Mathematical study on kinetics of hematopoietic stem cells – theoretical conditions for successful transplantation†

Shinji Nakaokaa* and Kazuyuki Aiharab

a FIRST, Aihara Innovative Mathematical Modelling Project, Japan Science and Technology Agency, Institute of Industrial Science, University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan; b Institute of Industrial Science, University of Tokyo, Tokyo, Japan

(Received 28 January 2011; final version received 11 May 2011)

Numerous haematological diseases occur due to dysfunctions during homeostasis processes of blood cell production. Haematopoietic stem cell transplantation (HSCT) is a therapeutic option for the treatment of haematological malignancy and congenital immunodeficiency. Today, HSCT is widely applied as an alternative method to bone marrow transplantation; however, HSCT can be a risky procedure because of potential side effects and complications after transplantations. Although an optimal regimen to achieve successful HSCT while maintaining quality of life is to be developed, even theoretical considerations such as the evaluations of successful engraftments and proposals of clinical management strategies have not been fully discussed yet.

In this paper, we construct and investigate mathematical models that describe the kinetics of hematopoietic stem cell self-renewal and granulopoiesis under the influence of growth factors. Moreover, we derive theoretical conditions for successful HSCT, primarily on the basis of the idea that the basic reproduction number $R_0$ represents a threshold condition for a population to successfully grow in a given steady-state environment. Successful engraftment of transplanted haematopoietic stem cells (HSCs) is subsequently ensured by employing a concept of dynamical systems theory known as ‘persistence’. On the basis of the implications from the modelling study, we discuss how the conditions derived for a successful HSCT are used to link to experimental studies.

Keywords: HSC (haematopoietic stem cell); HSCT (hematopoietic stem cell transplantation); G-CSF (Granulocyte colony stimulating factor); severe congenital neutropenia (SCN); cyclic neutropenia (CN)

1. Introduction

The homeostatic production of blood cells, including platelets, granulocytes and lymphocytes, is controlled by regulation mechanisms such as the transition of hematopoietic stem cells (HSCs) from quiescence to cell proliferation, and decisions in cell division between self-renewal and differentiation. HSCs are situated in specific microenvironments referred to as the stem cell niche, and their populations are maintained by complex cellular and molecular mechanisms [58]. The stem cell niche comprises stromal cells such as fibroblasts, reticular cells, endothelial cells

*Corresponding author. Email: snakaoka@rcai.riken.jp
†Dedicated to Professor Y. Takeuchi’s 60th birthday.
This is a paper based on a talk given at the 3rd China-Japan Colloquium of Mathematical Biology held in Beijing, October 2010.

ISSN 1751-3758 print/ISSN 1751-3766 online
© 2012 Taylor & Francis
http://dx.doi.org/10.1080/17513758.2011.588343
http://www.tandfonline.com
and osteoblasts [58,78]. Self-renewal of HSCs is believed to occur inside the bone marrow, and the stem cell niche is essential for maintaining the self-renewal capacity of HSCs [68]. HSC quiescence is regulated by thrombopoietin (TPO) and its receptor Mpl [29]. The mobilization of HSCs from the bone marrow into peripheral circulation is controlled by the granulocyte-colony stimulating factor (G-CSF) [40]. Despite the recent extensive progress in HSC biology, dynamic regulation mechanisms underlying the proliferation, quiescence and differentiation of HSCs are largely unknown.

A variety of haematological diseases occur due to dysfunctions during homeostasis processes of blood cell production. Abnormal regulation of blood cell proliferation can cause haematological malignancy. Some haematological diseases also occur as a consequence of congenital immunodeficiency. The population of neutrophils, a type of granulocyte cell, is highest among white blood cells (45–75%) [45]. A patient with neutropenia exhibits an abnormally low number of neutrophils (below 500 cells/ml, as compared with the value of < 2000 cells/ml for normal individuals) [12,45]. Under neutropenia conditions, a patient has a high propensity for bacterial infection [26]. Severe congenital neutropenia (SCN) is an autosomal genetic disorder that occurs soon after birth and continues chronically. Mutation in the ELANE (or ELA2) gene is related to approximately 40–60% of patients with severe SCN and cyclic neutropenia (CN); the latter exhibits periodic oscillations in the neutrophil number [44,45,49]. Recombinant human G-CSF (rhG-CSF) can be used as a therapeutic drug for patients with SCN and CN. Although it is not possible to completely cure congenital neutropenia, the use of G-CSF temporally recovers the number of neutrophils [10].

Haematopoietic stem cell transplantation (HSCT) has been performed as a treatment for some types of haematological malignancy and congenital immunodeficiency [11]. Peripheral blood stem cell transplantation is the most commonly used method; here, stem cells are collected from peripheral blood after G-CSF injection to promote HSC mobilization from bone marrow to blood. The engraftment of transplanted HSCs typically occurs 10–20 days after transplantation [39], and the engraftment result is decided by the absolute neutrophil count [48]. Despite clinical successes, HSCT can be a risky procedure because of potential side effects and complications after transplantation. The occurrence of complications due to the use of high-dose chemotherapy and G-CSF has been reported [40]. Graft-versus-host disease (GVHD) can occur after transplantation, and the transplanted immune cells attack recipient’s tissues [11]. Although it is necessary to develop an optimal regimen for achieving successful HSCT while maintaining quality of life, even theoretical considerations such as evaluations of successful engraftment and proposals of clinical management strategies have not been fully discussed yet.

In contrast to extensive studies on understanding molecular profiles of HSCs, the kinetics of HSCs is less known, possibly because experimental manipulations of HSCs are typically difficult and long-lasting both for in vitro and in vivo assays. Mathematical modelling approaches to estimate the kinetic parameters of HSCs can be helpful to elucidate the homeostasis of blood cell production. Since the number of HSCs in a body of healthy individuals is maintained at an almost constant number, only limited information can be obtained from steady-state conditions. Therefore, the addition of considerable amounts of perturbation has to be effective to obtain the necessary kinetic profiles of HSCs. A possible experimental manipulation for realizing perturbed states is to transplant HSCs into immunodeficient mice and observe mature cell counts such as neutrophils in peripheral blood. Mathematical modeling of dynamical haematological diseases such as cyclic neutropenia is another option for estimating the kinetics of HSCs. Since G-CSF is used both in the treatment of CN and as a pre-treatment option to HSCT, a model of haematopoiesis and granulopoiesis in response to G-CSF should be formulated.

The purpose of this paper is to construct mathematical models of haematopoiesis and granulopoiesis under the influence of growth factors such as G-CSF. Our primary focus is on deriving the theoretical conditions that determine the threshold value for the successful engraftment of
transplanted HSCs. Mathematical validation is realized by using the basic reproduction number $R_0$ that provides a threshold condition for a population to successfully grow in a given steady-state environment [15] and by employing a concept of dynamical systems theory known as ‘persistence’ [35]. The organization of this paper is as follows. In the next section, mathematical models are formulated by delay differential equations (DDEs). In Section 3, we derive the theoretical conditions for successful HSCT. In Section 4, we discuss how the derived conditions for successful HSCT can be used to link to experimental studies. The basic properties of solutions to the DDEs, such as nonnegativeness and ultimate boundedness are summarized in Appendix A.1.

2. Model formulation

The mathematical model to be formulated comprises three ingredients, $F$ representing the concentration of a growth factor; $S$, the population number of hematopoietic stem cells in G0-phase and $N$, the total number of cells in granulocyte lineage. Since most of the mature granulocyte cells comprise neutrophils, hereafter, we identify granulocyte lineage as the population of pre-mature and mature neutrophils. Several types of growth factors are known to influence the maintenance of self-renewal, differentiation with specific lineages and quiescence of HSCs. G-CSF is known as an important growth factor to induce granulopoiesis (see [71] and the references therein). Moreover, G-CSF promotes the mobilization of HSC from bone marrow to peripheral blood [7,51]. This property can be applied to collect peripheral blood stem cells before transplantation. Therefore, it is reasonable to assume that the growth factor $F$ indicates G-CSF. Hence, we implicitly specify the growth factor $F$ as G-CSF but maintain a constant notation.

