*Cytotechnology* **28:** 157–162, 1998.



# Population balance model of *in vivo* neutrophil formation following bone marrow rescue therapy

L. K. Nielsen[1]*, J. G. Bender[2], W. M. Miller[3] & E. T. Papoutsakis[3]
[1] *Department of Chemical Engineering, The University of Queensland, Brisbane, QLD 4072, Australia*
*E-mail: LarsN@cheque.uq.edu.au*
[2] *Nexell Therapeutics Inc, Irvine, CA, U.S.A.*
[3] *Department of Chemical Engineering, Northwestern University, IL, U.S.A.*

Received 26 August 1998; accepted 26 August 1998

*Key words:* bone marrow rescue therapy, *ex vivo* expansion, *in vivo* model, neutropenia

**Abstract**

In this paper, we develop a simple four parameter population balance model of *in vivo* neutrophil formation following bone marrow rescue therapy. The model is used to predict the number and type of neutrophil progenitors required to abrogate the period of severe neutropenia that normally follows a bone marrow transplant. The estimated total number of 5 billion neutrophil progenitors is consistent with the value extrapolated from a human trial. The model provides a basis for designing *ex vivo* expansion protocols.

*Abbreviations:* HDT – high dose therapy; BMT – bone marrow transplantation; G-CSF – Granulocyte-Colony Stimulating Factor.

## Introduction

Traditional chemo- and radio-therapies are dose limited due to haematological toxicity (Maraninchi, 1993). Employing 'bone marrow' transplantation (BMT) to restore haematopoiesis (blood cell formation) after therapy enables the use of high dose therapies (HDTs), i.e., therapies using 2–10 times the normal dose.

The haematopoietic tissue used for BMTs is increasingly precursor cells collected from the patient prior to HDT through mobilisation and aphaeresis (Figure 1a). The precursor cells include so-called stem cells capable of fully restoring the haematopoietic system. Mobilisation is typically achieved using low dose chemotherapy followed by daily cytokine treatment with Granulocyte-Colony Stimulating Factor (G-CSF). After approximately 10 days, the blood is enriched in precursor cells, which are collected in the mononuclear cell fraction through aphaeresis. The collected cells can be further enriched for precursor cells through CD34 sorting (precursors contain the CD34 surface marker, while other mononuclear cells do not). The final product is frozen and stored. After recovery from the mobilisation process, patients undergo HDT followed by transplantation of the stored cells (Figure 1b). After transplantation it is now common to use cytokine treatment – in particular G-CSF – to accelerate engraftment.

Despite significant improvements in BMT, patients suffer a 5–10 day period with severe neutropenia (less than 500 neutrophils per $\mu$L) (Figure 2). Neutropenia is associated with a high risk of infection, a significant cause of acute morbidity and mortality after BMT. It has been speculated that additional transplantation of more mature neutrophil progenitors may abrogate this problem and that these progenitors could be produced by *ex vivo* expansion of (part of) the haematopoietic tissue used in the BMT.

Initial human trials using cells expanded under non-optimised conditions have not led to significant reduction in neutropenia (Brugger et al., 1995; Williams et al., 1996). These trials, however, did

* Author for all correspondence.

