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Stochastic modeling of stress erythropoiesis using a two-type age-dependent branching process with immigration

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Abstract

The erythroid lineage is a particularly sensitive target of radiation injury. We model the dynamics of immature (BFU-E) and mature (CFU-E) erythroid progenitors, which have markedly different kinetics of recovery, following sublethal total body irradiation using a two-type reducible age-dependent branching process with immigration. Properties of the expectation and variance of the frequencies of both types of progenitors are presented. Their explicit expressions are derived when the process is Markovian, and their asymptotic behavior is identified in the age-dependent (non-Markovian) case. Analysis of experimental data on the kinetics of BFU-E and CFU-E reveals that the probability of self-renewal increases transiently for both cell types following sublethal irradiation. In addition, the probability of self-renewal increased more for CFU-E than for BFU-E. The strategy adopted by the erythroid lineage ensures replenishment of the BFU-E compartment while optimizing the rate of CFU-E recovery. Finally, our analysis also indicates that radiation exposure causes a delay in BFU-E recovery consistent with injury to the hematopoietic stem/progenitor cell compartment that give rise to BFU-E. Erythroid progenitor self-renewal is thus an integral component of the recovery of the erythron in response to stress.

Keywords

Cell kinetics; Age-dependent branching process; Immigration; Erythropoiesis; BFU-E; CFU-E

1 Introduction

Hematopoietic stem cells, characterized by multipotentiality and the capacity to self-renew, give rise to all circulating blood cells in adult organisms by the sequential transition of cells from progenitor to precursor compartments. Adult humans synthesize more than 200 billion new red blood cells (RBC) every day. This robust process of cellular expansion and maturation is characterized by the transition of erythroid lineage-committed progenitors to maturing precursors in the bone marrow that ultimately enucleate in mammalian species to generate oxygen-carrying RBCs. To rapidly recover from blood loss or the transient suppression of erythropoiesis, RBC production acutely expands - a process termed stress erythropoiesis.

Erythroid progenitors, present primarily in the bone marrow of mammals, are defined by their capacity to generate clonal colonies of RBCs in semisolid media. Immature erythroid progenitors in the mouse, termed burst-forming units-erythroid (BFU-E), generate large colonies that take 7-10 days to form in vitro (Stephenson, 1971). Late-stage erythroid progenitors, termed colony-forming units-erythroid (CFU-E), form small colonies of RBCs at 2 days of culture. CFU-E subsequently mature into morphologically recognizable erythroid precursors that undergo 3-4 maturational cell divisions as they accumulate hemoglobin, decrease cell size, and condense their nuclei (Bessis, 1977). RBCs are ultimately generated following the enucleation of late-stage precursors (Figure 1).

Acute loss of circulating RBCs, either from bleeding or hemolysis, leads to hypoxic signals that result in increased level of the cytokine erythropoietin (EPO). CFU-E and immature erythroid precursors are exquisitely dependent on EPO for their survival and increased EPO levels lead to the survival of more CFU-E and increased production of RBCs (Koury and Bondurant, 1990; Socolovsky, 2007). Similarly, the administration of exogenous EPO causes an acute increase in the number of CFU-E and expansion of the erythron. We have recently developed a murine model of stress erythropoiesis using sublethal total body irradiation and find that recovery of the erythron is characterized by the specific overproduction of late-stage erythroid progenitors (Peslak et al, 2011; 2012). These data suggest that stress erythropoiesis is characterized by the transient self-renewal of both late-stage and immature erythroid progenitors.

Previous models of the kinetics of erythropoiesis have been developed by Loeffler and Wichmann (1980), Loeffler et al (1989), Bélair et al (1995), Mahaffy et al (1998), Banks et al (2004), Ackleh et al (2006), Crauste et al (2008, 2010), and Fischer et al (2012), to name a few. These studies were focused on stress erythropoiesis, the role of self-renewal in the recovery process, and, more recently, the role of macrophage islands on the regulation of erythropoiesis. A detailed review of models of erythropoiesis can be found in Crauste et al (2008).

In this paper we focus on the initial phase of the recovery of erythropoiesis in response to stress, and develop a stochastic model of the kinetics of the BFU-E and CFU-E progenitor compartments. The model distinguishes these two cell types, and it allows cells to divide, differentiate or die at the end of their life-span. It allows also for the duration of the life-span

to follow arbitrary, non-necessarily exponential, distributions. This assumption of age-dependence achieves a description of the cell cycle that is similar in spirit to that obtained by age-structured differential equation models. Finally, an immigration process, formulated as a Poisson process, is included to describe the influx of newly formed BFU-E arising from the differentiation of upstream stem/progenitor cells.

We use this model to gain quantitative insights into the dynamics of BFU-E and CFU-E in mice following sublethal total body irradiation. In particular, we are interested in studying the ability of erythroid progenitors to increase self-renewal during the early phase of the recovery. This question was approached by Crauste et al (2008), who concluded that progenitor self-renewal is a key component of the recovery of stress erythropoiesis. Their study was performed in the context of anemia, and the authors assessed their hypotheses about erythroid progenitors using observations that were made in the erythrocyte compartment. In particular, erythroid progenitors were not distinguished in their model based on their maturation level. Here, we use experimental data on the BFU-E and CFU-E erythroid progenitor compartments using functional colony-forming assays. Our analysis confirms that self-renewal plays a key role in accelerating the recovery of stress erythropoiesis. It suggests also that the probability of self-renewal increased more in CFU-E than in the less mature BFU-E. Thus, the propensity of erythroid progenitors to modulate self-renewal appears to be stage-dependent. We also find that more immature hematopoietic stem/progenitor cells may be damaged by radiation exposure.