Let $F(t)$ denote the concentration of the growth factor $F$ at time $t$. The dynamics of the growth factor without loss by consumption from cells is given by

$$\frac{dF}{dt}(t) = g(F(t)).$$  

(1)

We assume that $g$ is continuously differentiable with respect to $F$, and further, that there exists a positive constant $K$ such that

$$g(K) = 0, \quad g(F) > 0 \text{ for } 0 < F < K \quad \text{and} \quad g(F) < 0 \text{ for } F > K. \quad (2)$$

The above assumption includes the following function, known as the resource-type function [72]:

$$g(F) := \sigma_F \left(1 - \frac{F}{K}\right).$$  

(3)

Here $\sigma_F$ denotes the constant supply rate of the growth factor $F$. In the case of G-CSF, the supply is provided by many types of cells such as epithelial cells in response to inflammatory cytokines [4], or exogenously injected from peripheral blood as recombinant human G-CSF (rhG-CSF) [26]. In general $\sigma_F$ is not constant but time dependent if a periodic injection schedule is administrated.

Every solution of Equation (1) under a positive initial condition $F(0) > 0$ satisfies

$$\lim_{t \to \infty} F(t) = K$$  

(4)

as $t \to \infty$. If the degradation rate of the growth factor $F$ is specified as a constant $d_F$, $d_F = \sigma_F / K$, and hence, $K = \sigma_F / d_F$. Two major forms of rhG-CSF to treat neutropenia are filgrastim and polyethylene glycol (PEG)-filgrastim [70]. Since the PEG form has a considerably longer half-life, differences in drug types are represented as differences in parameter values [70].
Let $\gamma_i(F)$ denote the per capita consumption rate of the growth factor $F$ by HSCs ($i = S$) or pre-mature and mature neutrophils ($i = L$), respectively. We assume that $\gamma_i$ is continuously differentiable and satisfies

$$\frac{d}{dF} \gamma_i(F) > 0 \quad \text{and} \quad \gamma_i(0) = 0.$$  

(5)

There are three possibilities for the status of daughter HSCs depending on whether they maintain self-renewal capacity or not [43,57] (see Figure 1 for schematic explanation) as follows:

Type-1 (symmetric self-renewing division): Two daughter cells maintain self-renewal capacity.

Type-2 (asymmetric division): One of the daughter cells differentiates and loses, but the other maintains self-renewal capacity.

Type-3 (symmetric differentiating division) Two daughter cells lose self-renewal capacity and commit to progenitors by differentiation.

Let $p$ denote the probability that when an HSC undergoes cell division, the self-renewal capacity is inherited to a daughter cell. Correspondingly, $1 - p$ is the probability that a daughter cell loses the self-renewal capacity to commit to a progenitor cell. $\rho_S$ and $\rho_L$ denote the expected number of identical and committed daughter HSCs resulting from the cell division of a single HSC, respectively. By definition, it immediately follows that $\rho_S := 2p^2 + 2p(1 - p) = 2p$ and $\rho_L := 2p(1 - p) + 2(1 - p)^2 = 2(1 - p)$. Although the ratio of asymmetric to symmetric division seems to be constant in some situations [42], the fate of daughter cells can be influenced not only by intrinsic-factors but also by extrinsic signals [43]. For example, in [79], it was shown that the frequency of asymmetric cell division can be altered by the expression of leukemogenic proteins. Chronic infection can lead to HSC exhaustion, i.e. a decrease in the number of HSCs caused by excessive replicative stress [2]. Chronic exposure to external signals such as type-I interferons can shift the balance of cell division toward a high propensity to differentiation [67]. Therefore, we consider a situation wherein the growth factor $F$ operates as a promoter for cell differentiation. A microenvironmental niche for HSCs plays a crucial role in the regulation of cell proliferation, survival and differentiation [68]. Attachment to niche cells in bone marrow has been suggested as a mechanism for the maintenance of the self-renewal capacity of HSC [57]. Since G-CSF promotes commitment to granulocyte lineage as well as mobilization of HSCs from bone marrow to peripheral circulation, it is possible that G-CSF promotes differentiating cell division (see [28] and the references therein). In the model ingredient, we replace $p$ with $p(F)$, which is now a decreasing function of $F$. It immediately follows that $\rho_S(F)$ is decreasing and $\rho_L(F)$ is increasing in $F$ as follows:

$$\frac{d}{dF} \rho_S(F) < 0 \quad \text{and} \quad \frac{d}{dF} \rho_L(F) > 0.$$  

(6)

A specific form of $\rho_S$ is given as an example in Section 3.

To formulate the self-renewal division of HSCs, we assume that an HSC is classified either in the resting-phase $S$ ($G_0$- or quiescent- phase) or in the proliferation-phase $P$ ($G_1/S/G_2/M$-phases).

![Diagram of hematopoietic stem cell division](image)
Let $\lambda$ denote the rate of transition from the resting-phase to the proliferation-phase. We assume that the transition rate depends on the concentration of growth factor $F$. From a mathematical point of view, $\lambda(F)$ is an increasing function of $F$. At least for the mice case, this assumption can be justified with G-CSF as the growth factor, since the administration of G-CSF significantly increases cell cycle entry by quiescent HSCs [73]. Further, the transition from the proliferation-phase to the resting-phase includes cell division. The transition period roughly corresponds to the cell cycle period. Although G-CSF might accelerate the cell cycle of HSC, we merely assume that the period is constant (see Section 4 for a more detailed discussion). Let $\tau_S$ denote the cell cycle period. Note that we have implicitly neglected the existence of heterogeneity in a HSC population in terms of the cell division turn-over rate [22]. Loss of cells occurs as a result of cell death. We assume that the death rate of HSCs in the resting- and proliferation-phases is identical and given as a constant $d_S$ (see Figure 2 for an illustration of HSC self-renewal division).

All the above assumptions lead to the following mathematical model:

\[
\frac{d}{dt} F(t) = g(F(t)) - \gamma_S(F(t))S(t),
\]

\[
\frac{d}{dt} S(t) = \rho_S(F(t - \tau_S))\lambda(F(t - \tau_S))e^{-d_S\tau_S}S(t - \tau_S) - \{d_S + \lambda(F(t))\}S(t). \tag{E0}
\]

The initial condition for model Equation (E0) is given by

\[
(\phi_F, \phi_S) \in C \equiv C([-\tau_S, 0]; \mathbb{R}_+^2), \quad \phi_F(\sigma) \geq 0, \quad \phi_S(\sigma) \geq 0, \quad -\tau_S \leq \sigma \leq 0. \quad (I_0)
\]

Note that the well-known G0-model proposed in [52] can be deduced from model Equation (E0) (see Appendix A.3). We have implicitly imposed that the growth factor $F$ is mixed well in bone marrow, peripheral blood and tissues.

We then extend model Equation (E0) to incorporate the population dynamics of neutrophils. The formulation characteristic due to delay (differential) equations is that the population density of neutrophils is represented by the history of $S$. This is a natural consequence of the fact that all the cells in the granulocyte lineage result from a HSC population by cell proliferation and differentiation.