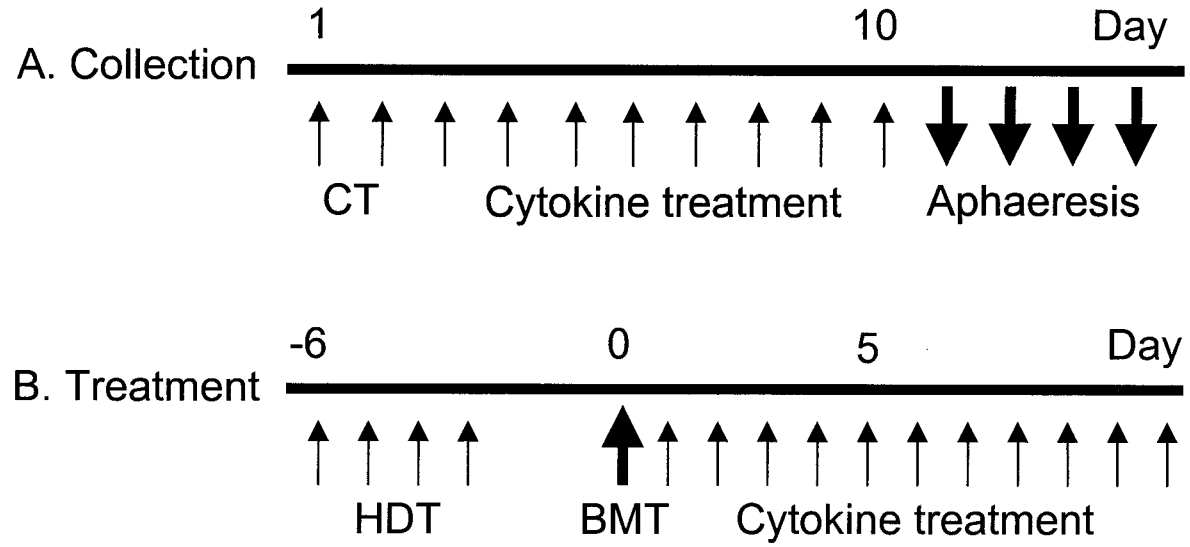


*Figure 1.* Collection of hematopoietic tissue (A) and treatment (B). See text for details. CT: Chemotherapy; HDT: High Dose Therapy; BMT: Bone Marrow Transplant.

not consider exactly what type of precursors and how many were required to abrogate neutropenia. Haematopoietic tissue was simply cultured for 10–12 days in a medium suitable for neutrophil formation.

Scheding et al. (1996) used a model of haematopoiesis to address the question of what type of precursors and how many are required to abrogate neutropenia. They concluded that ~40 billion neutrophil progenitors (see later) were required to abrogate neutropenia (in this case defined as less than 100 neutrophils per $\mu$L) in a normal patient. This estimate, however, is questionable.

Firstly, the basis for their model is an elaborate model of *homeostasis* in *mice*. Modelling the homeostatic response to a disturbance is very different to modelling engraftment following high dose chemotherapy. It corresponds to trying to model start up behaviour in a chemical plant with a model developed around steady state. The proposed pseudo-control loops are questionable, as is the proposed lumping of a naturally distributed process into 6 stages. These inadequacies of the model were highlighted by the need to assume 'reduced mitotic responsiveness' to make the model fit standard engraftment.

Secondly, the model was reparameterised for human neutrophil formation based on a very limited set of data, including some inaccurate data. For example, they use a neutrophil transit time of 5 hr based on data from the 60s, while a more recent publication – from the same group of people – suggests a transit time of ~24 hr and discusses the discrepancy (Steinbach et al., 1979). The parameters used by Scheding et al. led to the conclusion that under normal conditions neutrophil precursors alone amount to 800 billion cells (400 mL packed cell volume assuming a diameter of ~10 $\mu$m), which appears excessive given that the total system is estimated to contain 'only' 500–1000 billion cells (Koller and Palsson, 1993).

Finally, the simple initial condition used (a fixed reduction in all bone marrow populations) does not reflect the expected initial condition for a patient undergoing high dose therapy followed by BMT.

In this paper, no attempt is made to predict the full kinetics of engraftment. Rather, we use a simple population balance approach to estimate the potential benefit of transplanting additional *ex vivo* expanded neutrophil progenitor cells. The benefit of this approach is that the assumptions made become totally transparent, highlighting where additional information is required.

## Theory

### *Model*

Neutrophil development can be divided into a proliferative stage (P; CFU-G, blasts, promyelocytes, and proliferating myelocytes), a non-proliferative maturation stage (NP; non-proliferating myelocytes, metamyelocytes, and bands), and three stages for segment

