Novel Strategies for Granulocyte Colony-Stimulating Factor Treatment of Severe Prolonged Neutropenia Suggested by Mathematical Modeling

Eliezer Shochat and Vered Rom-Kedar

Abstract

Purpose: To improve the effectiveness of granulocyte colony-stimulating factor (G-CSF) treatment in high-risk neutropenic patients.

Experimental Design: We study G-CSF effects on chemotherapy-induced neutropenia by expanding a simple mathematical model of neutrophil dynamics in the blood. The final model is fitted and validated using published clinical data of neutrophil response to chemotherapy and standard s.c. G-CSF protocol (SG; filgrastim 5 μg/kg/d), single pegylated (pegG; pegfilgrastim 100 μg/kg), and continuous infusion (CG; filgrastim 10 μg/kg/d). The interpatient variability is studied by Monte-Carlo simulation of pegG compared with SG and placebo.

Results: The effect G-CSF support on neutropenia depends on the neutrophil count at the nadir. Three distinct neutropenia grades are identified: G1 (300×10^3-500×10^3 cells/mL), G2 (50×10^3-300×10^3 cells/mL), and G3 ($\leq 50 \times 10^3$ cells/mL). For many G2 patients, the G-CSF levels required for recovery are not attainable by the standard regimen, whereas the sustained pegG and CG seem to be significantly more effective. For G3 patients, G-CSF support alone is not sufficient and additional clinical approaches should be considered. The results presented here are robust and are only slightly affected by population variability.

Conclusions: The model captures the G-CSF-neutrophil dynamics of severe chemotherapy-induced neutropenia. Our results clarify and complement the current American Society of Clinical Oncology recommendations for G-CSF administration in neutropenia: High sustained G-CSF levels are needed to treat severe neutropenia and may be achieved by either CG or pegG. The potential effect of sustained G-CSF on severe neutropenia should be studied within a framework of a prospective randomized clinical trial.

Prolonged severe neutropenia is common in hematologic and solid malignancies that are treated with intensive chemotherapy regimens. Severe neutropenia may lead to potentially hazardous delays in treatment and life-threatening bacterial and fungal infections (1). In such patients, in addition to vigorous antibiotics treatment, standard s.c. granulocyte colony-stimulating factor (G-CSF) daily injections (SG), useful for neutropenia prevention (2) are often used (3). However, the yield of SG in already severely neutropenic patients is debatable, as many patients remain dangerously neutropenic for significant durations despite the G-CSF support (4). A novel G-CSF moiety, pegfilgrastim (pegG), was recently introduced to the clinic (with double the molecular weight and an attenuated systemic clearance; ref. 5). pegG regimen attains sustainable G-CSF levels $\geq$10,000 pg/mL for $\approx$4 days (peak levels $\geq$100,000 pg/mL). Notably, pegG administration correlates with a reduced frequency of febrile neutropenia in breast cancer patients (5) and a shorter duration of neutropenia in high-dose chemotherapy (6), but no pegG trial for neutropenic fever had been reported.

In fact, the American Society of Clinical Oncology current clinical guidelines do not advise a routine use of G-CSF in neutropenia and fever (2). Smith et al. (2) recommend that "G-CSF should be considered in patients with fever and neutropenia who are at high risk for infection-associated complications" and suggest that the severe prolonged neutropenia ($N \leq 100 \times 10^3$ cells/mL; i.e., $N \leq 0.1 \times 10^9$ cells/L) is a major risk factor. The guidelines conclude that "predictive models are needed to better identify high-risk patients who may benefit from the addition of adjunctive CSFs." Here we use a mathematical model of G-CSF-neutrophil interaction to identify those patients that may benefit from G-CSF administration. Such identification is made possible by introducing a new parameter, the acute marrow capacity (AMC), which denotes the maximal neutrophil flux that can be induced by G-CSF during the nadir.

Authors' Affiliation: Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Rehovot, Israel

Received 3/28/08; revised 6/2/08; accepted 6/19/08.

Grant support: The Arthur and Rochelle Belfer Institute of Mathematics and Computer Science.

Note: V. Rom-Kedar holds the Estrin Family Chair of Computer Science and Applied Mathematics.

Dedicated to the memory of Lee Segel—our teacher, colleague, and a dear friend.

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Requests for reprints: Eliezer Shochat, Iatromatics, 50/12 Ben Yehuda St. Netanya 42305, Israel. Phone/Fax: 972-9-834-2939; E-mail: eliezer.shochat@iatromatics.com or Vered Rom-Kedar, Computer Science and Applied Mathematics, Weizmann Institute of Science, P.O. Box 26, Rehovot 76100, Israel, E-mail: vered.rom-kedar@weizmann.ac.il.

doi:10.1158/1078-0432.CCR-08-0807

Materials and Methods

We study the effect of G-CSF support on the neutrophil dynamics following chemotherapy. We translate the corresponding clinical notions into mathematical terms and analyze the system behavior in various G-CSF regimens. We then use simulations and clinical data to validate the resulting model and arrive at medical insights that suggest new treatment strategies.

The basic *GN* model that describes the natural G-CSF (G)-neutrophil (N) dynamics in the blood was recently developed (7). The basic model fits well a range of clinical observations about the effect of standard G-CSF regimen on neutrophil count with a "normal bone marrow."

Here, we extend the model to describe the effect of various G-CSF administration regimens on the neutrophil count with "chemotherapy damaged bone marrow." We apply the extended model to study the potential effect of three clinically important G-CSF protocols [pulsed (SG), pegylated (pegG), and continuous (CG)]. The variability in the patient population is studied analytically by probabilistic methods and by Monte-Carlo simulations. In this study, we focus on the acute post-chemotherapy phase and do not attempt to model the long-term bone marrow dynamics.

The model. The detailed mathematical formulation of the model describing the *GN* dynamics following chemotherapy is provided in Supplementary data. Briefly, the following assumptions enter the model: The endogenous G-CSF production is increased as the neutrophil level decreases (as following chemotherapy), and the systemic G-CSF is cleared by the neutrophil consumption and by renal clearance. The neutrophil flux increases in response to an increased G-CSF concentration, and the neutrophils are cleared at a constant rate. These propositions are based on the medical evidence described in ref. 7. Here, we propose that the neutrophil flux may be temporarily reduced by the chemotherapy. Thus, we introduce here a time-dependent factor, BNF(t), which represents here the basic (G-independent) flux of neutrophils from the bone marrow to the blood. Normally BNF(t) is essentially constant as in ref. 7. We assume hereafter that chemotherapy makes it plummet to some low value (Bnadir) for a few days (Tstop - Tstart) till its recovery. The BNF(t) fit of the clinical data indicates that it is treatment specific (see Figs. 1 and 2 and Supplementary data for the functional form and the fitted