Let the variable $a$ denote the age of cells at the present time $t$, which have committed to the granulocyte lineage at time $t - a$. Progenitor cells in the granulocyte lineage still possess the ability to have limited number of cell divisions. Let $\alpha(a)$ denote the amplification ratio in granulopoiesis. Since G-CSF promotes the proliferation of granulocyte progenitors, $\alpha$ might depend on the concentration of the growth factor $F$ as well. For simplicity, however, we neglect this effect. Note that at the beginning of the commitment, the amplification ratio must be unity. Moreover, it is natural to assume the existence of a maximum amplification ratio. These assumptions can be formulated as follows. There exists a positive constant $A_M$ such that

\[
\frac{d}{da} \alpha(a) > 0, \quad \alpha(0) = 1 \quad \text{and} \quad \lim_{a \to \infty} \alpha(a) = A_M > 1. \tag{9}
\]

![Figure 2. Schematic diagram for self-renewal division of HSCs. The activated HSC in the resting-phase enters the cell cycle (proliferation-phase) with the rate of transition $\lambda(F)$. The cell cycle period is assumed to be a constant $\tau_S$. At the end of the cell cycle, a surviving cell in the proliferation period (multiplied by $e^{-d_S\tau_S}$) generates one or two daughter cells with self-renewal capacity. The expected number of such cells is given by $\rho_S(F)$.](image)
Let $\mathcal{F}_L$ denote the survival probability of neutrophils. As introduced in Section 1, the proliferation and survival of a neutrophil are regulated by G-CSF. Hence the survival of cells in the granulocyte lineage might depend on the concentration of the growth factor $F$. We use the symbol $F_t$ to denote the history of the growth factor relative to $t$, or, in other words, to denote the function

$$\sigma \mapsto F(t + \sigma), \quad \sigma \leq 0.$$ 

If the death rate of granulocyte progenitor cells is given by $\mu_L(a, F_t)$, then $\mathcal{F}_L(a, F_t)$ is represented by [19]

$$\mathcal{F}_L(a, F_t) := \exp \left[ - \int_0^a \mu_L(F_t(-\sigma)) \, d\sigma \right]. \tag{10}$$

Pre-mature neutrophils mobilize from bone-marrow to peripheral blood and tissues after maturation. Assume that it takes a constant time $\tau_N$ for cells to mature after a daughter of an HSC is committed to the granulocyte lineage. In patients with severe congenital neutropenia, maturation arrest is observed at the promyelocyte stage in granulopoiesis [45]. Since the injection of G-CSF can decrease the maturation period [69], $\tau_N$ depends on the concentration of the growth factor $F$.

In such a situation, maturation delay is variable. Although it is possible to incorporate variability in neutrophil maturation, in this paper, we simply assume that $\tau_N$ is constant.

The total number of neutrophil progenitors $N_P$ – in bone marrow – is given by

$$N_P(t) := \int_0^{\tau_N} \rho_L(F_t) \lambda(F_t) \alpha(a) \mathcal{F}_L(a, F_t) S(t - a) \, da. \tag{11}$$

The number of mature neutrophils in peripheral blood and tissues $N_M$ is given as follows:

$$N_M(t) := \int_{\tau_N}^{\infty} \rho_L(F_t) \lambda(F_t) \alpha(a) \mathcal{F}_L(a, F_t) S(t - a) \, da. \tag{12}$$

Equation (12) is known as the absolute neutrophil count (ANC) [45], which can be observed from the collected peripheral blood.

Put $N(t) := N_P(t) + N_M(t)$. To complete the model formulation, it is only sufficient to modify the first equation of model Equation (E0) by adding feedback effects via the consumption of the growth factor by neutrophils:

$$\frac{d}{dt} F(t) = g(F(t)) - \gamma_S(F(t)) S(t) - \gamma_L(F(t)) N(t). \tag{13}$$

The full model comprises Equation (13) for $F$, the second equation of model Equations (E0), and (11) and (12). Note that both Equations (11) and (12) are subordinate to the variables $F$ and $S$. Hence, the dynamics of the full model is completely described by two variables $F$ and $S$ with their histories.

We conclude this section by deriving a special case of the full model. Assume that the death rate of neutrophils is constant and the normalized amplification ratio $\alpha(a)/(A_M - 1) - 1$ is given by a cumulative distribution function of the exponential distribution with rate parameter $d_M$. More precisely,

$$\mathcal{F}_L(a, F_t) := e^{-d_M a} \quad \text{and} \quad \alpha(a) = 1 + (A_M - 1)(1 - e^{-d_M a}). \tag{14}$$
Straightforward calculations yield that the full model is reduced to the following system of DDEs:

\[
\begin{align*}
\frac{d}{dt} F(t) &= g(F(t)) - \gamma_S(F(t))S(t) - \gamma_L(F(t))(N_1(t) - N_2(t)), \\
\frac{d}{dt} S(t) &= \rho_S(F(t))\lambda(F(t))e^{-\frac{dS}{\tau_S}}S(t) - \{dS + \lambda(F(t))\}S(t), \\
\frac{d}{dt} N_1(t) &= -dLN_1(t) + AM\rho_L(F(t))\lambda(F(t))S(t), \\
\frac{d}{dt} N_2(t) &= -(dL + dM)N_2(t) + (AM - 1)\rho_L(F(t))\lambda(F(t))S(t),
\end{align*}
\]

\[\text{(E1)}\]
(see Appendix A.2 for the derivation of model Equation (E1)).

3. Threshold conditions for successful engraftment

In this section, we derive a threshold value known as the basic reproduction number \(R_0\) that determines the engraftment outcome, i.e., whether it is successful.

3.1. Basic reproduction number

From Equation (2), equilibrium \(E_u := (K, 0)\) representing unsuccessful transplantation always exists. Let \(E^* := (F^*, S^*)\) denote a positive equilibrium of model Equation (E0).

The explicit value of \(F^*\) is obtained from the second equation of model Equation (E0) as a root of the following equation:

\[
\rho_S(F^*)\lambda(F^*)e^{-\frac{dS}{\tau_S}} - \{dS + \lambda(F^*)\} = 0.
\]  

(15)

Define \(\psi(a, F)\) as

\[
\psi(a, F) = \rho_S(F)\lambda(F)e^{-\frac{dS}{\tau_S}}e^{-\frac{1}{\sigma_0}\{dS + \lambda(F)\}d\sigma}.
\]

(16)

For constant \(\bar{F}\), \(\psi(a, \bar{F})\) is given as follows:

\[
\psi(a, \bar{F}) = \rho_S(\bar{F})\lambda(\bar{F})e^{-\frac{dS}{\tau_S}}e^{-\frac{1}{\sigma_0}\{dS + \lambda(\bar{F})\}a}.
\]

(17)

Define \(r(F)\) as

\[
r(F) := \int_{0}^{\infty} \psi(a, F) \, da.
\]

(18)

Equation (15) is then equivalent to \(r(F^*) = 1\). From the first equation of model Equation (E1), the explicit value of \(S^*\) is generically given by

\[
S^* = \frac{g(F^*)}{\rho_S(F^*) + \gamma_L(F^*)\int_{0}^{\infty} \rho_L(F^*)\lambda(F^*)\alpha(a)F_L(a, F^*)\, da}.
\]

(19)

Note that \(F^* < K\). Otherwise, \(g(F^*) < 0\), and hence, \(S^* < 0\).

Let us consider a situation wherein HSCs cannot maintain their population if there is no growth factor \(F\). This assumption translates into \(r(0) < 1\). Since \(\rho_S(F)\) is decreasing but \(\lambda(F)\) is increasing in \(F\), the following four generic possibilities are present, as illustrated in Figure 3:
Figure 3. Four possibilities for the existence of positive equilibria $F^*$. The depicted curves are represented by the following specific example: $p(F) = \frac{1}{2}(1 + \sigma F) + \frac{1}{2}$ and $\lambda(F) = mF/(\theta + F) + b$ ($m = 5$, $\theta = 2$, $b = 0.1$, $d_S = 0.1$ and $\tau_S = 3$). The bold-, dashed-, dotted- and dashed-dotted-lines correspond to (i) $\sigma = 0.1$ (weak induction of differentiation), (ii) $\sigma = 1.0$ (moderate induction of differentiation), (iii) $\sigma = 2.0$ (strong induction of differentiation), and (iv) $\sigma = 5.0$ (exhaustion), respectively.

Case-1 (weak induction of differentiation) $y = r(F)$ is monotone increasing and has unique intersection with $y = 1$ on $(0, K]$.

Case-2 (moderate induction of differentiation) $y = r(F)$ has a single-hump and unique intersection with $y = 1$ on $(0, K]$.

Case-3 (strong induction of differentiation) $y = r(F)$ has a single-hump and two intersections with $y = 1$ on $(0, K]$.

Case-4 (exhaustion) $y = r(F)$ is under the line $y = 1$, and hence, no intersection with $y = 1$ exists on $(0, K]$.