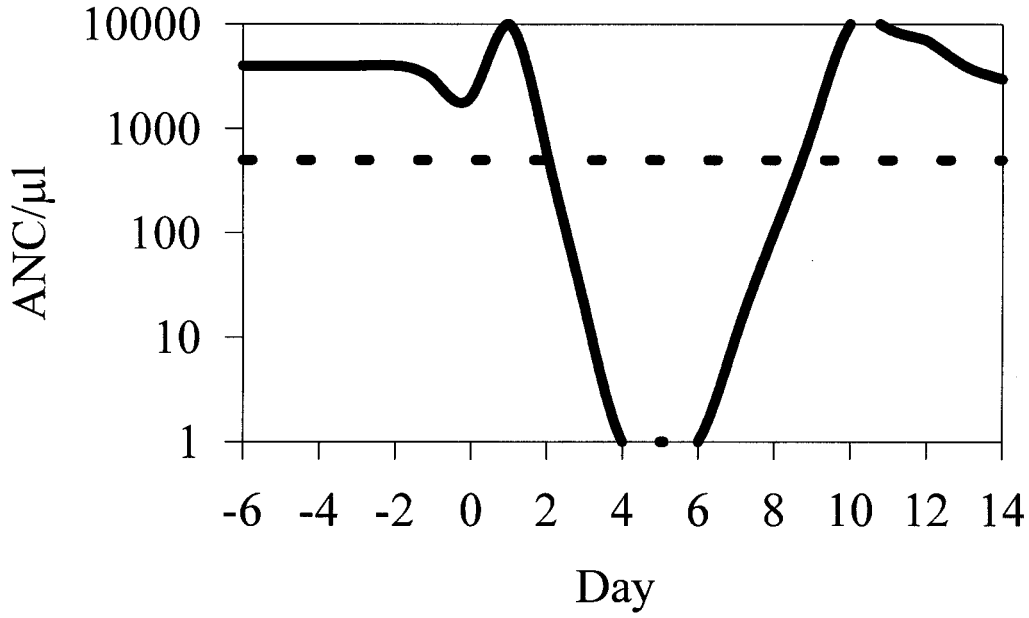


*Figure 2.* Idealised transplantation curve. The neutrophil count (ANC) typically drops slightly after therapy and before the BMT (day 0). The day after BMT – particularly when using G-CSF for recovery – the neutrophil number typically increases significantly, before dropping rapidly to below detection level. In a successful transplant, engraftment commences around day 6–10 and the period with severe neutropenia (dotted line) ends around day 8–12.

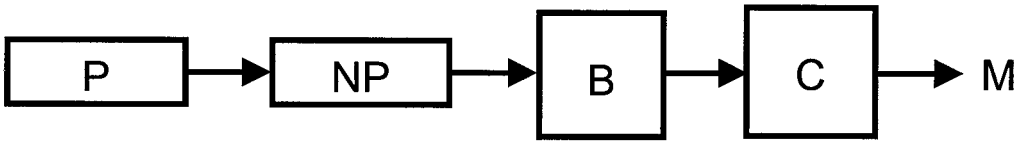


*Figure 3.* Model of neutrophil development (see text).

forms (neutrophils): bone marrow stage (B), circulation stage (C), and marginated stage (M) (Figure 3).

The P and NP stages are distributed stages, i.e., there is a deterministic progression through these stages. In contrast, labelling studies of the transit time of the circulating segment forms show the characteristic exponential decay (rather than linear) corresponding to an undistributed stage (Athens, 1969). In an undistributed stage, a cell has no 'memory' of how long it has been in a stage and can potentially leave the stage immediately after entering it. The three segment form stages will all be treated as undistributed.

The undistributed stages are described with ordinary differential equations

$$\frac{dB}{dt} = r_{NP}^{out} - \frac{B}{\tau_B} \quad (1)$$

$$\frac{dC}{dt} = \frac{B}{\tau_B} - \frac{C}{\tau_C} \quad (2)$$

where $r_{NP}^{out}$ is the rate of cells leaving the non-proliferative precursor stage, $\tau_B$ is mean transit time for the bone marrow segment form stage, and $\tau_C$ is mean transit time for the circulating segment form stage. We do not consider the marginated stage as the kinetics are not well understood due to the inherent difficulty of sampling tissue.