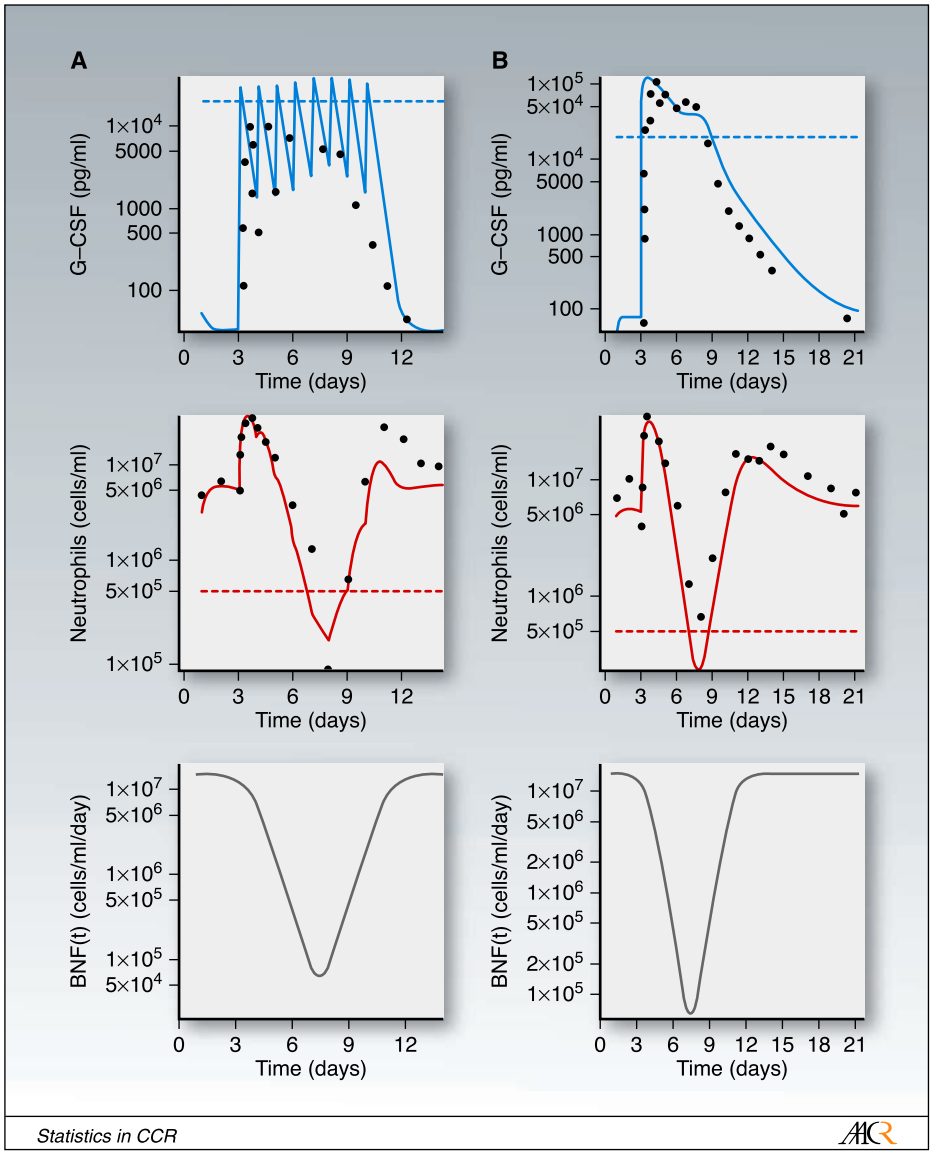

Fig. 1. The effect of SG and pegG in lung cancer patients on the averaged *GN* dynamics during conventional chemotherapy protocol – fitted (*A*) and predicted (*B*) dynamics. Data points from Holmes et al. (9); solid lines, simulation of the mathematical model; dashed blue line, the critical *G* level for the G2 group; dashed red line, the neutropenia threshold ($N_{tr} = 500 \times 10^3$ cells/mL). The chemotherapy is administered at day 0. *A,* SG regimen with fitted BNF(t). Note the rapid systemic clearance of SG and the resulting *G* oscillations (such oscillations are indeed observed in the clinical setting when both morning and afternoon measurements were taken; ref. 23). The insufficiency of SG to provide critical *G* levels for low BNF(t) is apparent. BNF(t) is fitted as described in Materials and Methods: Tstart - Tstop = 8 d; the fitted bone marrow decay/recovery rates are $\beta_1 \approx \beta_2 \approx 0.1$ [1/d] and Bnadir = 1.5×10^4 cell/mL/d ($n = 24$). *B,* model prediction for pegG ($n = 46$). BNF(t) is identical to *A*. All other parameters are taken from Supplementary Table S1.

parameter values). Summarizing, the model has the following components:

$$\begin{cases} \begin{bmatrix}\text{rate of change}\\ \text{of G}\end{bmatrix} = \begin{bmatrix}\text{injected G}\end{bmatrix} + \begin{bmatrix}\text{production}\\ \text{(N dependent)}\end{bmatrix} - \begin{bmatrix}\text{renal}\\ \text{clearance}\end{bmatrix} - \begin{bmatrix}\text{consumption}\\ \text{by N}\end{bmatrix} \\ \begin{bmatrix}\text{rate of change}\\ \text{of G}\end{bmatrix} = \begin{bmatrix}\text{Basic Neutrophil}\\ \text{Flux BNF}(t)\\ \text{(chemotheraphy}\\ \text{dependent)}\end{bmatrix} \begin{bmatrix}\text{G}-\text{dependent}\\ \text{factor}\end{bmatrix} - \begin{bmatrix}\text{senescence and}\\ \text{transport to}\\ \text{tissue}\end{bmatrix} \end{cases}$$

The mathematical analysis of the model leads to an important revelation. There is a single index, which we call the acute marrow capacity (AMC), that overwhelmingly dominates the neutrophil dynamics of patients and determines their neutropenia grade. Roughly, AMC measures the maximal reduction in the neutrophil level from the typical normal value of $N^* = 5 \times 10^6$ cells/mL under the largest possible G-CSF effect:

$$AMC = \frac{\begin{bmatrix}\text{maximal enhancement}\\ \text{of the N flux by G}\end{bmatrix}\begin{bmatrix}\text{minimal value of BNF}(t)\end{bmatrix}}{N^*\begin{bmatrix}\text{N clearance rate}\end{bmatrix}} \qquad \text{(A)}$$

The detailed derivation and the precise definition of AMC appear in Supplementary data.

Fitting and validation by clinical data. To show that the model adequately describes cases in which both chemotherapy and G-CSF treatments are applied, we first parameterize the model using clinical data of several therapeutic regimens as training sets. We then validate the model by predicting (without any parameter fitting) the *GN* dynamics of an independent clinical data set.

The effect of SG and pegG on the neutrophils is fitted to the clinical data set of patients with normal marrow reported by Wang et al. (8) and Johnston et al. (5), respectively; see Supplementary data for values. The chemotherapy effect [the parameters of BNF(t)] is found from the SG post-chemotherapy data set of Holmes et al. (ref. 9; Fig. 1A). The fully parameterized model successfully predicts the effect of pegG on the post-chemotherapy *N* dynamics; see Fig. 1B.

Population variability. The bone marrow response to treatments varies among patients (10), and the parameters of the basic *GN* model are patient dependent as well (see ref. 7 for estimates of the parameter range in the basic *GN* model). Ideally, we should strive to estimate the specific parameters in the patient population under a specific treatment protocol to provide confidence limits to the parameters. As only the published averaged clinical data sets are available to us, such direct estimates cannot be derived here. We compensate for the direct fitting error estimates by performing a detailed sensitivity analysis and extensive Monte-Carlo simulations.

Both the analysis and the simulations reveal a surprisingly simple result: Whereas the dimensional model has 18 patient-dependent parameters (see Supplementary tables), the beneficial effect of G-CSF treatment mainly depends on a single nondimensional combination of just three of these parameters, the AMC. In the Monte-Carlo simulations we set this ratio to a fixed low value by choosing Bnadir to depend on other parameters that are all log-normally distributed. It follows that the resulting Bnadir is log-normally distributed as well, yet it is not independent of the other parameters (see Supplementary data).

