Artificial inflation of pharmacokinetic difference between two granulocyte colony-stimulating factor (G-CSF) drug products by non-compartmental analysis

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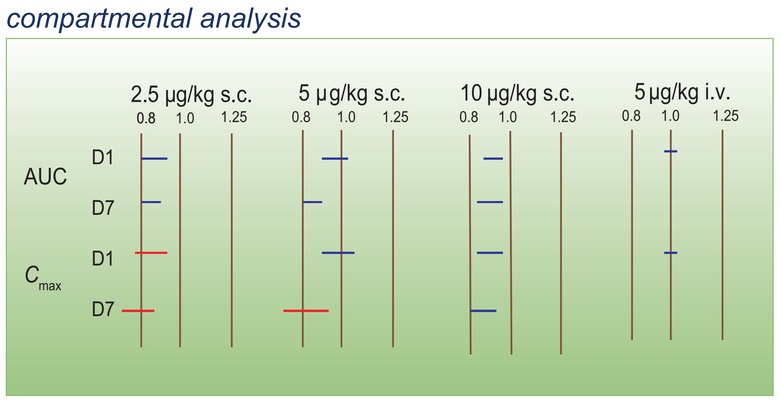
Background

- Granulocyte colony-stimulating factor (G-CSF) is an endogenous protein also administered as a drug to stimulate the proliferation of neutrophils in oncology patients after chemotherapy.
- Its pharmacokinetics (PK) exhibit a non-linear behavior through a target-mediated drug disposition process.
- Conventional bioequivalence analysis between Zarzio® (EP2006) and Neupogen® (two formulations of recombinant human G-CSF) showed that their C_{max} and AUC ratios were decreasing between a single and repeated administration at 2.5 and 5 µg/kg, but not at 10 µg/kg daily dose.
- The aim of the modeling analysis was to assess whether the drift in these ratios when doses are decreased or repeated could be explained by the mechanisms underlying the well known pharmacokinetic non-linearity for this drug.

Methods

- About 6000 plasma concentration–time records were evaluated from the rich sampling profiles of 112 healthy male and female volunteers in three cross-over studies.
- G-CSF was administered as repeated s.c. daily administration for one week of 2.5, 5 and 10 µg/kg doses and a single i.v. dose of 5 µg/kg.
- Pharmacodynamic (PD) data (blood absolute neutrophil count [ANC]) were available for the same time frame.
- G-CSF (unbound to neutrophils) was measured in serum using a validated ELISA. The limit of quantitation was 39 pg/mL.
- PK/PD parameters were obtained through non-linear mixed effect regression using NONMEM version VI (Globomax Corp., Hanover, MD, USA).[1]
- The results of the conventional bioequivalence analysis are presented in **Figure 1**.

Figure 1. *90% CI of AUC and C_{max} ratios from the bioequivalence ANOVA comparing Zarzio® (test) and Neupogen® (reference). Metrics obtained from non-compartmental analysis*

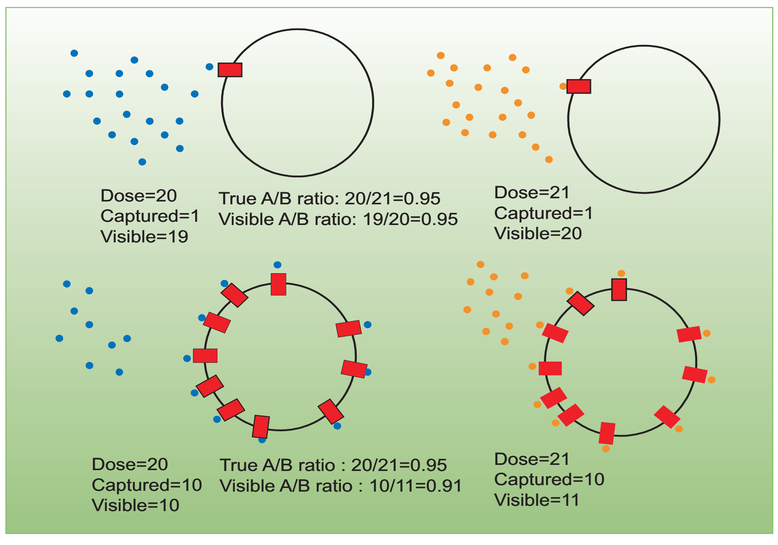


- 90% CI of the AUC and C_{max} ratios appear to drift away from unity when the s.c. dose is decreased or repeated.
- We hypothesized that this observation was due to the target-mediated drug disposition phenomenon inflating a small pre-existing difference in the systemically available amount of the drugs as described in **Figure 2.**
- To check whether our hypothesis was operating for the G-CSF within the therapeutic dose and concentration ranges, an estimation of the unbound amount of the two drugs was necessary.
- Assuming a rapid binding equilibrium between the free drug and the free receptor to form a drug–receptor complex, the unbound amount of the drug can be computed from the estimated drug-receptor complex amount using the following equation derived from the law of mass action.