The non-proliferative stage acts as a delay between the proliferative stage and the segment forms. If the transit time is $\tau_{NP}$, we have that

$$r_{NP}^{out}(t) = r_{NP}^{in}(t - \tau_{NP}) \quad (3)$$

Progression through the proliferative stage will be defined in terms of residual number of divisions and the doubling time, $T_2$, which will be assumed constant throughout the stage. Thus, we can define relationships such as

*Table 1.* Parameters for myelopoiesis. Data are derived from Schmitz et al. (1993) and Price et al. (1996)

| | Normal | G-CSF stimulated |
|---|---|---|
| | (Days) | |
| $T_2$ | 1 | 0.5 |
| $\tau_{NP}$ | 5 | 2.5 |
| $\tau_B$ | 3 | 0.5 |
| $\tau_C$ | 1 | 1 |

*Table 2.* Estimated number and type of precursors required to produce a flux of $2.5 \times 10^9$ cells day$^{-1}$

| Division | $2^n$ | Effective period | Total cells |
|---|---|---|---|
| 0 | 1 | 1.0–2.5 | $3.8 \times 10^9$ |
| 1 | 2 | 2.5–3.0 | $6.3 \times 10^8$ |
| 2 | 4 | 3.0–3.5 | $3.1 \times 10^8$ |
| 3 | 8 | 3.5–4.0 | $1.6 \times 10^8$ |
| 4 | 16 | 4.0–4.5 | $7.8 \times 10^7$ |
| 5 | 32 | 4.5–5.0 | $3.9 \times 10^7$ |
| 6 | 64 | 5.0–5.5 | $2.0 \times 10^7$ |
| 7 | 128 | 5.5–6.0 | $9.8 \times 10^6$ |
| 8 | 256 | 6.0–6.5 | $4.9 \times 10^6$ |
| 9 | 512 | 6.5–7.0 | $2.4 \times 10^6$ |
| 10 | 1024 | 7.0–7.5 | $1.2 \times 10^6$ |
| 11 | 2048 | 7.5–8.0 | $6.1 \times 10^5$ |
| Total | | | $5.0 \times 10^9$ |

$$r_{NP}^{in}(t) = 2r_{P1}^{out}(t) = 2r_{P1}^{in}(t - T_2) = 4r_{P2}^{out}(t - T_2) = 4r_{P2}^{in}(t - 2T_2) = \ldots = 2^k r_{Pk}^{out}(t-[k-1]T_2) = 2^k r_{Pk}^{in}(t-kT_2) \quad (4)$$

where we have defined division as occurring upon exit of each substage, $r_{Pk}^{out}$ is the rate of cells leaving stage k, and $r_{Pk}^{in}$ is the rate of cells entering stage k.

*Parameters*

Under severe neutropenia and with co-stimulation from exogenous G-CSF, we expect neutrophil development to occur at near maximum rates. There are no data for the kinetics during engraftment, though it is well established that G-CSF accelerates engraftment (Price et al. 1996). We will use the kinetic data for healthy human adults stimulated with G-CSF (Table 1). The parameters have been rounded to nearest half-day, which is a reasonable indication of the variation between the data sets used. G-CSF causes both a decrease in doubling time and reduction in marrow transit time, while cycling time remains approximately constant. As a result, healthy individuals treated with high doses of G-CSF can have a circulating neutrophil count as much as 10 times greater than normal!

The fact that the G-CSF response is not counteracted by some negative regulation response lends credence to the use of parameters for healthy adults to describe neutrophil development during engraftment: the dominant G-CSF signal is the same in the two scenarios. One illustration that the G-CSF effect also dominates during engraftment was illustrated in Figure 2. Chemotherapy ablates the proliferative stages, but the neutrophil count is reasonably maintained during the 6 days up to the BMT drawing on the 8 days of neutrophil reserve in the non-proliferative and bone marrow segmented form stages. Immediately after BMT and onset of G-CSF treatment, the remaining reserve is purged producing neutrophil counts 2–3 times the normal level.

The use of an unchanged 1 day transit time for the circulating stage is more questionable. The kinetics of margination (neutrophils moving from circulation to tissue) is not well understood. One may speculate that after a period of neutropenia there could be a 'sponge effect' with tissue quickly 'absorbing' any circulating segment forms released from the bone marrow. A mechanism for this effect could be other cells (e.g., macrophages) signalling a need for neutrophils to fight infection. In any case, the effect of this parameter will be evaluated.