The log-normal probability density function

$$f(x;\mu,\sigma) = \frac{\exp\left(-\frac{[\ln(x)-\ln(\mu)]^2}{2\sigma^2}\right)}{\sqrt{2\pi}\,x\sigma}$$

is a plausible choice as many biological parameters are believed to follow it (11). For the unperturbed *GN* dynamics [in which BNF(t) is a fixed constant], the parameter means [$\ln(\mu)$] are taken as ln(values) of the fitted parameters to the clinical data sets of refs. 5 and 8. The specific σ are estimated by the 3 sigma rule (the estimated range of the parameters was found in ref. 7). For the remaining chemotherapy-related parameters that define BNF(t), we take hypothetical values that represent intermediate behavior between the high-dose chemotherapy and the conventional chemotherapy fitted values (Figs. 1 and 2). The variability in these parameters is taken to be rather small, $\sigma \approx 0.01$; for Tstart it is observed that the effect of chemotherapy occurs within only a few hours following *in vitro* exposure of marrow cells to cytotoxic agents (12). For the marrow decay rate, it is observed that the SD of the *N* descending slope after chemotherapy is only 5% to 6% (13). No direct evidence about the variability in the duration and recovery rate of the bone marrow is known to us. Here we have assumed their variability to be the same as for the onset time and the decay rate parameters. Noteworthy, taking a larger variability (e.g., $\sigma = 0.15$) for these parameters in the simulations leads to a recovery time span of 6 to 22 d. Such large variations are not observed in the clinic and in the literature. In fact, taking these parameters to be distributed with $\sigma \approx 0.01$, we obtain recovery time spans of 9 to 14 d, which is very similar to what is reported in the literature; see for example Fenk et al. (6). We list the mean and variances of all the parameters that enter the Monte-Carlo simulations in the Supplementary Table 2.

Fixing the ratio AMC in the Monte-Carlo simulation to the low value of 0.11 corresponds to targeting the new treatment strategy to a specific patient population: the lower G2 patients (see also Supplementary data, in which it is shown that a significant portion of patients with a neutrophil count at the nadir of $50 \times 10^3 - 300 \times 10^3$ cells/mL and a wide log-normal distribution of endogenous G-CSF have AMC in the range of 0.1, 0.13). For this G2 patient population, as explained in the article, we propose that the difference between sustained and pulsed regimen is maximal. To show this claim, we perform a Monte-Carlo simulation of three different treatment arms (placebo, SG, and pegG). In each treatment arm, the time course of *G* and *N* was simulated for 100 patients. For each patient, all parameters, except a fixed low AMC, are randomly chosen from the above-described hypothetical log-normally distributed population. For each simulated patient, the area under the threshold of N_{tr} (the $AUC_{500} = \max\{0, [N_{tr} - N(t)]\}dt$), a quantity that was suggested to be a good predictor of neutropenic fever (14), was calculated. We estimate the distribution of the AUC_{500} in each of the treatment arms and compare between them using the Kolmogorov-Smirnov test.

Results

We find that the neutropenic patients may be categorized according to their AMC values into three distinct grades that respond differently to G-CSF applications. A group of patients that does not adequately respond to SG but will benefit from sustained G-CSF levels is thus identified. A method for assigning an AMC value to a patient is described. These results are shown to be robust to patient variability.

Neutrophil dynamics in chemotherapy. To gain intuition about the *GN* dynamics under chemotherapy, consider first the case in which the basic neutrophil flux rate is low and constant so that BNF(t) = Bnadir (as explained below, this occurs in the nadir period following high-dose chemotherapy).

For a fixed BNF(t), SG only transiently perturbs the system and both *G* and *N* readily equilibrate back to a fixed value (G_{eq}, N_{eq}); see Fig. 3. Notice that the *G* elimination rate controls the equilibration time. Whereas for i.v. injection this elimination rate is governed by the natural *G* clearance, for s.c. *G* it is dominated by the slow absorption. This phenomena is especially noticeable for the pegG data: The clearance rate of pegG is essentially determined by its very small absorption rate (see Figs. 2 and 3 and the estimated values of the absorption rates listed in Supplementary data).

Figure 3 shows that the effectiveness of G treatment depends on the neutropenia severity and on the specific G protocol. Whereas for a very low basic neutrophil flux (Fig. 3C; AMC = 0.07) neither SG nor pegG reverses the neutropenia, for the intermediate damage pegG and CG maintain the neutrophil levels above N_{tr} for more than 5 days (Fig. 3B; AMC = 0.12). We see that provided AMC $\geq$ 0.1, holding the G level at a high fixed value of >10,000 pg/mL stabilizes the neutrophil levels above N_{tr}. On the other hand, we see that if AMC $\leq$ 0.1, G-CSF alone cannot reverse the neutropenia at the nadir. See Supplementary data for the mathematical explanation of this phenomenon.

The SG is characterized by a fast (several hours) G-CSF disappearance from the system (see Fig. 1A) and is not sufficient to maintain the critical G levels required for patients with severely depleted marrow (e.g., with AMC = 0.12; see Fig. 3B). Yet, as shown in the figure, such patients may be helped by maintaining high G levels by CG, by bidaily injections, or by