Let us assume that the growth factor $F$ is in a steady state. The basic reproduction number $R_0$ is defined as the average number of daughter cells with self-renewal capacity that are reproduced by a single HSC within an expected remaining period in the resting-phase. $R_0$ is regarded as the threshold value for a successful transplantation, since on an average, the population of transplanted HSCs grows over generations, if $R_0 > 1$, while the population declines if $R_0 < 1$. From a mathematical point of view, $R_0$ is defined as the spectral radius of the next generation matrix defined for the linearized system of the main model around an equilibrium point [46]. In our model, $R_0$ is given by

$$R_0 := r(K) = \frac{\rho_S(K)\lambda(K) e^{-d_S\tau_S}}{d_S + \lambda(K)}. \tag{20}$$

Since the shape of $r(F)$ can be single-humped, $F = K$ does not necessarily attain the maximum value of $r(F)$ on $[0, K]$. In Case-2, there exists $F_{\text{max}} \in (0, K]$ such that $r(F)$ attains its maximum value at $F = F_{\text{max}}$. The corresponding threshold quantity is denoted by $R_{\text{max}}$ and given as

$$R_{\text{max}} := r(F_{\text{max}}) = \frac{\rho_S(F_{\text{max}})\lambda(F_{\text{max}}) e^{-d_S\tau_S}}{d_S + \lambda(F_{\text{max}})}. \tag{21}$$
Note that $R_0 = R_{\text{max}}$ in Case 1 while $R_0 < R_{\text{max}}$ in Case 2. In the following two subsections, we will show that the basic reproduction number $R_0$ defines the threshold value for successful engraftment in Case 1 (weak induction of differentiation). However, in Case 2 (moderate induction of differentiation), engraftment is successful if $R_0 > 1$ but the alternative quantity $R_{\text{max}}$ is the threshold value for unsuccessful engraftment. In Case 3 (strong induction of differentiation), there are generically two positive equilibria. Determining the theoretical conditions is difficult because even though $R_0 < 1$, there is still the possibility of successful engraftment in a density-dependent manner; if a considerable amount of HSCs is initially transplanted, then positive feedback effects can support the engraftment of transplanted HSCs. Since experimental validations of such speculations are difficult, we leave this problem unsolved in this paper. Hence, in the following subsections, we assume that there exists at most one root of Equation (15) in the interval $(0, K]$.

### 3.2. Unsuccessful engraftment

If $R_0 < 1$ or $R_{\text{max}} < 1$ when it exists, then there is no positive equilibrium of model Equation (E0) (see curve (iv) of Figure 3). Correspondingly, we obtain the following result for unsuccessful HSCT (see Table 1).

**Theorem 3.1** Assume that $R_0 < 1$ or $R_{\text{max}} < 1$ when it exists. Every solution of model Equation (E0) then converges to $E_u = (K, 0)$ as $t \to \infty$.

**Proof** It is sufficient to prove that

$$\lim_{t \to \infty} S(t) = 0.$$  \hspace{1cm} (22)

In fact, from the first equation of model Equation (E0), $F(t) \to K$ as $t \to \infty$.

Note that for any $\varepsilon > 0$, there exists $T(\varepsilon) > 0$ such that for any $t \geq T(\varepsilon)$,

$$0 < F(t) \leq K + \varepsilon$$  \hspace{1cm} (23)

(see the proof of Proposition A.2). We assume $\varepsilon$ to be sufficiently small to ensure $\psi(a, F_t) \leq \psi(a, F_{\text{max}})$ for each $a \geq 0$ and $t \geq T(\varepsilon) + \tau_S$ (see Figure 3 for graphical representation). Define $G_0(t)$ by $G_0(t) := \int_t^{\infty} \psi(a, F_t)S(t - a) \, da$ and $\Psi(a)$ by $\Psi(a) \equiv 0$ for $0 \leq a < \tau_S$ and $\Psi(a) := \psi(a, F_{\text{max}})$ for $a \geq \tau_S$. For $t \geq T(\varepsilon) + \tau_S$, the equation for $S$ in the integral form is evaluated as follows (see Equation (16)):

$$S(t) = \int_{\tau_S}^{\infty} \psi(a, F_t)S(t - a) \, da$$

$$= G_0(t - T(\varepsilon)) + \int_{\tau_S}^{t - T(\varepsilon)} \psi(a, F_t)S(t - a) \, da$$

$$\leq G_0(t - T(\varepsilon)) + \int_{\tau_S}^{t - T(\varepsilon)} \psi(a, F_{\text{max}})S(t - a) \, da$$

$$= G_0(t - T(\varepsilon)) + \int_{0}^{t - T(\varepsilon)} \Psi(a)S(t - a) \, da$$

$$= G_0(t - T(\varepsilon)) + \int_{T(\varepsilon)}^{t} \Psi(a - T(\varepsilon))S(t - (a - T(\varepsilon))) \, da.$$
### Threshold conditions for (un)successful engraftment of HSCT.

| Condition | Successful (Theorem 3.2) | Unsuccessful (Theorem 3.1) |
|-----------|--------------------------|--------------------------|
| $R_0 < 1$ or $R_{\text{max}} < 1$ | $R_0 > 1$ |

Let $S_M(t) \geq 0$ denote the solution of the following linear renewal equation:

$$S_M(t) = G_0(t) + \int_0^t \Psi(a) S_M(t-a) \, da$$

under the initial condition $S_M(\sigma) = S(\sigma + T(\varepsilon)) \geq 0$. Note that

$$\max\{R_0, R_{\text{max}}\} = \max\{r(K), r(F_{\text{max}})\} = \int_0^\infty \Psi(a) \, da < 1.$$  

Hence, $S_M(t)$ approaches zero as $t \to \infty$ [46]. Since all the solutions of model Equation (E0) under the above initial condition are nonnegative (see Appendix A.1), the comparison theorem for the Volterra integral equations [32, Chapter 12] implies that $S(t) \to 0$ as $t \to \infty$. This completes the proof.

### 3.3. Successful engraftment

In this section, we derive theoretical conditions to ensure successful engraftment of transplanted HSCs. Model Equation (E0) is called ‘uniform persistent’ if there exists $\varepsilon_i > 0$ ($i = 1, 2$) such that

$$\liminf_{t \to \infty} F(t) \geq \varepsilon_1 \quad \text{and} \quad \liminf_{t \to \infty} S(t) \geq \varepsilon_2.$$  

The concept of persistence was first introduced for ecological models to formulate an idea that all species do not go extinct. We apply the mathematical theory of uniform persistence to model Equation (E0) (see [35,41] and [72, Appendix D]). The prerequisites to prove the uniform persistence of model Equation (E0) are summarized in Appendix A.4. If we apply the result in [35] to model Equation (E0), then the ultimate boundedness for solutions should hold. Therefore, we include the conditions for ultimate boundedness (see Proposition A.2 for details). Since white blood cells are significantly reduced in a patient’s body before transplantation, it is reasonable to assume that no neutrophils exist until the cells arising from donor-derived HSC are observed in several tissues (2–4 weeks after transplantation). Hence it may be reasonable to assume that there is no consumption of growth factor $F$ by neutrophils during the process of engraftment. Hence we assume $\gamma_L(F) \equiv 0$ and derive the theoretical conditions for successful engraftment for model Equation (E0). Next, the result for the uniform persistence of model Equation (E1) are derived as a corollary of the following theorem (see Table 1).

**Theorem 3.2 (uniform persistence)** Assume that conditions of Proposition A.2 hold. Model Equation (E0) is uniformly persistent if and only if $R_0 > 1$.

**Proof** The existence of a positive equilibrium $E^*$ is a necessary condition for model Equation (E0) to be uniformly persistent (see [83, Theorem 1.3.7]). Note that there exists at least one positive equilibrium of model Equation (E0) if $R_0 > 1$. Hence the necessity is automatically satisfied. In order to apply Theorem A.3 to semiflow $\Phi$ generated by the solution of model Equation (E0) under initial condition Equation (I0), we set $X_0 = C([-\tau_S, 0], \mathbb{R}_+^2)$. Assumption (i) in Theorem A.3 requires that $\Phi(t)$ is asymptotically smooth; this holds true for a fairly general class of DDEs.
with a finite delay (see [34, P.90, Theorem 6.1 and pp. 113–115], [33]). From Proposition A.2, the solutions of model Equation (E0) are ultimately bounded. In other words, \( \Phi \) is point dissipative and hence assumption (ii) holds. The boundary of \( X_0 \) is defined by
\[
\partial X_0 := \{(\phi_F, \phi_S) \in X_0 : \phi_F(\sigma) > 0, \ \phi_S(\sigma) \equiv 0, \ -\tau_S \leq \sigma \leq 0\}. \tag{25}
\]
Assumption (iii) requires that boundary \( \partial X_0 \) is invariant under \( \Phi \). To prove this assumption, we consider the stable set of \( E_u = (K, 0) \), denoted by \( W^+(\{E_u\}) \). We claim that \( W^+(\{E_u\}) = \partial X_0 \).