## Results and discussion

Our objective is to transplant a sufficient number of proliferative and non-proliferative progenitors from the expanded graft to transiently maintain ANC$>$500 $\mu L^{-1}$ until engraftment of the unexpanded graft. The neutrophil reserve will supply a sufficient number of neutrophils for one to two days after the BMT and the unexpanded graft is effective from around day 8. Thus, we need the expanded graft to supply an adequate flux out the non-proliferative stage from day 1 to day 8.

The required flux, $r_{NP}^{out}$, can be determined from the desired circulating cell count (500 $\mu L^{-1}$), the blood

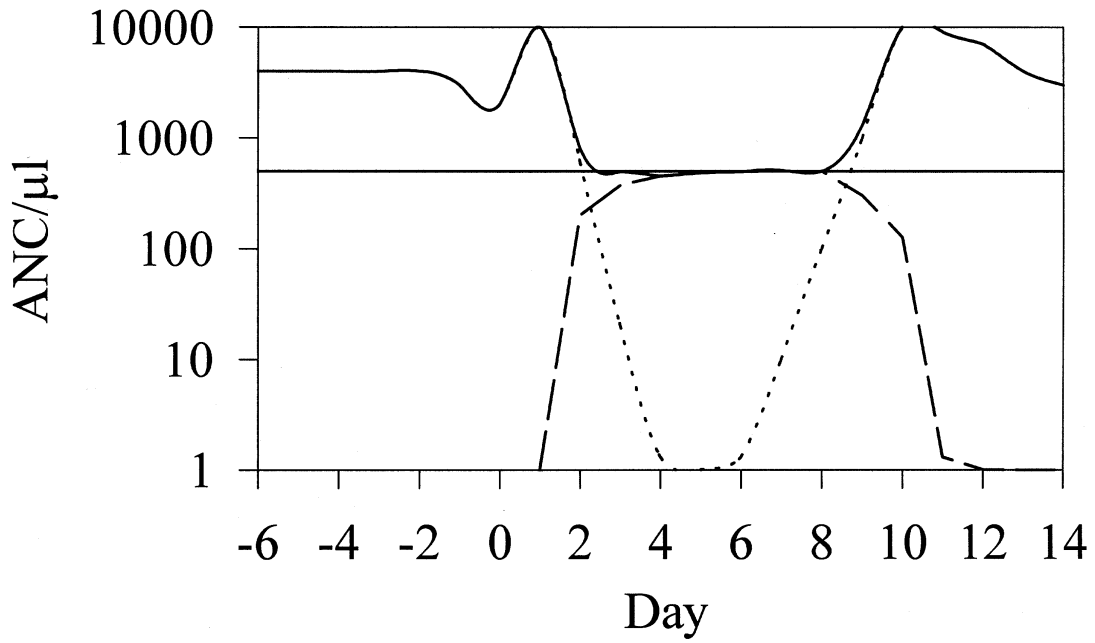


*Figure 4.* Simulated response with expanded cells added. Expanded cells (dashed), unexpanded cells (dotted), and total (solid).

volume ($5 \times 10^6$ $\mu$L), and the blood transit time (1 day):

$$\begin{aligned} r_{NP}^{out} &= \text{desired count} \times \text{blood volume/transit time} \\ &= 500\ \mu\text{L}^{-1} \times 5 \times 10^6\ (1\ \text{day})^{-1} \\ &= 2.5 \times 10^9\ \text{cells day}^{-1} \end{aligned} \quad (5)$$

A total of 5 billion precursors is required to generate this flux from day 1 to day 8 (Table 2). Of these cells, 2/3 are non-proliferative precursors needed to maintain the flux from day 1 to day 2.5. In estimating the numbers in Table 2, a fixed transit time was assumed for bone marrow segmented forms ($\tau_B$ = 0.5 day). Figure 4 shows the dynamics predicted using equations (1) and (2) (i.e., allowing for the natural variation around the mean, $\tau_B$ = 0.5 day, in transit time) together with the idealised normal response curve. The figure illustrates that by transplanting precursor cells that are 1 day away from reaching segmented form, a flux is generated that reaches the desired level of $2.5 \times 10^9$ new circulating segment forms per day approximately 2 days after transplantation, i.e., at the time where the neutrophil reserve is rapidly being depleted.