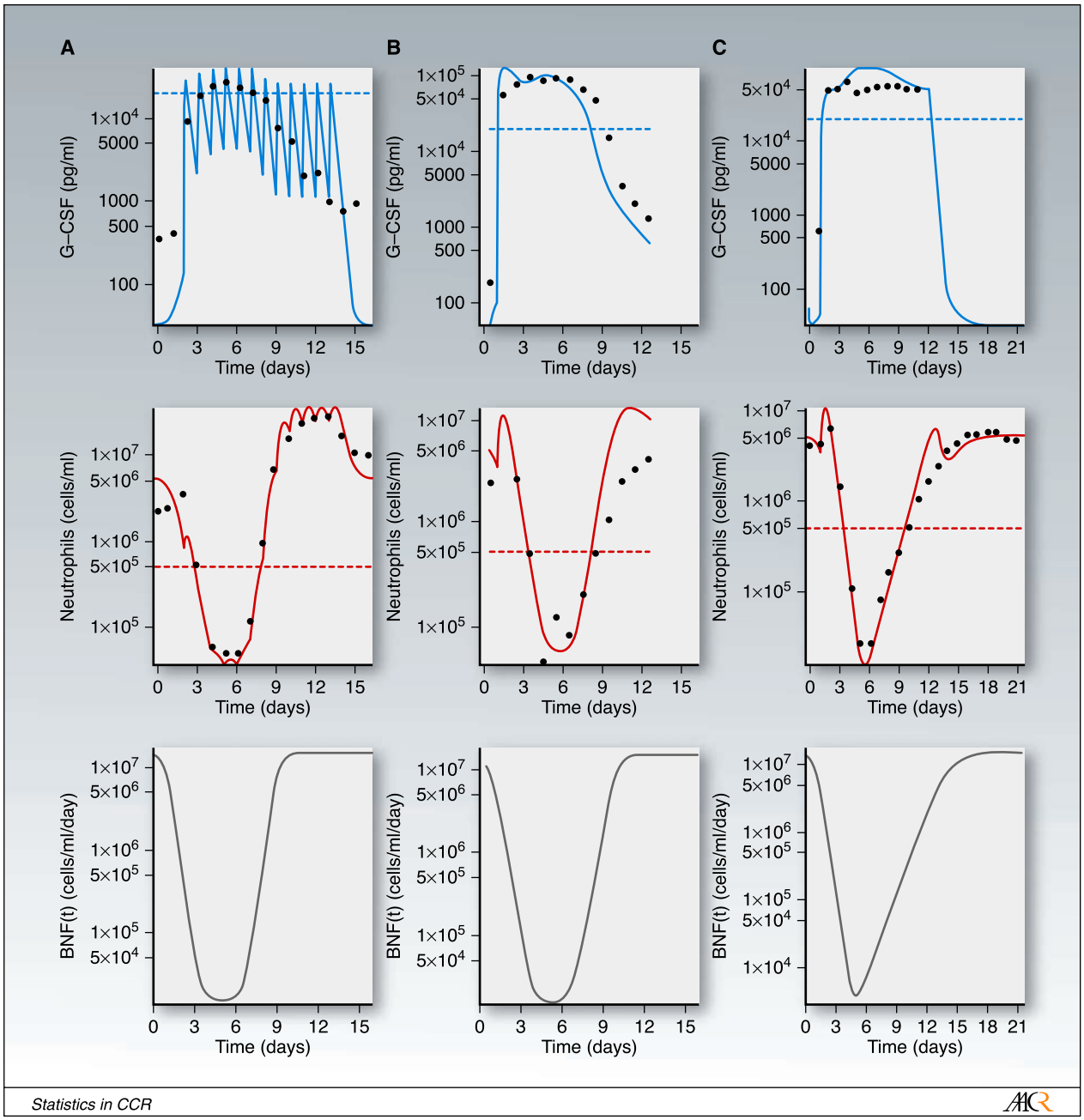

Fig. 2. Averaged G-CSF-neutrophil dynamics in several high-dose chemotherapy protocols. BNF(t) is fitted for each clinical data set individually as described in Materials and Methods. Other parameters are as in Fig. 1. Post – high-dose chemotherapy time series followed by multipulsed (s.c.) G (*A*), single pegG (*B*), and continuous G (*C*). *A,* multipulsed (s.c.) G. The chemotherapy is administered at days (-6, -3) and stem cells are transfused at day 0. G-CSF application is indicated by blue bar. Data adapted from Testa et al. (24); Tstart - Tstop = 8 d, $\beta_1 \approx \beta_2 \approx 0.166$[1/d], and Bnadir = 15×10^3 cells/mL/d; AMC = 0.01 (n = 9). *B,* single pegG. Data adapted from Fenk et al. (6); Tstart - Tstop = 9 d, $\beta_1 \approx \beta_2 \approx 0.133$[1/d], and Bnadir = 15×10^3 cells/mL/d; AMC = 0.01 (n = 21). *C,* continuous G. Data adapted from Layton et al. (19) and Sheridan et al. (25); Tstart - Tstop = 13 d, $\beta_1 \approx 0.133$[1/d], $\beta_2 \approx 0.05$[1/d], and Bnadir = 1.5×10^3 cells/mL/d; AMC = 0.001 (n = 3).

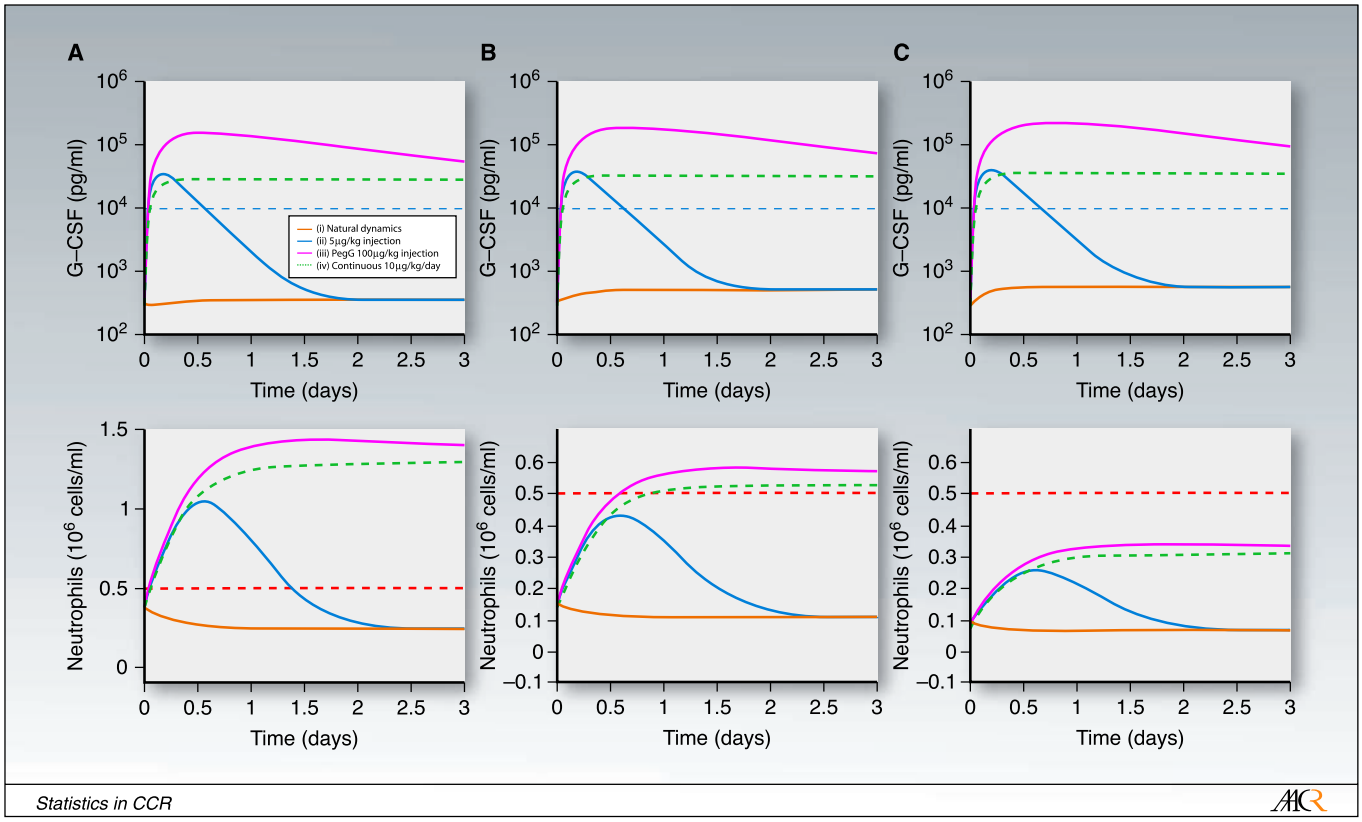

Fig. 3. Simulating *GN* dynamics at the nadir following chemotherapy. *A* to *C,* patients with increasing degrees of severity in the bone marrow damage are modeled by taking low constant values of the basic neutrophil flux [BNF(t) = Bnadir]. For each patient, four different G-CSF regimens are simulated: placebo-natural *GN* dynamics (*orange*), a single GCSF injection (*blue*), a pegG injection (*magenta*), and continuous *G* infusion (*green*). *A,* G1 recovering patient (Bnadir = 450×10^3 cell/mL/d, AMC = 0.3). *B,* G2 salvageable patient (Bnadir = 180×10^3 cell/mL/d, AMC = 0.12). *C,* G3 feeble patient (Bnadir = 105×10^3 cell/mL/d, AMC = 0.07). Note the insufficient neutrophil response despite the staggering G-CSF levels (80,000 pg/mL for pegG) in this very severe marrow damage case. For the pegG injections, due to the slow clearance, the neutrophil and G-CSF levels equilibrate only after 15 d. Noteworthy, this observation from the fit of our model to cancer patient data (5) is in line with a recent pharmacokinetic-pharmacodynamic analysis of the pegG effect published by Roskos et al. (26) for healthy volunteers. All other parameters are as in Supplementary Table S1.

pegG. These are exactly the regimens that guarantee a safety margin of G levels >10,000 pg/mL at all times.