The model proposed here is a two-type reducible age-dependent branching process with immigration, which can be viewed as a stochastic analog of a partial differential equation model. The theoretical properties of age-dependent branching processes have been mostly studied in the single type case (Sevastyanov, 1957; Jagers, 1968; Yanev, 1972a, 1972b; Pakes, 1972; Radcliffe, 1972; Pakes and Kaplan, 1974; Kaplan and Pakes, 1974; Vatutin, 1977; Olofsson, 1996; Yakovlev and Yanev, 2006; Hyrien and Yanev, 2012, 2013), but they do not appear to have been used in analyses of biological data. We note that the developed model builds on previous work on a two-type Bellman-Harris process (without immigration) by Jagers (1969).

We determine the asymptotic behavior of the process as time increases, extending earlier results from Hyrien and Yanev (2012). Such analytical results are important because they facilitate the rapid evaluation of distributional properties of the cellular system over arbitrarily long time periods without having to resort to computationally intensive simulations using discrete agent-based models. Secondly, they enable the identification of the parameters that govern the long-term behavior of the process, thereby simplifying the parametric structure of the model. Such simplifications are particularly useful when fitting the model to experimental data. Finally, no general asymptotic theory exists for multi-type reducible branching processes such that every particular case is interesting from a mathematical standpoint.

This article is organized as follows. Section 2 is a preamble to the paper that introduces the model and biological findings in non-technical terms. Section 3 describes the biological background and experimental data that will guide the choice of our modeling assumptions.

The model, justification of modeling assumptions, and its properties are presented in Section 4. An application to study the kinetics of erythroid progenitors following total body irradiation is described in Section 5. Additional discussions about our results and modeling assumptions are given in Section 6. The Appendix contains the theoretical derivations of the asymptotic behavior of the moments of the process.

2 Preamble

From a biological standpoint, this paper is concerned with studying the effects of radiation exposure on the dynamics of erythropoiesis. Erythropoiesis is the process used by our body to produce, maintain and repair its supply of red blood cells. It involves a complex system of sequentially maturing cells that begins with the differentiation of hematopoietic stem cells and culminates with the release of circulating red blood cells (Fig. 1). In between, cells are found at progressive stages of maturation. These include the lineage-committed erythroid progenitors, termed BFU-E (burst-forming units-erythroid) and CFU-E (colony-forming units-erythroid) that are each capable of forming colonies of erythroid cells when cultured in semisolid media with cytokines. CFU-E then give rise to maturing erythroblasts that accumulate hemoglobin, the protein used to carry oxygen and carbon dioxide. We have previously examined the kinetics of loss and recovery of these various erythroid cells and postulated that the recovery of the erythroid lineage might involve the ability of the erythroid progenitors (BFU-E and CFU-E) to transiently self-renew.

To investigate this question, we develop a mathematical model of the dynamics of the BFU-E and CFU-E compartments schematized in Fig. 2. Unlike differential equation models, our model describes population dynamics at the single cell level and allow cell fate and lifespan to vary randomly between cells. It belongs to a class of models, referred to as continuous-time branching processes, that is increasingly used to study the dynamics of cellular and other biological systems (Jagers (1975); Yakovlev and Yanev (1989); Kimmel and Axelrod (2002), Haccou, Jagers and Vatutin (2005)). In our model, the lifespan of each cell may have an arbitrary probability distribution; such models are called age-dependent, in opposition to Markov processes which rest on the assumption of exponentially distributed life-span which may not be realistic for describing cell kinetics. When a cell completes its lifespan, it either divides, dies, or differentiates. Each of these events occurs with a given probability specified by the model. The model also allows upstream progenitor cells to turn into BFU-E at random points in time. In the usual terminology of branching processes, this influx of cells is referred to as an immigration process. We formulate it as a Poisson process.

From a mathematical standpoint, the contribution of this paper is to present transient and asymptotic properties of the above model. The combination of an age-dependent branching process with two types of cells and of the immigration process creates substantial technical challenges. The tools that we use to study properties of our model belong to renewal theory. An excellent introduction to the field can be found in Feller's (1971) textbook. Also, a result useful to derive the asymptotic behavior of the numerous renewal-type equations that appear in the study of our model is provided in the Appendix.

Fitting the model to experimental data leads to several conclusions concerning the dynamics of BFU-E and CFU-E. The first one is that both of these cell types adapt their kinetics during the days that follow radiation exposure to speed up the recovery process. In particular, they both become more likely to undergo self-renewal than under normal erythropoiesis. Importantly, each cell type has its own way of modulating its kinetics: BFU-E divided with probability ~ 0.5 , whereas CFU-E divided with a probability greater than 0.5. BFU-E and CFU-E have probabilities of self-renewal that are less than 0.5 under normal steady-state conditions. Another finding is that the recovery of BFU-E and CFU-E did not start until around 2.18 days following radiation exposure, suggesting that the dynamics of upstream progenitor cells were also disrupted by radiation exposure. The following sections of this paper detail the experimental and mathematical steps that were necessary to reach these findings.

