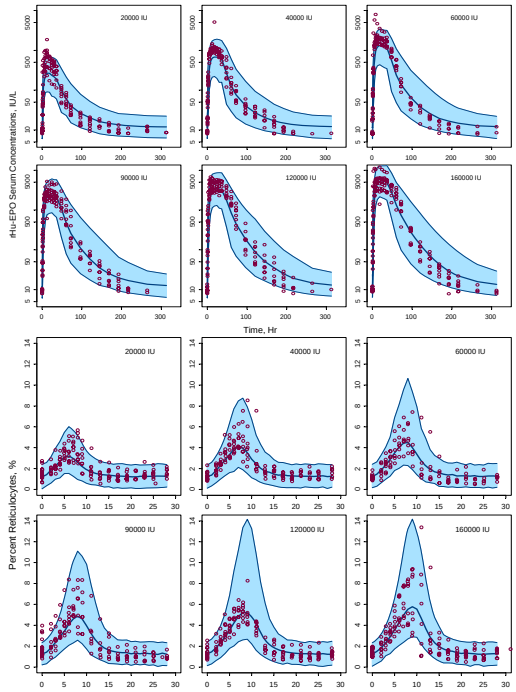


***Fig. 2.*** *Time course of the rHuEPO serum concentrations(upper) and the percentage of reticulocyte counts (lower). The lines and shaded area represent the median and 90% prediction interval from the posterior predictive check.*

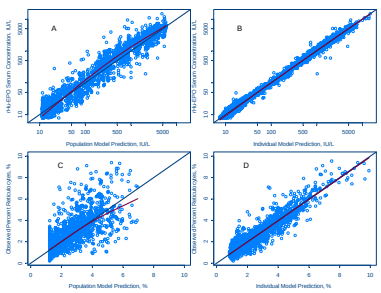


***Fig. 3.*** *Diagnostics plots for the PK model (panel A and B) and PD model (panels C and D).*

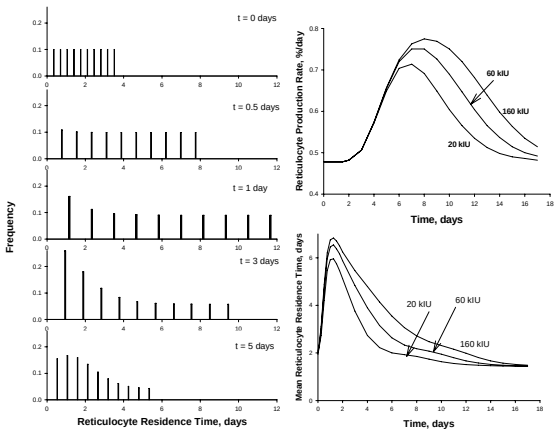


***Fig. 4.*** *Age distributions of circulating reticulocytes at various points of times for a typical subject who received a subcutaneous rHuEPO dose of 20 kIU. The jth bar represent the Rj reticulocyte compartment that are assumed to have the same age j·MRT(t) at time t.*

***Fig. 5.*** *Mean age of circulating reticulocytes (upper panel) and reticulocyte release rate (lower panel) as functions of time for at typical subject who received a subcutaneous rHuEPO doses of 20, 60, and 160 kIU.*

## Conclusions

- rHuEPO both stimulates the production of the marrow progenitor cells and transiently increases the mean transition time of the circulating reticulocytes.
- Both effects contribute to the increase in the reticulocyte counts in a dose-dependent manner.
- The change in the reticulocyte maturation time cannot be solely explained by the release to the circulation of the stress immature reticulocytes.

## Acknowledgments


This study was supported by Johnson & Johnson Pharmaceutical Research & Development, A Division of Janssen Pharmaceutica, NV, Beerse, Belgium, and in part by the National Institute of General Medical Sciences, National Institutes of Health Grant GM 57980.


## References


1. Major A, Bauer C, Breymann C, Huch A, Huch R, rh-Erythropoietin stimulates immature reticulocyte release in man. *Br. J. Haematol.* 87:605-8 (1994).
2. Al-Huniti NH, Widness JA, Schmidt RL, Veng-Pedersen P, Pharmacodynamic analysis of changes in reticulocyte subtype distribution in phlebotomy-induced stress erythropoiesis. *J. Pharmacokinet. Pharmacodyn.* 32:359-76 (2005).
3. Krzyzanski W, Perez-Ruixo JJ. An assessment of recombinant human erythropoietin effect on reticulocyte production rate and lifespan distribution in healthy subjects. *Pharm. Res.* 24:758-771 (2007).
4. Olsson-Gisleskog P, Jacqmin P, Perez-Ruixo JJ, Population pharmacokinetics meta-analysis of recombinant human erythropoietin in healthy subjects. *Clin. Pharmacokinet.* 46: 159-173 (2007).
5. Harker LA, Roskos LK, Marzec UM, Carter RA, Cherry JK, Sundell B, Cheung EN, Terry D, Sheridan W, Effects of megakaryocyte growth and development factor on platelet production, platelet life span, and platelet function in healthy human volunteers. *Blood* 95:2514-22 (2000).