In the previously mentioned considerations, the basic neutrophil flux BNF(t) was considered constant. Figure 1 supports the hypothesis that transient chemotherapy effects can be adequately represented by a fitted time-dependent BNF(t), which drops to a minimal value Bnadir, remains low for a while (Tstop - Tstart), and then naturally recovers (see also Fig. 2). Noteworthy, in profound chemotherapy-induced neutropenia, the marrow is depleted (15), and the fitted BNF(t) is essentially constant at its lowest level Bnadir for several days (e.g., Fig. 2A and B—notice that the scale is logarithmic). Hence, the time-dependent system may be compared with one having a piecewise constant BNF(t) with solutions that are "glued" from the corresponding pieces of constant BNF(t) solutions. These arguments suggest, and Fig. 4 shows, that at the nadir phase of severe neutropenia, with no G-CSF treatment, the neutrophil level converges to a quasi-equilibrium state (*orange*). Furthermore, at the nadir, SG only transiently elevates this level (*blue*). A significant change occurs if G is held fixed at a high value (*green, magenta*). Then, N does not decrease toward the natural equilibrium point of the system but instead is forced to increase (in ~16 hours; see Fig. 3) toward a significantly higher point. This is the point at which the neutrophil flux, driven by the high (fixed) G-CSF level, exactly balances the neutrophil elimination rate (*dashed green curves* in Figs. 3 and 4). The resulting neutrophil level is calculated as N = AMC × N^*, and implies that the best treatment strategy is to keep G-CSF high as long as BNF(t) is still low.

Figures 1 and 2 (that include clinical data sets) show that the above description of the N response to the various G protocols is also applicable to the transient BNF(t) that appears in the clinical setting.

Specific clinical scenarios. To determine the value of AMC in a chemotherapy patient with a newly diagnosed neutropenia, the clinician should first identify, according to the treatment history, whether the patient arrives at the nadir (s1), while the marrow is still depleting (s2), or at the marrow recovery stage (s3). These three main scenarios correspond to three different phases of the transient behavior of the *GN* system:

s1. When the patient arrives at the nadir so the neutrophils and the G had already settled to their quasi-equilibrium value, it can be shown that AMC $\approx \frac{k_G + G}{k_G/k_{NEF} + G} \frac{N}{N*}$ where $k_G \approx 5{,}000$ pg/mL and $k_{NEF} \approx 10$ are parameters (that may be patient dependent as discussed next). Accordingly, if G and N are known, then AMC may be estimated by this formula. If the G measurement is not available (as is often the case), we propose to use the averaged value. We find that patients with a given

low value of N in a population with averaged $G \approx 1{,}500$ pg/mL (16) have:

$$\mathrm{AMC} \approx 3.35 \frac{N}{N^*} \qquad \text{(B)}$$

with a small variance that is proportional to N/N^* (even when G has a wide log-normal distribution; see Supplementary data). Noteworthy, the variability in k_{NEF} turns out to have only a minor effect: for $k_{\mathrm{NEF}} \varepsilon$ [8–16] and $k_{\mathrm{G}} \varepsilon$ [3,000–6,000 pg/mL] the prefactor in Eq. B becomes [2.5–4.2]. The treatment

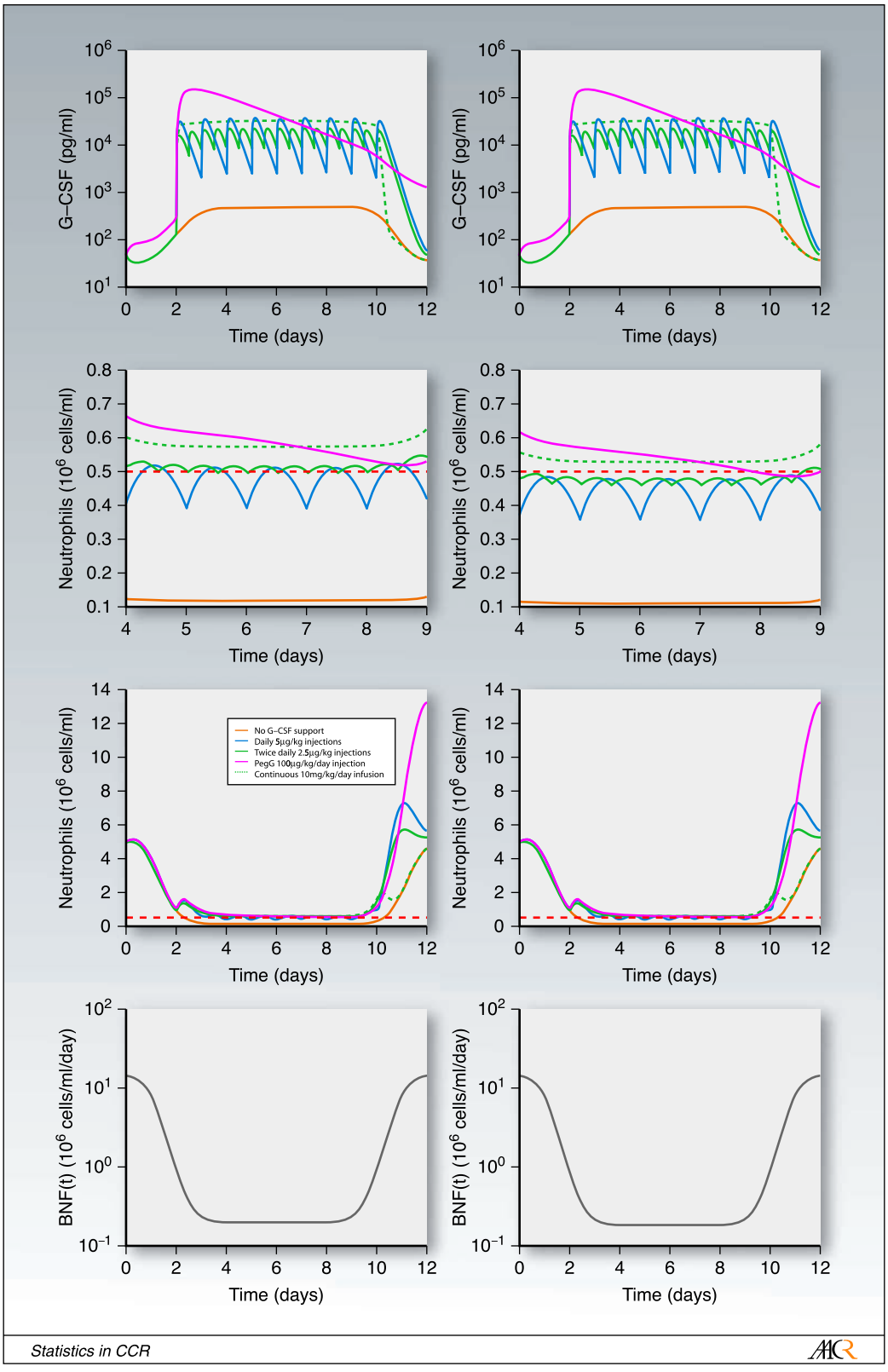

Fig. 4. Simulating treatments by the four different G-CSF regimens in two hypothetical prolonged G2 neutropenic patients, with Tstart - Tstop = 10 d, $\beta_{1\text{-}2}$ = 0.166 [1/d], AMC = 0.13 (*left*), and AMC = 0.12 (*right*). Without treatment, both patients would have severe prolonged neutropenia (*orange*). For the first patient (*left*), a sequence of 5 μg/kg daily (s.c.) injections (*blue*) leaves the patient neutropenic for a few hours everyday. Yet, two 2.5 μg/kg daily (s.c.) injections (*solid green*), a continuous 10 μg/kg/d infusion (*dashed green*), or a single pegG injection (*magenta*) will do. For the second patient (*right*), who is on the lower G2 grade, only the sustained regimens work.