3 Stress erythropoiesis following radiation exposure

3.1 Colony-forming unit assays

We have generated an experimental model of stress erythropoiesis using a single dose of sublethal total body irradiation, in which we estimate the size of the BFU-E and CFU-E compartments using colony-forming assays in irradiated mice compared to sham-irradiated control mice. To perform these assays, single cell suspensions from the bone marrow are cultured *in vitro* with cytokines in semi-solid media. The numbers of BFU-E and CFU-E are estimated by enumerating the number of erythroid (red) colonies at a pre-determined time point. BFU-E are quantified by counting the number of large erythroid colonies 7 days after plating cells in media supplemented with 2 U/mL rhEPO, 0.02 $\mu\text{g/mL}$ IL-3 and IL-6, and 0.12 $\mu\text{g/mL}$ SCF (Peprotech, Rocky Hill, NJ), whereas CFU-E are quantified by counting colonies 2 days after plating cells in media supplemented with 0.3 U/mL rhEPO (Amgen, Thousand Oaks, CA). The number of colonies scored by these assays likely underestimate the total number of BFU-E and CFU-E, but they provide information about the size of the erythroid progenitor compartments in exposed mice relative to control mice.

3.2 Preliminary observations from experimental data

Exposure of C57Bl/6 adult mice to 4 Gy total body irradiation causes the rapid loss of greater than 95% of all erythroid progenitors and precursors in the bone marrow by 2 days post-irradiation (Peslak et al, 2011). Recovery of the erythron is characterized by a slow, linear expansion of BFU-E, which even at two weeks post-irradiation have not returned to normal levels in the bone marrow (Peslak et al, 2012). In marked contrast, CFU-E transiently, and remarkably, expand to $\sim 250\%$ of normal levels at 6 days post-radiation. These CFU-E then give rise to a wave of maturing erythroid precursors at days 7-9 in the marrow that subsequently enucleate and emerge as new RBCs in the bloodstream, leading to the recovery from anemia that develops following irradiation (Peslak et al, 2012). Thus we observed three sequential phases in the kinetics of erythroid progenitors following sublethal irradiation: 1- cell death (day 0 to day 2); 2- recovery (day 2 to day 6); 3- stabilization of the population of CFU-E (days 6-10).

We found that the acute expansion in CFU-E following sublethal irradiation is dependent on a spike in EPO levels that occurs following the development of anemia (Peslak et al, 2012). However, the massive expansion in CFU-E numbers, particularly in the setting of low BFU-E numbers, suggested that more than CFU-E survival may be effected by EPO. Here, we ask whether the acute expansion in CFU-E numbers at 4-6 days following sublethal irradiation (Figure 2) reflects a transient capacity for CFU-E to undergo self-renewal cell divisions *in vivo*. Thus, this paper focuses on the recovery phase of the erythroid progenitor compartments, and leaves aside observations made during the stabilization phase, which is fundamentally different from the first two phases and will be studied in a subsequent paper.

4 A two-type branching process with immigration

4.1 Modeling assumptions

We model the dynamics of the populations of BFU-E and CFU-E using a two-type age-dependent branching process with immigration. To simplify notation, we shall sometimes refer to BFU-E and CFU-E as type-1 and type-2 cells, respectively, and write $Z_1(t)$ and $Z_2(t)$ for the numbers of BFU-E and CFU-E at time t . Put $\mathbf{Z}(t) = \{Z_1(t), Z_2(t)\}$. The time origin ($t = 0$) is the time at which radiation exposure occurred. We assume that:

(A1) At any time $t \geq 0$, the population consists of two types of BFU-E and CFU-E: those that were born before and those that were born after radiation exposure. To reflect this distinction between cells, we express $\mathbf{Z}(t)$ as:

$$\mathbf{Z}(t) = \mathbf{Z}^-(t) + \mathbf{Z}^+(t) \quad (1)$$

where $\mathbf{Z}^-(t) := \{Z_1^-(t), Z_2^-(t)\}$ and $\mathbf{Z}^+(t) := \{Z_1^+(t), Z_2^+(t)\}$, and where $Z_k^-(t)$ and $Z_k^+(t)$ are the numbers of type- k cells born before and born after radiation exposure, respectively. The cell counts $\mathbf{Z}^-(t)$ and $\mathbf{Z}^+(t)$ are likely weakly dependent because radiation exposure disrupted the course of cell kinetics, causing it to be “reset”. Moreover the cells that contribute to $\mathbf{Z}^-(t)$ are different from those that contribute to $\mathbf{Z}^+(t)$; they have common ancestors, however. Thus it is reasonable to assume that the processes $\{\mathbf{Z}^-(t), t \geq 0\}$ and $\{\mathbf{Z}^+(t), t \geq 0\}$ are independent. It follows from the definition of $\mathbf{Z}^+(t)$ that $\mathbf{Z}^+(0) = (0, 0)$, and we assume that $\mathbf{Z}^-(0)$ is a random vector with finite expectation $E\{\mathbf{Z}^-(0)\} = (\eta_1, \eta_2)$ and

$$\text{variance-covariance matrix } \text{Var}\{\mathbf{Z}^-(0)\} = \begin{pmatrix} \sigma_{10}^2 & \rho_0 \\ \rho_0 & \sigma_{20}^2 \end{pmatrix}.$$

We assume that all type- k cells exposed to radiation either die before completing their cycle and disintegrate, or migrate out of the bone marrow. This assumption is motivated by the experimental observation that the populations of BFU-E and CFU-E were almost entirely depleted by day 2 (Fig. 3). We formalize this assumption by modeling $Z_1^-(\cdot)$ and $Z_2^-(\cdot)$ as non-Markovian pure death processes:

(A2) Every type- k cell ($k = 1, 2$) exposed to radiation disappears from the population with probability one after a random duration that follows a distribution with cumulative distribution function (c.d.f.) $F_k(t)$, $t \geq 0$.