In fact, every solution of model Equation (E0) starting at any point in \( \partial X_0 \) must satisfy
\[
\frac{d}{dt} F(t) = g(F(t)) \quad \text{and} \quad S(t) \equiv 0, \ F(0) > 0. \tag{26}
\]
Since \( R_0 > 1, d_s + \lambda(K) < \rho_S(K)\lambda(K)e^{-d_s\tau_s} \). From Equation (26), for sufficiently small \( \epsilon \), there exists \( T(\epsilon) > 0 \) such that
\[
K - \epsilon < F(t) < K + \epsilon \quad \text{and} \quad 0 < S(t) < \epsilon \tag{27}
\]
for all \( t > T(\epsilon) + \tau_S \). From Equation (27), the following inequality:
\[
d_s + \lambda(K + \epsilon) < \rho_S(K + \epsilon)\lambda(K - \epsilon)e^{-d_s\tau_s} \tag{28}
\]
holds for sufficiently small \( \epsilon \). The second equation of model Equation (E0) is evaluated as follows:
\[
\frac{d}{dt} S(t) > -(d_s + \lambda(K + \epsilon))S(t) + \rho_S(K + \epsilon)\lambda(K - \epsilon)e^{-d_s\tau_s}S(t - \tau_S), \quad t \geq T(\epsilon) + \tau_S.
\]
Let \( S_m(t) \) denote a solution of the following linear DDE:
\[
\frac{d}{dt} S_m(t) = -(d_s + \lambda(K + \epsilon))S_m(t) + \rho_S(K + \epsilon)\lambda(K - \epsilon)e^{-d_s\tau_s}S_m(t - \tau_S) \tag{29}
\]
with initial function \( S_m(\sigma) = S(\sigma) \) for \( \sigma \in [T(\epsilon) - \tau_S, T(\epsilon)] \). From the comparison theorem for DDEs [32, Chapter 13], \( S(t) > S_m(t) \) for all \( t > T(\epsilon) \). From Equation (28), the zero solution of Equation (29) is unstable (see Appendix A.5). Hence, there exists a particular initial condition and time \( \tilde{t} > T(\epsilon) + \tau_S \) such that \( S_m(\tilde{t}) \geq \epsilon \). This contradicts Equation (27). Hence, \( W^+(\{E_u\}) \cap \text{int}X_0 = \emptyset \). This completes the proof.

It is possible to similarly prove ultimate boundedness for solutions of model Equation (E1) provided that the solutions of model Equation (E0) are ultimately bounded. Moreover, we can show that the sufficient conditions for model Equation (E1) to be uniformly persistent are the same as those for model Equation (E0). Thus, we obtain the following result.

**Corollary 3.3** Assume that conditions of Proposition A.2 hold. Model Equation (E1) is uniformly persistent if and only if \( R_0 > 1 \).

The results obtained in this section are summarized as follows.
4. Discussion

We constructed mathematical models of haematopoiesis and granulopoiesis under the influence of growth factors such as G-CSF. The theoretical conditions for (un)successful engraftment of transplanted HSCs were derived as Theorems 3.1 and 3.2. The basic idea behind these theoretical conditions was obtained by combining two concepts – the basic reproduction number $R_0$ that defines a threshold value for the population of transplanted HSCs to grow with various generations on an average and uniform persistence that ensures no population extinction. The present paper only discusses the possibility of successful engraftment of HSCT. Many aspects of this paper remain to be discussed in subsequent works. We have postulated several assumptions to formulate models Equations (E0) and (E1). It is necessary to experimentally validate the derived theoretical conditions and underlying assumptions in the model formulation. One possible validation method is to cultivate HSCs under *in vitro* conditions such as repopulation assays (see quantitative study for repopulation assays [77]). A cell line HL60 is a leukaemic cell for which it is possible to induce granulocyte differentiation in *in vitro* experiments. Hence, cultivation of the HL60 cell line can be used to investigate granulopoiesis in *in vitro* experiments [8]. Another possible experimental validation method is to transplant the collected HSCs into immunodeficient mice and observe mature cell counts in peripheral blood circulation.

Further, the presented mathematical work can be generalized to include more realistic situations. First, assumptions to ensure uniform boundedness in Proposition A.2 can be relaxed if we apply more flexible theorems for uniform persistence (see [74, Theorem 4.6]). Secondly, model Equation (E1) that includes the equation for granulopoiesis can be used as a quantitative mathematical model to estimate the kinetic parameters of HSCs from the sampled absolute neutrophil counts. Predictions of successful HSCT will be more reliable if other lineages such as lymphocyte and myeloid cell lines are obtained from peripheral blood. It is necessary to extend model Equation (E0) to include more variables representing several lineage cell lines in the hematopoietic system [27]. Third, since the number of HSCs collected from peripheral blood can be small, demographic stochasticity may play a significant role in the HSCT failure. Analyses of stochastic processes and simulations would be necessary to consider more realistic situations. The basic reproduction number $R_0$ can be defined for the Galton–Watson branching process that describes the growth and extinction of a population consisting of a finite number of cells. It has already been shown that $R_0$ essentially provides identical threshold values as those in deterministic systems [14]. Fourth, the main model Equation (E0) can be generalized to consider other various functional forms for the kinetic states of cells, such as survival, proliferation and differentiation. An alternative mathematical model can be formulated based on the theory of physiologically structured population models [56,66]. The use of delay equations, or in other words, a coupled system comprising DDEs and renewal equations is one possible method of representing the dynamics of physiologically structured populations. Note that age/size-structured population models have been formulated by hyperbolic partial differential equations (PDEs). In fact, age-structured population models are used to describe the dynamics of haematopoiesis [50,64]. One potential problem of using the formulation with PDEs is that for some transport equations, a nonlocal nonlinearity under a boundary condition may result in non-uniqueness of the solution [17]. For delay equations, mathematical theories for the well-posedness of solutions, (in)stability and bifurcation have already been developed [13,16,18].

The existence/development of useful packages for numerical analyses is desirable to investigate physiologically structured population models. The Escalator Boxcar Train is a numerical method for investigating the dynamics of physiologically structured population models that can be used to implement numerical simulations for models Equations (E0) and (E1) [65,66]. However, to our knowledge, there are few useful packages available for renewal (Volterra integral) equations to implement numerical simulation and bifurcation analyses, although several numerical schemes for
simulations have already been developed [6,59]. In contrast, user-friendly packages for numerical bifurcation analyses, such as DDE-BIFTOOLS [24] and TRACE-DDE [5], are available for DDEs. Hence, the development of useful numerical analysis packages for delay equations is an important work.

Model Equation (E1) can be used to investigate the dynamics of granulopoiesis in patients with cyclic neutropenia (CN). The importance of delayed negative feedback in granulopoiesis has been highlighted in seminal works on CN [10,25,36]. Mathematical models have been used to represent cyclic oscillations in absolute neutrophil counts (see comprehensive reviews for mathematical modeling and analysis, [10,25,36]). Although mutation in ELANE gene is predominant in human severe congenital neutropenia (SCN), mice expressing a neutrophil elastase mutation have normal granulopoiesis [30]. A mouse model that resembles human SCN has not been developed yet. There exists an experimental report stating that growth factor independent-1 (Gfi-1) blocks murine granulopoiesis, and this can be one of the key genes to induce SCN and CN for mice [80]. However, it is unclear whether mechanisms of Gfi-1 to induce neutropenia are shared between mice and humans. Hence, differences between human and murine granulopoiesis must be identified to construct a murine model of SCN and CN.