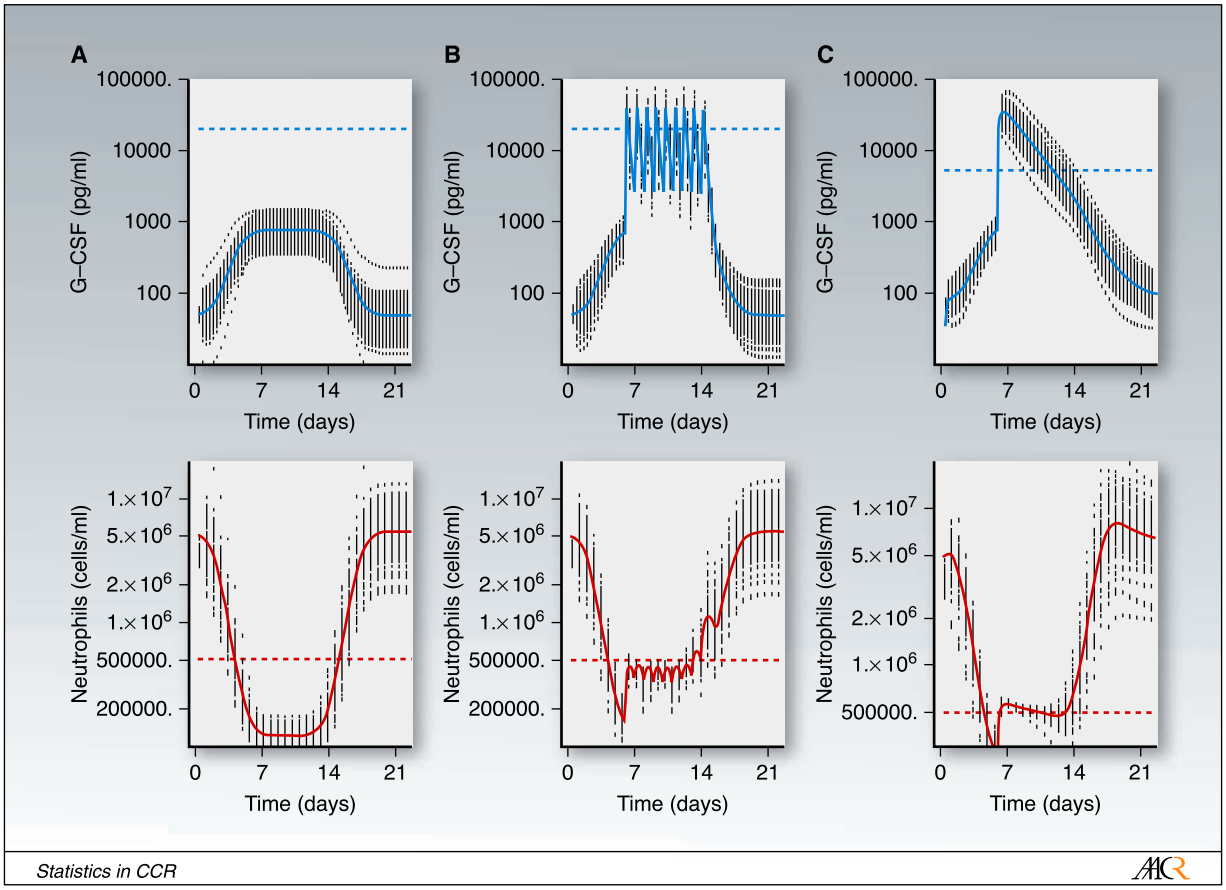

Fig. 5. Clinical trial simulation of neutropenia treatment by placebo (*A*) and two G-CSF regimens: the standard multipulsed (s.c.) *G* (*B*) and a single pegG (*C*). AMC = 0.11 and all other parameter values are independent log-normally distributed with $\sigma \approx 1.2$; mean values are as in Supplementary Table S2. Tstop - Tstart = 15 d and $\beta_{1,2} = 0.08$ [1/d]. Note the decrease in neutrophil level variability at the nadir for the pegG group. We note that log-normal distribution is a plausible choice of biological parameters (11). We take the parameter distributions to be independent. We note that the dynamics of the model with averaged parameter values seems to be similar to the averaged dynamics over an ensemble of parameters (thus, here, parameter fitting from the published averaged clinical data sets may be justified).

strategy for this case is explained in the "New grading of neutropenic patients in the s1 scenario" section.

s2. A more subtle case to consider is when the patient arrives with severe neutropenia ($N \leq 300 \times 10^3$ cells/mL) while the marrow is still depleting (usually during the first week after chemotherapy). In this case, it is not possible to estimate AMC (yet, it is always smaller than $k_{NEF}N/N^*$). Here it is best to fix *G* beyond 10,000 pg/mL until the neutrophil level settles (when the nadir is reached). Then, due to the high *G* levels that are held, AMC $\approx N/N^*$.

s3. Finally, consider the case at which the patient arrives while the marrow is recovering (during the third week after chemotherapy) yet the neutropenia is still severe (so $N \leq 300 \times 10^3$ cells/mL). Here SG should suffice.

New grading of neutropenic patients in the s1 scenario. We see that the potential for neutrophil recovery by G-CSF is determined by the AMC value of the patient.

Specifically, a patient arriving with a prolonged neutropenia (s1 scenario) should be assigned, according to the *N* blood counts, to one of the following three grades: G1 are the favorable patients, with $N \approx 300 \times 10^3$ to 500×10^3 cells/mL [AMC ε (0.13, 1)]. These may do with little *G* intervention. G2 are the salvageable patients, with AMC ≈ 0.12. These patients require $G_{Crit} \gtrsim 10{,}000$ pg/mL at all times [including patients with $50 \leq N \leq 300 \times 10^3$ cells/mL in this category suffices; a patient arriving with an *N* count out of this range has a small probability of having an AMC ε (0.1, 0.13); see Supplementary data]. Recall that the G-CSF critical level G_{Crit} for the G2 group cannot be maintained by SG (see Fig. 1). Finally, G3 are the feeble patients, with $N < 50 \times 10^3$ cells/mL (in fact, if $N < 30 \times 10^3$ cells/mL, then AMC ≤ 0.1 even for $k_{NEF} = 16$). The neutropenia in this group is not salvageable by G-CSF alone (see Fig. 2). Notably, the {G2, G3} category includes all high-dose chemotherapy patients and a significant ≈2% of adjuvant breast cancer patients (17). These are the patients for which the current treatment regimen should be altered.

Indeed, a portion of the G2 group (i.e., patients with $N \leq 100 \times 10^3$ cells/mL) is identified as high risk in the current American Society of Clinical Oncology guidelines (2) and thus should be considered for G-CSF support by these recommendations. Yet, we predict that SG, which is useful in neutropenia prevention (acting efficiently on the G1 grade; ref. 18), will not be useful in this case of G2 neutropenia. On the other hand, we predict that the critical level of G-CSF (G_{Crit}) that is required for these patients can be readily obtained by the alternative clinically available G-CSF regimens. For the favorable G2 patients ($N \approx 300 \times 10^3$ cells/mL), an efficient and economical two 2.5 μg/kg daily (s.c.) injections may suffice (see Fig. 4). For

intermediate G2 patients, two 5 μg/kg daily (s.c.) injections should be sufficient. For the lower borderline G2 patients, the required high G-CSF concentrations can be obtained by either CG [a more demanding protocol (19) that is seldom used] or pegG.

No improvement in the neutrophil counts beyond N_{tr} within 24 hours would indicate a G3 grade. For such patients, a larger G-CSF dose may not have a significant immediate effect on the neutrophils, and additional measures for the treatment of the severe neutropenia [whole-blood (20) or neutrophils (21) infusions] could be considered.