The size of the populations of BFU-E and CFU-E both reached a nadir around day 2 after radiation exposure and began to recover shortly thereafter (Peslak et al, 2011; 2012). This recovery indicates that upstream hematopoietic stem/progenitor cells did not completely die out and thus were less sensitive to radiation exposure than the BFU-E and CFU-E compartments. By dividing and differentiating, these stem/progenitor cells generated new BFU-E, which eventually led to the recovery of the BFU-E and CFU-E compartments. The processes $Z_1^+(t)$ and $Z_2^+(t)$ describe the regeneration of BFU-E and CFU-E over time. We propose to model $Z^+(t)$ as a two-type age-dependent branching process with immigration. The model consists of a Poisson process which describes the arrival (or immigration) of new BFU-E by differentiation of upstream progenitor cells, and of a two-type Bellman-Harris process modeling the populations of BFU-E and CFU-E generated by each BFU-E arising from the differentiation of upstream progenitors. The two-type Bellman-Harris process is defined by Assumptions (A3-A7):

(A3) Upon completion of its life-span, every BFU-E either dies with probability p_0 , or differentiates into one CFU-E with probability p_2 , or divides into two new BFU-E with probability $p_1 = 1 - p_0 - p_2$ (we shall refer to such divisions as self-renewing divisions). Put $\xi = (\xi_1, \xi_2)$, where ξ_1 and ξ_2 denote the number of BFU-E and the number of CFU-E generated by any BFU-E at the end of its lifespan. The probability generating function (p.g.f.) of ξ is

$$\begin{aligned} h_1(s_1, s_2) &:= E(s_1^{\xi_1} s_2^{\xi_2}) & |s_k| \leq 1, k=1, 2 \\ &= p_0 + p_1 s_1^2 + p_2 s_2, \end{aligned}$$

and we set $a_1 := E\xi_1 = 2p_1$ and $a_2 := E\xi_2 = p_2$ to denote the expected number of BFU-E and CFU-E produced by a single BFU-E. We introduce also the second order moment $a_{11} := E[\xi_1(\xi_1 - 1)] = 2p_1$, and notice that $a_{11} = a_1$, $a_{22} := E[\xi_2(\xi_2 - 1)] = 0$ and $a_{12} := E(\xi_1\xi_2) = 0$.

(A4) The life-span of every BFU-E (referring here either to the mitotic cycle duration or to the time necessary for the cell to die or to differentiate into a CFU-E, calculated from its birth) is described by a r.v. τ_1 with c.d.f. $G_1(t) := P(\tau_1 \leq t)$ that satisfies the regularity condition $G_1(0+) = 0$ to prevent explosion of the process. Write $\mu_1 := E(\tau_1)$ and $\sigma_1^2 := Var(\tau_1)$ for its expectation and variance. A general class of distributions that is well suited for applications is the non-central gamma distribution with c.d.f.

$$G_1(t) = \Gamma(t; \nu_1, \beta_1, \delta_1) = \int_0^t \frac{\beta_1^{\nu_1}}{\Gamma(\nu_1)} (x - \delta_1)^{\nu_1-1} e^{-\beta_1(x-\delta_1)} dx \quad (t \geq 0)$$

where $\nu_1 > 0$, $\beta_1 > 0$ and $\delta_1 \geq 0$ are the shape, scale and shift parameters of the distribution. The (central) gamma distribution ($\delta_1 = 0$) is a classical choice for modeling the duration of cell lifespan (Yakovlev and Yanev, 1989; Kimmel and Axelrod, 2002; Hyrien et al, 2006; Hyrien and Zand, 2008). It reduces to the exponential distribution when $\nu_1 = 1$, in which case the model is Markovian. This particular case is treated in detail in Section 4.4. Parameter values satisfying $\nu_1 = 1$ and $\delta_1 > 0$ reflect assumptions which define the Smith-Martin model (Smith and Martin, 1973). The non-central gamma distribution prevents the

lifespan from lasting less than δ_1 units of time, thereby achieving a more realistic description of the cell lifespan than the gamma distribution. This refinement comes at the cost of an additional parameter in the model.

(A5) Upon completion of its lifespan, every CFU-E either divides into 2 new CFU-E with probability q_1 , or it exits the mitotic cycle either to die or to differentiate into one or two pro-erythroblasts with probability $q_0 = 1 - q_1$. Write $h_2(s_2) := 1 - q_1 + q_1 s_2^2$, for the associated p.g.f.. Let $b_2 := E\eta_2 = 2q_1$ denote the expected number of CFU-E produced by any CFU-E upon completion of its lifespan. Define also the second order factorial moment: $b_2 := E[\eta_2(\eta_2 - 1)] = 2q_1$. Notice that $b_{22} = b_2$.

(A6) The life-span of every CFU-E is described by a r.v. τ_2 with c.d.f. $G_2(x) := P(\tau_2 \leq x)$ that satisfies $G_2(0+) = 0$. For example, one could assume that τ_2 follows also a gamma distribution with shape, scale, and shift parameters $\nu_2 > 0$, $\beta_2 > 0$ and $\delta_2 \geq 0$. Let $\mu_2 := E(\tau_2)$ and $\sigma_2^2 := Var(\tau_2)$ for the expectation and variance of the lifespan of CFU-E.