The estimates in Table 2 and Figure 4 are based on a transit time of one day for circulating neutrophils, which as mentioned may or may not reflect the situation during engraftment. The estimates are inversely related to the transit time, i.e., if the true transit time is 0.5 day the total number of expanded cells required is 10 billion. The true transit time can only be established through *in vivo* studies, e.g., using tritiated thymidine (Price et al., 1996).

The estimate in Table 2 is consistent with a cell number extrapolated (to be submitted) from the University of Chicago/Baxter trial with expanded cells (Williams et al., 1996). Although the trial failed to demonstrate total abrogation of neutropenia, the relative neutropenic risk (defined as the integral time under 500 neutrophils per $\mu$L) did decrease with increasing expanded cell dose. Linear extrapolation led to an estimated requirement of ~20 billion expanded cells to overcome neutropenia (the highest actual dose was 14 billion). Of these cells, approximately a third are of the progenitor type discussed in this paper, leading to an estimate of 6–7 billion neutrophil precursors required.

The model provides a basis for deciding what types of progenitors are required for abrogating neutropenia and hence a basis for optimising the *ex vivo* expansion cultures. It may also be useful for optimising the HDT/BMT/G-CSF regimen used for treatment. If we can delay the initial onset of neutropenia by 1–2 days (e.g., reducing the clearance period between HDT and BMT or by delaying G-CSF treatment), the need for non-proliferative progenitors in the expanded

cell population disappears and thereby 2/3 of the cell requirements. Finally, the model highlights the effect of the four parameters introduced, thus identifying areas of further research.

## Acknowledgments

This work is supported by National Institutes of Health (U.S.A.) Grant R01 HL48276.

## References

Athens JW (1969) Granulocyte Kinetics in Health and Disease. In: Human Tumor Cell Kinetics, S Perry (ed.) Bethesda, MD: U.S. National Cancer Institute, 1969.

Brugger W, Heimfeld S, Berenson RJ, Mertelsmann R and Kanz L (1995) Reconstitution of hematopoiesis after high-dose chemotherapy by autologous progenitor cells generated *ex vivo*. N Engl J Med 333: 283–287.

Chatta GS, Price TH, Allen RC and Dale DC (1994) Effects of *in vivo* recombinant methionyl granulocyte colony-stimulating factor on the neutrophil response and peripheral blood colony-forming cells in healthy young and elderly adult volunteers. Blood 84: 2923–2929.

Koller MR and Palsson BO (1993) Tissue engineering: reconstitution of human hematopoiesis *ex vivo*. Biotech Bioeng 42: 909–930.

Maraninchi D (1993) The clinical consequences of haematological and non-haematological toxicity following bone marrow transplantation amd the possible impact of haematopoietic growth factors. Bone Marrow Transplantation, 11(2), 12–22.

Price TH, Chatta GS and Dale DC (1996) Effect of recombinant granulocyte colony-stimulating factor on neutrophil kinetics in normal young and elderly humans. Blood 88: 335–340.

Scheding S, Franke H, Brugger W, Kanz L and Schmitz S (1996) How many myeloid progenitors have to be transplanted to completely abrogate neutropenia after peripheral blood progenitor cell transplantation? Exp Hematol 24: 1044a.

Schmitz S, Franke H, Brusis J and Wichmann HE (1993) Quantification of the cell kinetic effects of G-CSF using a model of human granulopoiesis.

Steinbach KH, Schick P, Trepel F, Raffler H, Dohrmann J, Heilgeist G, Heltzel W, Li K, Past W, Van der Woerd-de Lange JA, Theml H, Fliedner TM and Begemann H (1979) Blut 39: 27–38.

Williams SF, Lee WJ, Bender JG, Zimmerman T, Swinney P, Blake M, Carreon J, Schilling M, Smith S, Williams DE, Oldham F and Van Epps D (1996) Selection and expansion of peripheral blood CD34+ cells in autologous stem cell transplantation for breast cancer. Blood 87: 1687–1691.