We note that here we focus on the immediate effects of G-CSF in the potentially lethal situation of severe neutropenia. The long-term effects of G-CSF regimens on the marrow processes were not modeled here.

Supporting clinical evidence. Analysis of reported clinical data sets of patients receiving high-dose chemotherapy supports the adequacy of the model with transiently depleted BNF(t) to describe the effect of G regimens, chemotherapy, and stem cell transplantation on the neutrophil counts (Fig. 2). Here BNF(t) is individually fitted depending on the specific treatment protocol while all the other model parameters are fixed (see Supplementary data). The observations made are as follows:

(a) The standard (pulsed) G-CSF regimen is not sufficient to maintain G-CSF levels beyond 10,000 pg/mL. Indeed, the simulated curves of G in Fig. 2A and the data points in Fig. 1A clearly show that everyday G drops to levels that are <4,000 pg/mL (due to the strong oscillations, the clinical data points of G depend sensitively on the time of the measurements).

(b) No G-CSF concentration is effective while the patient is in the G3 state. The neutrophil counts are only slightly changed by the G injections in the nadir period (Fig. 2A) and these counts remain low even when G is kept beyond the staggering 30,000 pg/mL levels as in Fig. 2B and C.

A Monte-Carlo simulation of G-CSF treatment. The results of the Monte-Carlo simulation of three treatment arms is shown in Fig. 5. One hundred G2 patients with a low and fixed AMC = 0.11 and a large variation in all the other parameters were simulated in each arm (see Materials and Methods). The average advantage of the sustained G-CSF treatment over the SG protocol for these patients is maintained even in this highly variant sample (noticeable on the logarithmic scale). This observation is confirmed and quantified in Fig. 6, where the probability distributions of the AUC_{500} (the area under the curve of the neutropenic state) in the three arms are compared. The Kolmogorov-Smirnov test clearly indicates that the AUC_{500} distribution in the pegylated pegG arm is significantly lower than that of the SG arm ($P \leq 0.001$). This simulation may be envisioned as a hypothetical clinical trial in which patients with low neutrophil counts corresponding to the lower G2 grade are randomized to pegG, SG, or placebo. Indeed, in Supplementary data, we show that the G2 patients (defined by their neutrophil counts at the nadir) have a high probability of having AMC in the (0.1, 0.13) range. Finally, we estimate the risk of infection (IR) in each treatment arm. Indeed, this risk is strongly correlated with both the extent and duration of neutropenia as characterized by the AUC_{500} (14). Crawford et al. (1) reported the risk of infectious episode as a function of the duration of the neutropenia (in days) in leukemia patients with neutrophil counts $\geq 100 \times 10^3$. From these data we calculate that the AUC_{500} at which the risk of infection is >20% is $\sim 2 \times 10^6$ days × cells/mL. We use the distribution of the AUC_{500} in our Monte-Carlo trial to calculate P, the right-tailed probability of having AUC $\geq$ AUC_{500} that represents the infection risk (Fig. 6). We find that almost all G2 patients in the control arm are at a significant risk for infection ($P = 0.99$; IR, 20%), so such patients should definitely be treated by some G-CSF regimen. Whereas the risk of infection for SG is only somewhat reduced ($P = 0.85$; IR, 17%), the pegG regimen cuts the risk dramatically to ($P = 0.01$; IR, 0). These effects are prominent for patients in this borderline G2 group (AMC $\approx$ 0.11), stressing the importance of the sustained regimen for these patients.

The robustness of these results to the demographic and clinical characteristics of the potential patients is further verified by repeating the Monte-Carlo simulations with different values

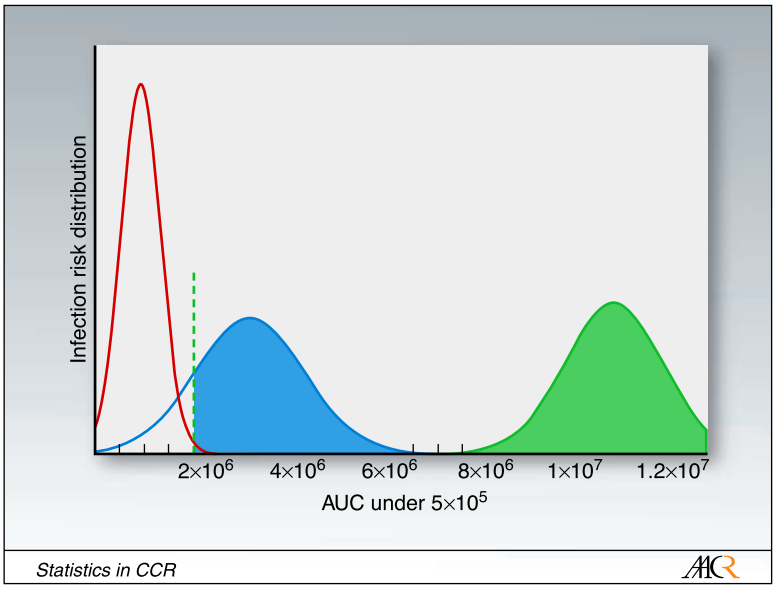

Fig. 6. Probability distributions for the area under the curve, AUC_{500}, which is correlated with the risk of infection, for the three arms of Fig. 5. The shaded areas provide the probability that $AUC_{500} \geq 2 \times 10^6$ [days × cells/mL], taken as the probability to have a risk of infection ≥20%. The risk of infection in the pegG arm (red shade) is significantly lower ($P \leq 0.001$, Kolmogorov-Smirnov test) than in the other two arms: the standard G-CSF injections arm (*blue shade*) and the placebo arm (*green shade*).

of the parameters mean (e.g., changing the patients' averaged weight to the female average of 60 kg)—no significant changes in the figures are observed as long as AMC is kept near its critical value of 0.11. Taking AMC values outside the (0.1, 0.13) range leads, as predicted, to completely different results: The SG treatment is sufficient for most patients with AMC $\geq$ 0.13, whereas no treatment is sufficient for most patients with AMC $\leq$ 0.1 (see Supplementary data).

Discussion

The current American Society of Clinical Oncology guidelines do not advise a routine use of standard SG G-CSF regimens in neutropenia. Our findings explain this advise: SG fails to significantly alter the *GN* dynamics of severe neutropenia (the G2,G3 groups). The guidelines further urge to identify high-risk patients who will benefit from G-CSF. Here we suggest a clinical grading system that identifies the patients that may belong to this class. Specifically, we suggest that the patients that can be categorized to the G2 group are both high risk and can benefit from the appropriate G-CSF regimens. Formally, the grading of a patient is determined by the patient's AMC level, the newly proposed indicator of the marrow capacity. We have shown that the neutrophil count at the nadir is a convenient, readily available, albeit somewhat approximate, indicator of the AMC. Better estimate of the AMC value for an individual neutropenic patient may be found if both the G-CSF and neutrophil blood levels are measured simultaneously (see Supplementary data). Choosing all patients with neutrophil counts of $N = 50 \times 10^3$ to 300×10^3 cells/mL (0.05×10^9-0.3×10^9 cells/L) at the nadir will include, with high confidence, all the patients that belong to this G2 group. For these patients, we propose to maintain a high level of G-CSF until a prompt neutrophil recovery is observed.

The findings reported here are robust to patient variability and fit well the relevant human data sets we have encountered. In the current study, the demographic and clinical characteristics of the potential patients are embedded in the wide distribution of the parameters of the Monte-Carlo simulations. Although, in a specific cohort, the patient characteristics may affect the results, we have shown that, overall, these will be rather insensitive to parameter variations.