(A7) Every cell evolve independently of all other cells.

Assumptions (A3-A7) define a two-type Bellman-Harris process embedded in the branching process with immigration. Write $X(t) := \{X_1(t), X_2(t)\}$ for the number of BFU-E and for the number of CFU-E at any time $t \geq 0$ under this process.

The recovery of the BFU-E and CFU-E compartment became observable around day 2 post-exposure, suggesting that the differentiation of erythroid progenitors into BFU-E might have been delayed by radiation exposure. Let T_0 denote the time at which the production of BFU-E from their upstream progenitors restarted following radiation exposure. This time is unobservable, but our experimental data suggest that the value of T_0 lies between day 0 and day 3. Values of T_0 close to 0 indicate that radiation exposure did not delay much the differentiation of upstream progenitors into BFU-E, and thus these progenitors were less affected by irradiation, and conversely for larger values of T_0 .

Possible reasons for a longer delay in the production of new BFU-E include the fact that upstream progenitors that are the closest to BFU-E (e.g., bipotential megakaryocyte erythroid progenitors) were also depleted by irradiation, causing this compartment to be momentarily unable to supply the pool of BFU-E with newly differentiated cells, but earlier precursors were more resistant. In this case, the value of T_0 represents the duration needed for these cells to differentiate and reach the pool of BFU-E from the time of radiation exposure. Another reason is that radiation exposure stopped some hematopoietic progenitors in their cycles without killing them, thereby delaying the production of new BFU-E. These alterations of precursor cell functions could also happen in combination.

Let $T_1, T_2, T_3, \dots$ denote a collection of ordered time points that represent the times at which megakaryocyte erythroid progenitors differentiate into BFU-E. We assume that a single BFU-E is produced at each of these time points. Define the immigration process:

$\Pi(t) := \sum_{l=1}^{\infty} \mathbf{1}_{\{T_l \leq t\}}$. This point process counts the number of upstream hematopoietic progenitors that differentiated into a BFU-E during the time period $[0, t]$. We assume that:

(A8) Conditional on T_0 , the inter-immigration times, $T_{l+1} - T_l$, $l = 0, 1, \dots$, are independent and follow an exponential distribution with common parameter r .

Assumption (A8) implies that, conditional on T_0 , the process $I(t - T_0)$ is a time-homogeneous Poisson process with instantaneous rate r . The first immigrant appears at time T_1 . The BFU-E that immigrates at time T_l initiates a Bellman-Harris process that obeys

Assumptions (A3-A7). We can therefore decompose $Z^+(t)$ as $Z^+(t) = \sum_{l=1}^{\infty} \mathbf{X}^{(l)}(t - T_l)$, where $\{\mathbf{X}^{(l)}(u), u \geq 0\}_{l=1}^{\infty}$ are independent and identically distributed copies of the two-type Bellman-Harris process $\mathbf{X}(t)$.

We assume that the rate of the immigration process $I(t)$ is first identically zero between the time of radiation exposure and T_0 , and then equal to r between T_0 and day 6. Thus, once upstream hematopoietic progenitors start to differentiate into BFU-E, they would do so at a constant rate over time up to day 6. It is possible that the influx of cells into the population of BFU-E increases over time due to the recovery of upstream hematopoietic progenitors. However, this assumption is made over a relatively short time period (T_0 –day 6), with T_0 to be estimated as 2.2 days. Moreover, as we shall see in Section 3.5, the assumption of a constant immigration rate is supported by the linear recovery of the BFU-E compartment as observed in our experiments. This assumption could be relaxed by modeling the immigration process as a time-inhomogeneous Poisson process, for example, as previously considered by Yakovlev and Yanev (2006) and Hyrien and Yanev (2012, 2013), but our goal here is to construct a parsimonious model that allows interpretation of our experimental data.

4.2 Simulations

We conducted simulations to investigate the behavior of the model under selected sets of parameter values that are biologically plausible. A summary of these simulations presented in Figure 4 illustrates the range of behaviors that is expected from the process. The color in each plot indicates an estimate of the distribution function of the population size at a given time point for the BFU-E compartment (first column from left) and for the CFU-E compartment (columns 2-4). In each case, we set $r = 0.1$, $F_1(\cdot) = I(\cdot; 5, 3, 0)$, $F_2(\cdot) = I(\cdot; 10, 2, 0)$, and $p_0 = 0$. The simulations were performed using different values of the probabilities of division p_1 and q_1 : $p_1 = 0.25$ (row 1), $p_1 = 0.5$ (row 2), $p_1 = 0.75$ (row 3); columns 2-4 show results obtained with $q_1 = 0.25$, $q_1 = 0.5$, and $q_1 = 0.75$, respectively. The values of p_2 and q_0 followed from the relationships $p_2 = 1 - p_1$ and $q_0 = 1 - q_1$.

Under normal conditions, the population of BFU-E and CFU-E are in steady state, which corresponds to the limit of the process when both p_1 and q_1 are smaller than 0.5 (panels A.1 and A.2). This is the only case where the system reaches stationarity. In all other cases, either one or both compartments keep on expanding over time. The expansion rate depends on the scenario, and we shall see later that it may be linear (panel B.1 and A.3), quadratic (panel B.2) or exponential (all remaining cases). Simulations performed using different parameter values led to observations that were qualitatively similar.