$$DR = 0.5\times\left(\left(TD+TR+K_d\times V\right)-\sqrt{\left(TD+TR+K_d\times V\right)^2-4\times TD\times TR}\right)$$

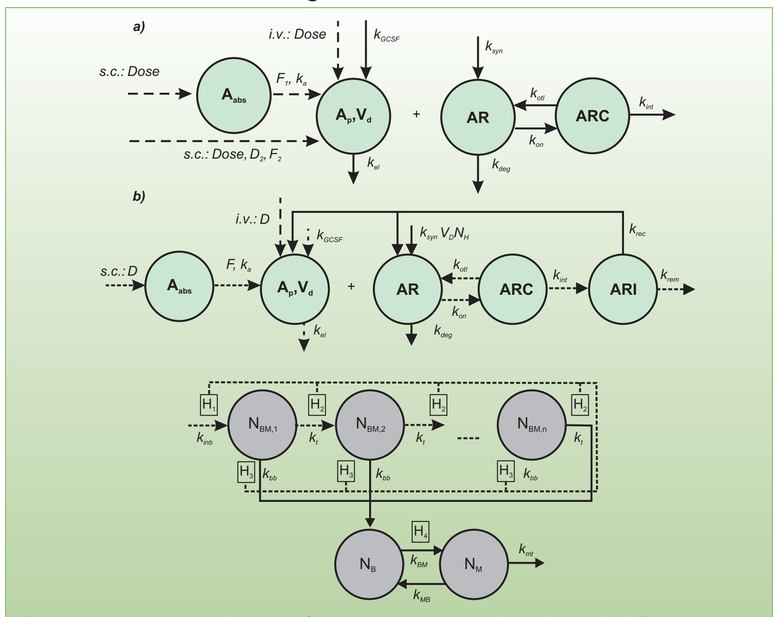
- Where DR, is the amount of drug–receptor complex; TD, is the total amount of drug; TR, is total amount of receptor; K_d, is the affinity constant; and V, is the volume of distribution. A semi-mechanistic model was developed to estimate K_d, V and TR[1].

Figure 2. *Schematic illustration of the inflation of a difference between the unbound (visible) amount of two drugs through the target-mediated drug disposition process when the number of receptors increase (i.e. after repeated administration)*



Results

Figure 3. (a) *Structural PK model used for the estimation of* K_d, V, *and* TR *after a single dose of* G-CSF. ***(b)*** *Structural* PK/PD *model used to estimate the change in* TR *with a repeated administration of the drug*



Abbreviations: A_{abs}: amount available for absorption, k_a: absorption rate constant; F_1, F_2: bio-availability through the first and zero order absorption respectively; D_2: virtual infusion time of the zero order absorption, k_{GCSF}: endogenous synthesis rate of G-CSF, A_p: amount of G-CSF in central volume; V_d: central volume of distribution; k_{el}: elimination rate constant; k_{syn}: endogenous synthesis rate of G-CSF receptor; AR: amount of receptor in central volume; k_{deg}: degradation rate constant of the receptor; k_{on}, k_{off}: association and dissociation rate constant of drug-receptor complex; ARC: amount of drug-receptor complex in central volume; k_{int}: internalization rate constant of drug-receptor complex; ARI amount of internalized drug-receptor complex; k_{rec} recycling constant of internalized complex; k_{rem} degradation rate constant of internalized complex; N_{BM}: bone marrow maturation compartment for neutrophils; N_B: blood neutrophil compartment; N_M: neutrophil margination compartment; k_{inb}: rate constant of precursor cells production; k_t: maturation transfer rate; k_{bb}: rate constant of release into blood; K_{BM}: margination rate constant; H_1-H_4: stimulation functions by G-CSF serum concentration

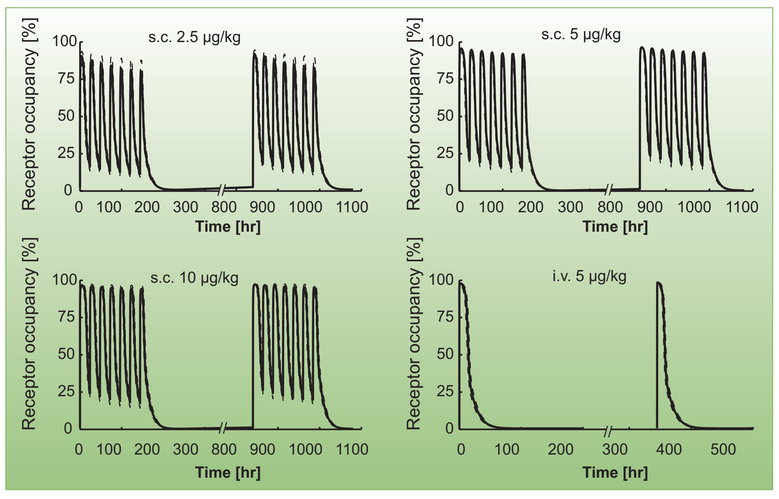
- Diagnostic plots showed that the PK model alone for the single dose administration fitted the data very well.
- PK/PD model was fitted on the full dataset with reasonably good diagnostics although some bias could still be detected, in particular the high G-CSF concentrations were somewhat under-predicted while the ANC was well fitted.
- It was used for estimating the increase in receptor numbers from Day 1 to Day 7 and simulate receptor occupancy.
- Unbound amount of Zarzio® (EP2006) and Neupogen® and their ratio after seven daily s.c. administration of 10, 2.5, and 1 µg/kg, assuming a 4% higher dose of Neupogen® and same bioavailability (70%) are shown in **Table 1**. The 4% difference is recovered in the unbound amount ratio at 10 µg/kg, but the ratio is drifting away from unity with lower doses. Drug and receptor amounts are expressed in µg and G-CSF µg equivalent, respectively.