The difference between mice and humans in the case of congenital neutropenia occurrence is a result of differences in the molecular and genetic basis as well as cell kinetics. It is still not clear whether and how differences in the kinetics of granulopoiesis between mice and humans play a role in the occurrence of CN. Numerous studies have implied that the elevated apoptosis rate in the developmental stages of neutrophils (in particular, at the promyelocyte stage) is the primary cause of severe neutropenia [31], and several mathematical investigations support this idea [3,9,37,38,53,54]. On the other hand, it is suggested that other mechanisms such as maturation arrest at the promyelocyte stage of granulopoiesis is the primary cause of cyclic neutropenia [20]. The quantification of kinetics in granulopoiesis is an important procedure for identifying the key mechanisms underlying the occurrence of CN. To quantify the kinetics of granulopoiesis, a few mathematical studies have already been applied to in vivo experiments [60,62].

G-CSF is used as a therapeutic option to recover the decreased number of neutrophils in patients with SCN and CN [9] or for post-chemotherapy treatment for cancers [61]. Considerable efforts have been devoted to describe the pharmacokinetic dynamics of G-CSF injection for post-chemotherapy to cancer [23,26,61,70,71,75] or treatment to SCN and CN [3,9,27,69]. Note that G-CSF can affect the neutrophil maturation period. Hence, the incorporation of variable maturation delay is realistic for investigating the generation mechanisms of cyclic neutropenia. Several studies have considered variable maturation delay in granulopoiesis [1,21,26,55]. Although the maturation period $\tau_N$ is formulated as a constant in model Equation (E1), we could consider delay equations with variable maturation delay.

Recent advances in understandings of molecular mechanisms underlying homeostasis of blood cell production show complicated views. Recent advances in our understanding of molecular mechanisms underlying the homeostasis of blood cell production show complicated perspectives. Although G-CSF is the most effective growth factor in granulopoiesis, the commitment process to the granulocyte lineage is governed by complex dynamical bio-molecular networks. A part of the mechanisms for the promotion of granulopoiesis has been identified in response to acute inflammation [76,82]; however, it is still largely unknown whether a mediator of direct negative feedback exists and plays a role in granulopoiesis. Granulocytes and monocytes differentiate from common myeloid progenitor cells. An interplay between G-CSF and CD137 genes can be a determinant of cell lineage specifications [47]. Therefore, delayed negative feedback may be formed not in a single lineage (granulopoiesis) but in a network of multiple lineages. Mathematical modelling approaches to describe multi-scale dynamics in haematopoiesis could provide insights for linking known molecular or genetic mechanisms with observed dynamical phenomena.
Acknowledgement

This research was partly supported by (i) Research Fellowships of the Japan Society for the Promotion of Science (JSPS) for Young Scientists, and (ii) Aihara Innovative Mathematical Modelling Project, JSPS through its ‘Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program).’

References

[1] M. Adimy, F. Crauste, M.L. Hbid, and R. Qesmi, Stability and hopf bifurcation for a cell population model with state-dependent delay, SIAM J. Appl. Math. 70 (2010), pp. 1611–1633.
[2] M.T. Baldridge, K.Y. King, and M.A. Goodell, Inflammatory signals regulate hematopoietic stem cells, Trends Immunol. 32 (2011), pp. 57–65.
[3] S. Bernard, J. Bélair, and M.C. Mackey, Oscillations in cyclical neutropenia: new evidence based on mathematical modeling, J. Theor. Biol. 223 (2003), pp. 283–298.
[4] N. Borregaard, Neutrophils, from marrow to microbes, Immunity 33 (2010), pp. 657–670.
[5] D. Breeda, S. Maset, and R. Vermiglio, TRACE-DDE: a tool for robust analysis and characteristic equations for delay differential equations, in Topics in Time Delay Systems: Analysis, Algorithms, and Control, Lecture Notes in Control and Information Sciences, vol. 388, Springer, Berlin, 2009.
[6] H. Brunner, Collocation Methods for Volterra Integral and Related Functional Differential Equations, Cambridge Monographs on Applied and Computational Mathematics, Cambridge University Press, Cambridge, 2004.
[7] C. Foley and M.C. Mackey, Dynamic hematological disease: a review, Math. Models Meth. Appl. Sci. 10 (2000), pp. 581–592.
[8] C. Foley and M.C. Mackey, Mathematical model for G-CSF administration after chemotherapy, J. Theor. Biol. 257 (2009), pp. 27–44.
[9] C. Foley and M.C. Mackey, Oscillations in cyclical neutropenia: new evidence based on mathematical modeling, J. Theor. Biol. 237 (2005), pp. 133–146.
[10] C. Foley and M.C. Mackey, Observations on the pathophysiology and mechanisms for cyclical neutropenia, Math. Model. Nat. Phenom. 1 (2006), pp. 45–69.
[11] E.A. Copelan, Hematopoietic stem-cell transplantation, N. Engl. J. Med. 354 (2006), pp. 1813–1826.
[12] D.C. Dale, R.E. Person, A.A. Bolyard, A.G. Aprikyan, C. Bos, M.A. Bonilla, L.A. Boxer, G. Kannourakis, C. Zeidler, K. Welte, K.F. Benson, and M. Horwitz, Mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia, Blood 96 (2000), pp. 2317–2322.
[13] O. Diekmann and M. Gyllenberg, Abstract Delay Equations Inspired by Population Dynamics in Functional Analysis and Evolution Equations, Birkhäuser, Basel, 2008.
[14] O. Diekmann and J.A.P. Heesterbeek, Mathematical Epidemiology Of Infectious Diseases: Model Building, Analysis And Interpretation, Wiley, New York, 2000.
[15] O. Diekmann, J.A.P. Heesterbeek, and J.A.J. Metz, On the definition and the computation of the basic reproduction ratio R0 in models for infectious diseases, J. Math. Biol. 35 (1990), pp. 503–522.
[16] O. Diekmann, S.A. van Gils, S.M.V. Lunel, and H.-O. Walther, Delay Equations: Functional, Complex, and Nonlinear Analysis, Applied Mathematical Sciences, vol. 110, Springer, Berlin, 1995.
[17] O. Diekmann, M. Gyllenberg, and H. Thieme, Lack of uniqueness in transport equations with a nonlocal nonlinearity, Math. Models Meth. Appl. Sci. 10 (2000), pp. 581–592.
[18] O. Diekmann, P. Getto, and M. Gyllenberg, Stability and bifurcation analysis of Volterra functional equations in the light of suns and stars, SIAM J. Math. Anal. 39 (2007), pp. 1023–1069.
[19] O. Diekmann, M. Gyllenberg, J.A.J. Metz, S. Nakaoka, and A.M. de Roos, Daphnia revisited: local stability and bifurcation theory for physiologically structured population models explained by way of an example, J. Math. Biol. 61 (2010), pp. 277–318.
[20] D. Dingli, T. Antal, A. Traulsen, and J.M. Pacheco, Progenitor cell self-renewal and cyclic neutropenia, Cell Prolif. 42 (2009), pp. 330–338.
[21] J. Dyson, R. Villella-Bressan, and G. Webb, A nonlinear age and maturity structured model of population dynamics: I. basic theory, J. Math. Anal. Appl. 242 (2000), pp. 93–104.
[22] H. Ema, K. Sudo, J. Seita, A. Matsubara, Y. Morita, M. Osawa, K. Takatsu, S. Takaki, and H. Nakauchi, Quantification of self-renewal capacity in single hematopoietic stem cells from normal and Lnk-deficient mice, Dev. Cell 8 (2005), pp. 907–914.
[23] C. Engel, M. Scholz, and M. Loeffler, A computational model of human granulopoiesis to simulate the hematotoxic effects of multicycle polychemotherapy, Blood 104 (2004), pp. 2323–2331.
[24] K. Engelborghs, T. Luzyanina, and D. Roose, Numerical bifurcation analysis of delay differential equations using DDE-BIFTOOL, ACM Trans. Math. Software, (2002), pp. 1–21.
[25] C. Foley and M.C. Mackey, Dynamic hematological disease: a review, J. Math. Biol. 58 (2009), pp. 285–322.
[26] C. Foley and M.C. Mackey, Mathematical model for G-CSF administration after chemotherapy, J. Theor. Biol. 257 (2009), pp. 27–44.
[27] C. Foley, S. Bernard, and M.C. Mackey, Cost-effective G-CSF therapy strategies for cyclical neutropenia: mathematical modelling based hypotheses, J. Theor. Biol. 238 (2006), pp. 754–763.