Our most important conclusion is that sustained high G-CSF levels (beyond 10,000 pg/mL) may be critical for the success of the treatment of severe neutropenia for patients having neutrophil counts of $N = 50 \times 10^3$ to 300×10^3 cells/mL at the nadir (the G2 patients). Such G-CSF-levels are not achieved by the standard daily G-CSF injections, but may be achieved by bidaily G-CSF injections (filgrastim 5 μg/kg/d), continuous G-CSF administration (filgrastim 10 μg/kg/d), or a single injection of pegfilgrastim 100 μg/kg. Whereas G-CSF sustained regimens may have rare long-term side effects (22), the potential for an efficient treatment of a highly dangerous infection-prone situation (1) should outweigh the risk. We note that our analysis focuses on the acute post-chemotherapy phase and not on the long-term bone marrow dynamics. The long-term dynamics may need to be considered if, for example, the patient is planned to have subsequent chemotherapy treatments.

The definitive clinical effect and the economic implications of the proposed treatment strategies are to be examined empirically. We strongly believe that a carefully designed randomized clinical trial comparing between the standard regimen and the sustained G-CSF regimen in high-risk severe neutropenia should be initiated. From the present analysis, we propose that such a trial should aim at patients with neutrophil counts of $N = 50 \times 10^3$ to 300×10^3 cells/mL at the nadir. Measuring the G-CSF levels at the nadir in these patients may allow for a better estimate of their AMC and a sharper distinction between the proposed three neutropenic grades.

Disclosure of Potential Conflicts of Interest

E. Schochat is on the Advisory Board of Teva Pharmaceutical Industries.

Acknowledgments

We thank Roy Malka, Solomon Stemmer, Edriss Titi, and Lyubomyr Zdomskyy for their insightful comments.

References

1. Crawford J, Dale DC, Lyman GH. Chemotherapy-induced neutropenia: risks, consequences, and new directions for its management. Cancer 2004;100:228–37.
2. Smith TJ, Khatcheressian J, Lyman GH, et al. 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. J Clin Oncol 2006;24:3187–205.
3. Bennett CL, Weeks JA, Somerfield MR, et al. Use of hematopoietic colony-stimulating factors: comparison of the 1994 and 1997 American Society of Clinical Oncology surveys regarding ASCO clinical practice guidelines. Health services research committee of the American Society of Clinical Oncology. J Clin Oncol 1999;17:3676–81.
4. Garcia-Carbonero R, Mayordomo JI, Tornamira MV, et al. Granulocyte colonystimulating factor in the treatment of high-risk febrile neutropenia: a multicenter randomized trial. J Natl Cancer Inst 2001;93:31–8.
5. Johnston E, Crawford J, Blackwell S, et al. Randomized, dose-escalation study of SD/01 compared with daily filgrastim in patients receiving chemotherapy. J Clin Oncol 2000;18:2522–8.
6. Fenk R, Hieronimus N, Steidl U, et al. Sustained G-CSF plasma levels following administration of pegfilgrastim fasten neutrophil reconstitution after high-dose chemotherapy and autologous blood stem cell transplantation in patients with multiple myeloma. Exp Hematol 2006;34:1296–302.
7. Shochat E, Rom-Kedar V, Segel L. G-CSF control of neutrophil dynamics in the blood. Bull Math Biol 2007;69:2299–338.
8. Wang B, Ludden TM, Cheung EN, Schwab GG, Roskos LK. Population pharmacokinetic-pharmacodynamic modeling of filgrastim (r-metHuG-CSF) in healthy volunteers. J Pharmacokinet Pharmacodyn 2001;28:321–42.
9. Holmes FA, Jones SE, O'Shaughnessy J, et al. Comparable efficacy and safety profiles of once-per-cycle pegfilgrastim and daily injection filgrastim in chemotherapy-induced neutropenia: a multicenter dose-finding study in women with breast cancer. Ann Oncol 2002;13:903–9.
10. Friberg LE, Henningsson A, Maas H, Nguyen L, Karlsson MO. Model of chemotherapy-induced myelosuppression with parameter consistency across drugs. J Clin Oncol 2002;20:4713–21.
11. Limpert E, Stahel WA, Abbt M. Log-normal distributions across the sciences: keys and clues. Bioscience 2001;51:342–52.
12. Erickson LC, Bradley MO, Ducore JM, Ewig RA, Kohn KW. DNA crosslinking and cytotoxicity in normal and transformed human cells treated with antitumor nitrosoureas. Proc Natl Acad Sci U S A 1980;77:467–71.
13. Sandstrom M, Lindman H, Nygren P, Lidbrink E, Bergh J, Karlsson MO. Model describing the relationship between pharmacokinetics and hematologic toxicity of the epirubicin-docetaxel regimen in breast cancer patients. J Clin Oncol 2005;23:413–21.
14. Minami H, Sasaki Y, Watanabe T, Makoto O. Pharmacodynamic modeling of the entire time course of leukopenia after a 3-hour infusion of paclitaxel. Jpn J Cancer Res 2001;92:231–8.

15. Fliedner TM, Graessle D, Paulsen C, Reimers K. Structure and function of bone marrow hemopoiesis: mechanisms of response to ionizing radiation exposure. Cancer Biother Radiopharm 2002;17:405–26.

16. Cebon J, Layton JE, Maher D, Morstyn G. Endogenous haemopoietic growth factors in neutropenia and infection. Br J Haematol 1994;86:265–74.

17. Shapiro CL, Recht A. Side effects of adjuvant treatment of breast cancer. N Engl J Med 2001;344:1997–2008.

18. Clark OA, Lyman GH, Castro AA, Clark LG, Djulbegovic B. Colony-stimulating factors for chemotherapy-induced febrile neutropenia: a meta-analysis of randomized controlled trials. J Clin Oncol 2005;23:4198–214.

19. Layton JE, Hockman H, Sheridan WP, Morstyn G. Evidence for a novel *in vivo* control mechanism of granulopoiesis: mature cell-related control of a regulatory growth factor. Blood 1989;74:1303–7.

20. Woll PJ, Thatcher N, Lomax L, et al. Use of hematopoietic progenitors in whole blood to support dose-dense chemotherapy: a randomized phase II trial in small-cell lung cancer patients. J Clin Oncol 2001;19:712–9.

21. Price TH, Raleigh A, Bowden RA, et al. Phase I/II trial of neutrophil transfusions from donors stimulated with G-CSF and dexamethasone for treatment of patients with infections in hematopoietic stem cell transplantation. Blood 2000;95:3302–9.

22. Hershman D, Neugut AI, Jacobson JS, et al. Acute myeloid leukemia or myelodysplastic syndrome following use of granulocyte colony-stimulating factors during breast cancer adjuvant chemotherapy. J Natl Cancer Inst 2007;99:196–205.

23. Chatta GS, Price TH, Allen RC, Dale DC. Effects of *in vivo* recombinant methionyl human granulocyte colony-stimulating factor on the neutrophil response and periph-eral blood colony-forming cells in healthy young and elderly adult volunteers. Blood 1994;84:2923–9.

24. Testa U, Martucci R, Scambia G, et al. Autologous stem cell transplantation: exogenous granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor modulate the endogenous cytokine levels. Blood 1997;89:2615–7.

25. Sheridan WP, Morstyn G, Wolf M, et al. Granulocyte colony-stimulating factor and neutrophil recovery after high-dose chemotherapy and autologous bone marrow transplantation. Lancet 1989;2:891–5.

26. Roskos LK, Lum P, Lockbaum P, Schwab G, Yang BB. Pharmacokinetic/pharmacodynamic modeling of pegfilgrastim in healthy subjects. J Clin Pharmacol 2006;46:747–57.