Table 1. *Computed unbound amount of EP2006, Neupogen® and their ratio from the semi-mechanistic PK/PD model*

Nominal dose	10 µg/kg		2.5 µg/kg		1 µg/kg	
Neup rel. dose 1.04	EP2006	Neup.	EP2006	Neup.	EP2006	Neup.
Total dose	700	728	175	182	70	72.8
Bioavailable dose	490	509.6	122.5	127.4	49	50.96
Total receptors	75	75	50	50	35	35
Complex amount	74.908	74.912	49.653	49.674	33.860	33.980
G-CSF fraction bound	0.153	0.147	0.405	0.390	0.691	0.667
G-CSF fraction unbound	0.847	0.853	0.595	0.610	0.309	0.333
Amount unbound	415.1	434.7	72.8	77.7	15.1	17.0
Ratio	0.955		0.937		0.892	

- Simulated time course of receptor occupancy after G-CSF administrations is given in **Figure 4**. The drug was administered in a sequence: Zarzio® followed by Neupogen® (For more details see PAGE poster[1]).

Figure 4. *Simulated time course of receptor occupancy after G-CSF administrations*



- A sensitivity analysis was performed due to possible biased estimation of receptor amount after 7 daily administrations.
- The ratio of unbound EP2006 and Neupogen® was computed as in **Table 1** with an estimated amount of receptors 1.5 fold higher. The drift in unbound amount ratios is still more important (**Table 2a**).

Table 2a. *Computed ratio of unbound EP2006 and Neupogen® with receptor number 50% higher than in **Table 1.***

Nominal dose	10 µg/kg		2.5 µg/kg		1 µg/kg	
Neup rel. dose 1.04	EP2006	Neup.	EP2006	Neup.	EP2006	Neup.
Total dose	700	728	175	182	70	72.8
Bioavailable dose	490	509.6	122.5	127.4	49	50.96
Total receptors	112.5	112.5	75	75	52.5	52.5
Complex amount	112.348	112.356	74.216	74.287	45.620	46.786
G-CSF fraction bound	0.229	0.220	0.606	0.583	0.931	0.918
G-CSF fraction unbound	0.771	0.780	0.394	0.417	0.069	0.082
Amount unbound	377.7	397.2	48.3	53.1	3.4	4.2
Ratio	0.951		0.909		0.810	

- Another PK/PD model was computed as in **Table 1** with an estimated amount of receptors 10-fold lower than in **Table 2a**, mimicking a severe neutropenia, the condition experienced by the target patient population of the drug. The small difference between the drugs is not inflated in this condition **(Table 2b)**.

Table 2b. *Computed ratio of unbound EP2006 and Neupogen® with receptor number 10 fold lower than in **Table 2a**.*

Nominal dose	10 µg/kg		2.5 µg/kg		1 µg/kg	
Neup rel. dose 1.04	EP2006	Neup.	EP2006	Neup.	EP2006	Neup.
Total dose	700	728	175	182	70	72.8
Bioavailable dose	490	509.6	122.5	127.4	49	50.96
Total receptors	11.25	11.25	7.5	7.5	5.25	5.25
Complex amount	11.238	11.239	7.467	7.468	5.190	5.192
G-CSF fraction bound	0.023	0.022	0.061	0.059	0.106	0.102
G-CSF fraction unbound	0.977	0.978	0.939	0.941	0.894	0.898
Amount unbound	478.8	498.4	115.0	119.9	43.8	45.8
Ratio	0.961		0.959		0.957	

Conclusion

- The inflation of a small pharmacokinetic difference when doses are decreased or repeated is a plausible hypothesis for a drift of C_{max} and AUC ratio away from unity when measured through the unbound G-CSF concentrations in healthy subjects.
- As suggested by the model, this inflation is due to the non-linear receptor-mediated drug disposition and would not be apparent in the target patient population due to the lower number of receptors.

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References

1. Krzyzanski W. *et al.* PAGE 2009, St. Petersburg, Russia, poster 1510.

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