4.3 Moments of the process

We introduce the probability generating functions (p.g.f.)

$$\varphi_1(t; s_1, s_2) = E \left\{ s_1^{X_1(t)} s_2^{X_2(t)} | \mathbf{X}(0) = (1, 0) \right\}$$

and

$$\varphi_2(t; s_2) = E \left\{ s_2^{X_2(t)} | \mathbf{X}(0) = (0, 1) \right\},$$

where $t \geq 0$ and $|s_k| \leq 1$, $k = 1, 2$. These generating functions characterize the distributions of the Bellman-Harris processes $\mathbf{X}(t)$ and $X_2(t)$ started from a single type-1 and a single type-2 cell, respectively. Setting $t = 0$ yields the boundary conditions $\varphi_1(0; s_1, s_2) = s_1$ and $\varphi_2(0; s_2) = s_2$. Under Assumptions (A3-A7), Jagers (1969) showed that $\varphi_1(t; s_1, s_2)$ and $\varphi_2(t; s_2)$ satisfy a system of nonlinear integral equations:

$$\begin{cases} \varphi_1(t; s_1, s_2) = \int_0^t h_1 \{ \varphi_1(t-u; s_1, s_2), \varphi_2(t-u; s_2) \} dG_1(u) + s_1 \{ 1 - G_1(t) \} \\ \varphi_2(t; s_2) = \int_0^t h_2(\varphi_2(t-u; s_2)) dG_2(u) + s_2 \{ 1 - G_2(t) \}. \end{cases} \quad (2)$$

Let $A_1(t) = E\{X_1(t) | \mathbf{X}(0) = (1, 0)\}$, $A_2(t) = E\{X_2(t) | \mathbf{X}(0) = (1, 0)\}$, and $B_2(t) = E\{X_2(t) | \mathbf{X}(0) = (0, 1)\}$ denote the expectations of $X_k(t)$, $k = 1, 2$, started either with a single type-1 cell or with a single type-2 cell. Expressions for these expectations are computed by differentiating $\varphi_1(t; s_1, s_2)$ and $\varphi_2(t; s_2)$ with respect to (w.r.t.) s_1 or s_2 . For example, the expected number of type-1 cells when the process begins with a single type-1 cell is:

$$A_1(t) = \frac{\partial}{\partial s_1} \varphi_1(t; s_1, s_2) \Big|_{s_1=s_2=1}.$$

differentiating both sides of the integral equations (2) yields a system of renewal-type equations satisfied by $A_1(t)$, $A_2(t)$ and $B_2(t)$:

$$\begin{cases} A_1(t) = a_1 \int_0^t A_1(t-u) dG_1(u) + 1 - G_1(t) \\ A_2(t) = a_1 \int_0^t A_2(t-u) dG_1(u) + a_2 \int_0^t B_2(t-u) dG_1(u) \\ B_2(t) = b_2 \int_0^t B_2(t-u) dG_2(u) + 1 - G_2(t). \end{cases} \quad (3)$$

We have the following initial conditions: $A_1(0) = 1$, $A_2(0) = 0$, and $B_1(0) = 1$. We remark also that these integral equations are valid if the (individual) moments a_1 , a_2 , b_2 , μ_1 , and μ_2 are finite.

Introduce next the second order moments of the embedded Bellman-Harris process:

$$A_{kl}(t) = E \left\{ X_k(t) [X_l(t) - \delta_{kl}] | \mathbf{X}(0) = (1, 0) \right\}, \quad k, l = 1, 2,$$

where $\delta_{kl} = 1$ if $k = l$ and $\delta_{kl} = 0$ otherwise, and

$$B_{22}(t) = E\{X_2(t)[X_2(t) - 1] | \mathbf{X}(0) = (0, 1)\}.$$

These moments are obtained by differentiating the p.g.f.s $\phi_1(t; s_1, s_2)$ and $\phi_2(t; s_2)$ twice:

$$A_{kl}(t) = \frac{\partial^2 \phi_1(t; s_1, s_2)}{\partial s_k \partial s_l} \Big|_{s_1=s_2=1}, \quad k, l=1, 2,$$

and

$$B_{22}(t) = \frac{\partial^2 \phi_2(t; s_2)}{\partial s_2^2} \Big|_{s_2=1},$$

yielding the renewal-type integral equations:

$$A_{11}(t) = a_1 \int_0^t A_{11}(t-u) dG_1(u) + a_{11} \int_0^t A_1^2(t-u) dG_1(u), \quad (4)$$

$$A_{12}(t) = a_1 \int_0^t A_{12}(t-u) dG_1(u) + a_{11} \int_0^t A_1(t-u) A_2(t-u) dG_1(u),$$

$$A_{22}(t) = a_1 \int_0^t A_{22}(t-u) dG_1(u) + a_2 \int_0^t B_{22}(t-u) dG_1(u) + a_{11} \int_0^t A_2^2(t-u) dG_1(u), \quad (5)$$

and

$$B_{22}(t) = b_2 \int_0^t B_{22}(t-u) dG_2(u) + b_{22} \int_0^t B_2^2(t-u) dG_2(u).$$

By assumption, the immigration process (t) is a Poisson process with instantaneous rate r . Hence, the expectation and the variance of the number of immigrants that entered into the population of BFU-E (that is, megakaryocyte erythroid progenitors that differentiated into BFU-E) during the time period $[0, t]$ are: $E\{I(t)\} = rt$ and $\text{Var}\{I(t)\} = rt$.