[28] E.C. Forsberg, E. Passegqué, S.S. Prohaska, A.J. Wagers, M. Koova, J.M. Stuart, and I.L. Weissman, Molecular signatures of quiescent, mobilized and leukemia-initiating hematopoietic stem cells, PLoS One 5 (2010), pp. e8785.

[29] C.A. de Graaf, M. Kauppi, T. Baldwin, C.D. Hyland, D. Metcalf, T.A. Willson, M.R. Carpintelli, G.K. Smyth, W.S. Alexander, and D.J. Hilton, Regulation of hematopoietic stem cells by their mature progeny, Proc. Natl Acad. Sci. USA 107 (2010), pp. 21689–21694.

[30] D.S. Grenda, S.E. Johnson, J.F. Mayer, M.L. McLemore, K.F. Benson, M. Horwitz, and D.C. Link, Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis, Blood 110 (2007), pp. 4179–4187.

[31] G. Gripenberg, S. Londen, and O. Staffans, Volterra Integral and Functional Equations, Encyclopedia of Mathematics and its Applications, Cambridge University Press, Cambridge, 1990.

[32] J.K. Hale, Asymptotic Behavior of Dissipative Systems, American Mathematical Society, Providence, RI, 1988.

[33] J.K. Hale and S.M.V. Lunel, Introduction to Functional Differential Equations, Springer, New York, 1993.

[34] J.K. Hale and P. Waltman, Persistence in infinite-dimensional systems, SIAM J. Math. Anal. 20 (1989), pp. 388–395.

[35] C. Haurie, D.C. Dale, and M.C. Mackey, Cyclical neutropenia and other periodic hematological disorders: a review of mechanisms and mathematical models, Blood 92 (1998), pp. 2629–2640.

[36] C. Haurie, D.C. Dale, R. Rudnicki, and M.C. Mackey, Modeling complex neutrophil dynamics in the grey collie, J. Theor. Biol. 204 (2000), pp. 505–519.

[37] T. Hearn, C. Haurie, and M.C. Mackey, Cyclical neutropenia and the peripheral control of white blood cell production, J. Theor. Biol. 192 (1998), pp. 161–181.

[38] M. Hertl, J.F.M., Paul S. Russell, and H. Yeh, Hematopoietic Stem Cell Transplantation (Merck manual), 2008.

[39] M. Horwitz, K.F. Benson, R.E. Person, A.G. Aprikyan, and D.C. Dale, Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis, Nat. Genet. 23 (1999), pp. 433–436.

[40] M.S. Horwitz, Z. Duan, B. Korkmaz, H.-H. Lee, M.E. Meallife, and S.J. Salipante, Neutrophil elastase in cyclic and severe congenital neutropenia, Blood 109 (2007), pp. 1817–1824.

[41] H. Inaba and H. Nishiura, The state-reproduction number for a multistage class age structured epidemic system and its application to the asymptotic transmission model, Math. Biosci. 216 (2008), pp. 77–89.

[42] D. Jiang and H. Schwarz, Regulation of granulocyte and macrophage populations of murine bone marrow cells by G-CSF and CD137 protein, Blood 109 (2007), pp. 1817–1824.

[43] M.C. Mackey, Cell kinetic status of haematopoietic stem cells, Cell Prolif. 34 (2001), pp. 71–83.

[44] M.C. Mackey, A.A.G. Aprikyan, and D.C. Dale, The rate of apoptosis in post mitotic neutrophil precursors of normal and neutropenic humans, Cell Prolif. 36 (2003), pp. 27–34.

[45] J.M. Mahaffy, J. Belair, and M.C. Mackey, Hematopoietic model with moving boundary condition and state dependent delay: applications in erythropoiesis, J. Theor. Biol. 190 (1998), pp. 135–146.

[46] J.A.J. Metz and O.D. (eds.), The Dynamics of Physiologically Structured Populations, Lecture Notes in Biomathematics, Springer, Berlin, 1986.

[47] S.J. Morrison and J. Kimble, Asymmetric and symmetric stem-cell divisions in development and cancer, Nature 441 (2006), pp. 1068–1074.

[48] S.J. Morrison and A.C. Spradling, Stem cells and niches: mechanisms that promote stem cell maintenance throughout life, Cell 132 (2008), pp. 598–611.

[49] T. Okayama, T. Matsuo, and M. Sugihara, Sinc-collocation methods for weakly singular Fredholm integral equations of the second kind, J. Comput. Appl. Math. 234 (2010), pp. 1211–1227. Proceedings of the Thirteenth International Congress on Computational and Applied Mathematics (ICCAM-2008), Ghent, Belgium, 7–11 July, 2008.

[50] R.A. Oostendorp, J. Audet, and C.J. Eaves, High-resolution tracking of cell division suggests similar cell cycle kinetics of hematopoietic stem cells stimulated in vitro and in vivo, Blood 95 (2000), pp. 855–862.
Appendix 1. Basic results

A.1. Nonnegativeness and boundedness

We present a result for the nonnegativeness for the solutions of model Equation (E0) under initial condition Equation (I0).

PROPOSITION A.1 Every solution of model Equation (E0) under initial condition Equation (I0) is nonnegative.

Proof. The proof is obtained by contradiction. Suppose there exists \( \tilde{t} > 0 \) such that \( F(\tilde{t}) = 0, F(t) > 0 \) for \( 0 \leq t < \tilde{t} \) and \( \frac{dF}{dt}(\tilde{t}) \leq 0 \). From the first equation of model Equation (E0), it follows that for \( t = \tilde{t} \),

\[
0 \geq \frac{dF}{dt}(\tilde{t}) = g(0) > 0.
\]

This is a contradiction. Hence \( F(t) \) is nonnegative. Note that the second equation of model Equation (E0) can be rewritten as

\[
S(t) = \int_{\tau_S}^{\infty} \rho_S(F(t)) \lambda(S(t)) e^{-d_S t_S} e^{-\int_{0}^{\tilde{t}} \int_{\tau_S}^{\infty} \rho_S(F(t')) \lambda(S(t')) dt'} \, dt' S(t-a) \, da. \tag{A1}
\]
Since all the elements in the integrand of Equation (A1) are non-negative, \( S(t) \) is nonnegative. This completes the proof.

Nonnegativeness for solutions of model Equation (E1) follows from Proposition A.1.

In Section 3, we assume that the transition rate \( \lambda \) from the resting-phase to the proliferation-phase is proportional to the per capita consumption rate of the growth factor \( F \). This assumption can be justified as follows. Assume that the number of G-CSF receptors on a cell surface is positively correlated with the G-CSF uptake rate. If the number of G-CSF receptors is directly linked to the strength of G-CSF signals for cell cycle activation, the rate of G-CSF uptake can be expected to be positively correlated with the rate of transition from the resting-phase to the proliferation-phase. Let \( \kappa \) denote the proportional constant between the rate of G-CSF uptake and the rate of transition from the resting-phase to the proliferation-phase. Then

\[
\lambda(F) = \kappa \gamma S(F). \tag{A2}
\]

We obtain the following result for the ultimate boundedness of the solutions of model Equation (E0):

**Proposition A.2** Assume that Equation (A2) holds. The solutions of model Equation (E0) are then ultimately bounded.

**Proof** The first equation of model Equation (E0) can be evaluated as follows:

\[
\frac{dF}{dt}(t) \leq g(F(t)).
\]

Since \( F = K \) is the asymptotically stable equilibrium of Equation (1), the comparison theorem for ordinary differential equations implies that for any \( \varepsilon > 0 \), there exists \( T(\varepsilon) > 0 \) such that for \( t - \tau S \geq T(\varepsilon) \),

\[
F(t) \leq K + \varepsilon.
\]

Define function \( V(t) \) by

\[
V(t) := 2 \kappa e^{-dS\tau S} F(t - \tau S) + S(t).
\]

Recall that \( \rho_S(F) \leq 2 \). The derivative of \( V \) along a solution of model Equation (E0) for \( t - \tau S \geq T(\varepsilon) \) is evaluated as follows:

\[
\frac{dV}{dt}(t) = 2 \kappa e^{-dS\tau S} [g(F(t - \tau S)) - \gamma S(F(t - \tau S))S(t - \tau S)]
\]

\[
+ \kappa \rho_S(F(t - \tau S)) \gamma S(F(t - \tau S))e^{-dS\tau S} S(t - \tau S) - (dS + \lambda(F(t)))S(t)
\]

\[
\leq -dS V(t) + 2 \kappa e^{-dS\tau S} dS F(t - \tau S) + 2 \kappa e^{-dS\tau S} g(F(t - \tau S))
\]

\[
- (2 - \rho_S(F(t - \tau S))) \kappa e^{-dS\tau S} S(F(t - \tau S)) S(t - \tau S)
\]

\[
\leq -dS V(t) + 2 \kappa e^{-dS\tau S} dS (K + \varepsilon) + 2 \kappa e^{-dS\tau S} g(F(K + \varepsilon)).
\]

By applying a theorem for uniform boundedness in [81], it is shown that \( S(t) \) is ultimately bounded. The proof for the ultimate boundedness of \( N(t) \) is straightforward from the fact that \( S(t) \) is bounded. This completes the proof.