Introduce next the p.g.f. $\Psi(t; s_1, s_2) = E\left\{s_1^{Z_1^+(t)} s_2^{Z_2^+(t)} | \mathbf{Z}^+(0) = (0, 0)\right\}$, and notice that $\Psi(0; s_1, s_2) = 1$. Using a result from Hyrien and Yanev (2012), we deduce that the p.g.f. $\Psi(t; s_1, s_2)$ assumes the expression:

$$\Psi(t; s_1, s_2) = \exp\left\{-r \int_0^t \{1 - \phi_1(u; s_1, s_2)\} du\right\}, \quad (6)$$

where $\phi_1(u; s_1, s_2)$ satisfies eqn. (2).

Define the expectation of $Z_k(t)$, $Z_k^-(t)$, and $Z_k^+(t)$ ($k = 1, 2$): $M_k(t) = E\{Z_k(t)\}$, $M_k^-(t) = E\{Z_k^-(t)\}$, and $M_k^+(t) = E\{Z_k^+(t)\}$.

By Assumption (A.2), $Z_k^-(t)$ is a pure death process, and

$$M_k^-(t) = \eta_{k0} \{1 - F_k(t)\}.$$

To obtain expressions for $M_k^+(t)$, $k = 1, 2$, we differentiate both sides of eqn. (6) w.r.t. s_1 and s_2 , which yields:

$$M_k^+(t) = r \int_0^t A_k(u) du, \quad k=1, 2, \quad (7)$$

where $M_k^+(0) = 0$. Thus, we see from eqn. (7) that the expectations $M_k^+(t)$, $k = 1, 2$, are proportional to the immigration rate. We deduce from eqn. (1) that $M_k(t)$ is the sum of $M_k^-(t)$ and $M_k^+(t)$:

$$M_k(t) = M_k^-(t) + M_k^+(t).$$

Introduce the variances $V_k(t) = \text{Var}\{Z_k(t)\}$, $V_k^-(t) = \text{Var}\{Z_k^-(t)\}$, and $V_k^+(t) = \text{Var}\{Z_k^+(t) | \mathbf{Z}^+(0) = (0, 0)\}$, $k = 1, 2$. It follows immediately from Assumption (A.1) that

$$\begin{aligned} V_k^-(t) &= E \left[\text{Var} \left\{ Z_k^-(t) | Z_k^-(0) \right\} \right] + \text{Var} \left[E \left\{ Z_k^-(t) | Z_k^-(0) \right\} \right] \\ &= [\eta_{0k} F_k(t) + \sigma_{k0}^2 \{1 - F_k(t)\}] \{1 - F_k(t)\}. \end{aligned}$$

In order to compute $V_k^+(t)$, define the second order moments

$$M_{kk}^+(t) = E \left\{ Z_k^+(t) [Z_k^+(t) - 1] | \mathbf{Z}^+(0) = (0, 0) \right\}.$$

Together with eqn. (6), the fact that

$$M_{kk}^+(t) = \frac{\partial^2 \Psi(t; s_1, s_2)}{\partial s_k^2} \Big|_{s_1=s_2=1}$$

yields the integral equation:

$$M_{kk}^+(t) = r \int_0^t A_{kk}(u) du + \left[r \int_0^t A_k(u) du \right]^2.$$

An expression for $V_k^+(t) = \text{Var}\{Z_k^+(t) | \mathbf{Z}^+(0) = (0, 0)\}$ follows directly from those of $M_k^+(t)$ and $M_{kk}^+(t)$ using the relationship:

$$\begin{aligned} V_k^+(t) &= M_{kk}^+(t) + M_k^+(t) - M_k^+(t)^2 \\ &= r \int_0^t A_{kk}(u) du + M_k^+(t). \end{aligned} \quad (8)$$

Finally, the independence between $Z_k^-(t)$ and $Z_k^+(t)$ (Assumption (A.1)) yields:

$$V_k(t) = V_k^-(t) + V_k^+(t).$$

The goal of the next sections is to derive expressions for the expectation and variance of the process. In particular, their explicit expressions are derived when the process is Markovian, and their asymptotic behaviors are obtained in the general case. Finally we propose to improve these asymptotic approximations by means of Markovian compensators.

4.4 Closed-form expressions for the moments in the Markovian case

The moments $M_k^+(t)$ and $V_k^+(t)$, $k = 1, 2$, take explicit expressions when the lifespan is exponentially distributed: $G_k(t) = 1 - \exp(-t/\mu_k)$, $t \geq 0$. Under this assumption, the process $\mathbf{Z}^+(t)$ is Markovian. In this section we derive expressions for these moments. We shall use them later on for improving asymptotic approximations to $M_k^+(t)$ in the non-Markovian case.