**A.2. Derivation of model Equation (E1)**

By substituting the specific form of \( \alpha(a) \) and \( \mathcal{F}_L(a, F) \) on the right-hand side of \( N(t) \), we find that

\[
N(t) = \int_0^\infty \rho_L(F_t) \lambda(F_t)(1 + (A_M - 1)(1 - e^{-dM^a})) e^{-dL a} S(t - a) d a
\]

\[
= \int_0^\infty \rho_L(F_t) \lambda(F_t) A_M e^{-dL a} S(t - a) d a - \int_0^\infty \rho_L(F_t) \lambda(F_t)(A_M - 1) e^{-(dL + dM^a)a} S(t - a) d a
\]

\[
=: N_1(t) - N_2(t).
\]

By changing variable \( t - a = \sigma \) and differentiating on both sides of \( N_1(t) \), we obtain

\[
\frac{d}{dt} N_1(t) = \frac{d}{d\sigma} \int_{-\infty}^\sigma \rho_L(F(\sigma)) \lambda(F(\sigma)) A_M e^{-dL(\sigma-a)} S(\sigma) d\sigma
\]

\[
= -dL N_1(t) + A_M \rho_L(F(t)) \lambda(F(t)) S(t).
\]

We can similarly obtain the differential equation for \( N_2(t) \).
A.3. Quasi-steady-state approximation

The G0-model proposed in [52] can be deduced from model Equation (E0) if the dynamics of the growth factor $F$ is approximated at the steady state. More precisely, we assume that $(d/dt) F(t) \approx 0$. From the first equation of model Equation (E0), it follows that

$$ S(t) = \frac{g(F(t))}{\gamma_S(F(t))} \quad (A3) $$

Since $\gamma_S(F)$ is increasing while $g(F)$ is decreasing in $F$, the right-hand-side of Equation (A3) is a decreasing function of $F$. Hence, there exists a function $h(S)$ such that

$$ F(t) = h(S(t)), $$

where $h$ is the inverse function of the right-hand side of Equation (A3). Moreover, we assume that HSCs always undergo symmetric division irrespective of the concentration of growth factor $F$; $p(F) \equiv 1$. By substituting $h(S(t))$ into the second equation of model Equation (E0), we have

$$ \frac{d}{dt} S(t) = 2\lambda(h(S(t) - \tau_S))e^{-\tau_S} S(t - \tau_S) - [d_S + \lambda(h(S(t)))] S(t). \quad (A4) $$

Equation (A4) corresponds to the G0-model, if $\lambda(h(S))$ has the form

$$ \lambda(h(S)) = \frac{\beta_0 \theta^n}{\theta^n + S^n}. \quad (A5) $$

Note that the periodic cycles in terms of the stem cell count can be observed for the G0-model without the existence of a delayed negative feedback from granulocyte lineage [52,63]. This is essentially because the density-dependent inhibitory effect in the form of Equation (A5) with intracellular delay in self-renewal divisions leads to delayed negative feedback effects.

A.4. Notation of uniform persistence

Let $X$ denote a metric space with metric $d$. By $X_0$, we denote a closed subset of $X$ with boundary $\partial X_0$ and interior $\text{int} X_0$. Let $\Phi(x, t) : X_0 \times \mathbb{R}_+ \to X_0$ denote a strongly continuous semiflow, that is, $(x, t) \to \Phi(t)x$ is continuous, $\Phi(0)x_0 = x_0$ and $\Phi(t) \circ \Phi(s) = \Phi(t+s)$ for $s, t \geq 0$. $\Phi$ is said to be uniformly persistent if there exists a positive number $\epsilon_0$ such that for all $x \in \text{int} X_0$,

$$ \lim_{t \to \infty} d(\Phi(x, t), \partial X_0) \geq \epsilon_0 > 0. $$

The boundary flow $\Phi_{\partial}$ is defined by the restriction of $\Phi$ to $\partial X_0 \times \mathbb{R}_+$. For $x \in A$, the omega limit set is denoted by $\omega(x)$. The stable set of the compact invariant set $A$ is defined as

$$ W^+(A) := \{ x \in A | x \in X, \omega(x) \neq \emptyset, \omega(x) \subset A \}. $$

The invariant set for the boundary flow is defined by $\Omega(\Phi_{\partial}) = \bigcup_{x \in X_0} \omega(x)$. A semiflow is said to be asymptotically smooth if for any bounded subset $U$ for which $\Phi(t)U \subset U$ for any $t \geq 0$, there exists a compact set $M$ such that $\text{dist}(\Phi(t)U, M) \to 0$ as $t \to \infty$. A semiflow is said to be point dissipative if there exists a positive constant $B$ such that for any solution of model Equation (E0) under initial condition Equation (I0), there exists $T_B > 0$ such that $|\Phi(t)x| < B$ for all $t > T_B$. A nonempty subset $M$ of $X$ is called an isolated invariant set if it is the maximal invariant set in some neighbourhood of itself. A boundary flow $\Phi_{\partial}$ is said to be isolated if there exists a covering $M = \bigcup_{i=0}^{k-1} M_i$ of $\Omega(\Phi_{\partial})$ by pairwise disjoint, compact, isolated invariant sets $M_0, M_2, \ldots, M_{k-1}$ for $\Phi_{\partial}$ such that each $M_i$ is also an isolated invariant set for $\Phi$. The boundary flow $\Phi_{\partial}$ is called acyclic if there exists some isolated covering $M = \bigcup_{i=0}^{k-1} M_i$ of $\Phi_{\partial}$ such that no subset of $M_i$’s forms a cycle.

**Theorem A.3** [35, Theorem 4.1.] Assume that (i) $\Phi(t)$ is asymptotically smooth, (ii) $\Phi(t)$ is point dissipative in $X_0$, (iii) boundary $\partial X_0$ is invariant under $\Phi$, and (iv) boundary flow $\Phi_{\partial}$ is isolated and acyclic with acyclic covering $M = \bigcup_{i=0}^{k-1} M_i$, $\Phi$ is then uniformly persistent if and only if

$$ W^+(M_i) \cap \text{int} X_0 = \emptyset \quad \text{for each } M_i \in M. \quad (A6) $$
A.5. Asymptotic stability of linear delay differential equation

We consider the following scalar linear DDE:

$$\frac{dx(t)}{dt} = -ax(t) + bx(t-\tau) \quad (A7)$$

under the following initial condition:

$$x(\sigma) = \phi(\sigma), \quad -\tau \leq \sigma \leq 0.$$ 

We assume that $a > 0$ and $b > 0$. As shown in the main text, the asymptotic stability for the zero solution of Equation (A7) is used to prove Theorem 3.2. In Theorem 3.2, the zero solution of Equation (A7) should be unstable. This is sufficient for only referring to the known result, since the asymptotic stability for the zero solution of Equation (A7) has already been solved completely.

**Proposition A.4** [34, p. 135 and Appendix A.5] The following statements hold:

1. Assume that $a > b$. The zero solution of Equation (A7) is then uniformly asymptotically stable.

2. Assume that $a < b$. The zero solution of Equation (A7) is then unstable.