When the life-spans are exponentially distributed, the system of nonlinear integral equations (2) can be transformed into a system of nonlinear differential equations:

$$\begin{cases} \frac{\partial}{\partial t} \varphi_1(t; s_1, s_2) = f_1(\varphi_1(t; s_1, s_2), \varphi_2(t; s_2)) \\ \frac{\partial}{\partial t} \varphi_2(t; s_2) = f_2(\varphi_2(t; s_2)), \end{cases}$$

– with $\phi_1(0; s_2) = s_2$ and $\phi_1(0; s_1, s_2) = s_1$, where $f_1(s_1, s_2) = [h_1(s_1, s_2) - s_1]/\mu_1$ and $f_2(s_2) = [h_2(s_2) - s_2]/\mu_2$ are the corresponding generator functions. The parameters $\alpha_1 = (a - 1)/\mu_1$ and $\alpha_2 = (b_2 - 1)/\mu_2$ coincide with the so-called Malthusian parameters of the process (see

Section 3.5 for a formal definition of these parameters). Since $\frac{\partial^2 \varphi_1}{\partial t \partial s_1} = \frac{\partial f_1}{\partial s_1} \frac{\partial \varphi_1}{\partial s_1}$ and

$\frac{\partial^2 \varphi_2}{\partial t \partial s_2} = \frac{\partial f_2}{\partial s_2} \frac{\partial \varphi_2}{\partial s_2}$, putting $s_1 = s_2 = 1$ yields the equations

$$\begin{cases} \frac{d}{dt} A_1(t) = \alpha_1 A_1(t) \\ \frac{d}{dt} B_2(t) = \alpha_2 B_2(t), \end{cases}$$

with $A_1(0) = B_2(0) = 1$. The solutions are $A_1(t) = e^{\alpha_1 t}$ and $B_2(t) = e^{\alpha_2 t}$.

Next, we have $\frac{\partial^2 \varphi_1}{\partial t \partial s_2} = \frac{\partial f_1}{\partial s_1} \frac{\partial \varphi_1}{\partial s_2} + \frac{\partial f_1}{\partial s_2} \frac{\partial \varphi_2}{\partial s_2}$. Setting $s_1 = s_2 = 1$ gives the linear differential equation

$$\frac{d}{dt} A_2(t) = \alpha_1 A_2(t) + \frac{\alpha_2}{\mu_1} e^{\alpha_2 t}.$$

With the initial condition $A_2(0) = 0$, the solution is:

$$A_2(t) = \begin{cases} a_2 t e^{\alpha_1 t} / \mu_1 & \text{if } \alpha_1 = \alpha_2 \\ \frac{a_2}{\mu_1(\alpha_1 - \alpha_2)} (e^{\alpha_1 t} - e^{\alpha_2 t}) & \text{if } \alpha_1 \neq \alpha_2. \end{cases}$$

We deduce from eqn. (7) expressions for the expectations $M_1^+(t)$ and $M_2^+(t)$:

$$M_1^+(t) = \begin{cases} rt & \text{if } \alpha_1 = 0 \\ \frac{r}{\alpha_1} (e^{\alpha_1 t} - 1) & \text{if } \alpha_1 \neq 0, \end{cases}$$

and

$$M_2^+(t) = \begin{cases} \frac{ra_2}{\alpha_1^2 \mu_1} (\alpha_1 t e^{\alpha_1 t} - e^{\alpha_1 t} + 1) & \text{if } \alpha_1 = \alpha_2 \neq 0 \\ \frac{ra_2}{2\mu_1} t^2 & \text{if } \alpha_1 = \alpha_2 = 0 \\ \frac{ra_2}{(\alpha_1 - \alpha_2)\mu_2} \left(\frac{e^{\alpha_1 t} - 1}{\alpha_1} - \frac{e^{\alpha_2 t} - 1}{\alpha_2} \right) & \text{if } \alpha_1 \neq \alpha_2, \alpha_1 \neq 0, \alpha_2 \neq 0 \\ \frac{ra_2}{\alpha_2 \mu_1} \left(\frac{e^{\alpha_2 t} - 1}{\alpha_2} - t \right) & \text{if } \alpha_1 \neq \alpha_2, \alpha_1 = 0, \alpha_2 \neq 0 \\ \frac{ra_2}{\alpha_1 \mu_1} \left(\frac{e^{\alpha_1 t} - 1}{\alpha_1} - t \right) & \text{if } \alpha_1 \neq \alpha_2, \alpha_1 \neq 0, \alpha_2 = 0. \end{cases}$$

To derive expressions for the variances, we consider the equation

$$\frac{\partial^3 \varphi_1}{\partial t \partial s_1^2} = \frac{\partial^2 f_1}{\partial s_1^2} \left(\frac{\partial \varphi_1}{\partial s_1} \right)^2 + \frac{\partial f_1}{\partial s_1} \frac{\partial^2 \varphi_1}{\partial s_1^2}. \text{ Putting } s_1 = s_2 = 1 \text{ yields the differential equation}$$

$$\frac{d}{dt} A_{11}(t) = \alpha_1 A_{11}(t) + \frac{a_{11}}{\mu_1} e^{2\alpha_1 t}$$

with $A_{11}(0) = 0$, which admits the following solution:

$$A_{11}(t) = \begin{cases} \frac{a_{11}}{\mu_1} t & \text{if } \alpha_1 = 0 \\ \frac{a_{11}}{\alpha_1 \mu_1} e^{\alpha_1 t} (e^{\alpha_1 t} - 1) & \text{if } \alpha_1 \neq 0. \end{cases}$$

The equation $\frac{\partial^3 \varphi_2}{\partial t \partial s_2^2} = \frac{\partial^2 f_2}{\partial s_2^2} \left(\frac{\partial \varphi_2}{\partial s_2} \right)^2 + \frac{\partial f_2}{\partial s_2} \frac{\partial^2 \varphi_2}{\partial s_2^2}$ evaluated at $s_1 = s_2 = 1$ gives

$$\frac{d}{dt} B_{22}(t) = \alpha_2 B_{22}(t) + \frac{b_{22}}{\mu_2} e^{2\alpha_2 t}.$$

With the initial condition $B_{22}(0) = 0$, its solution is

$$B_{22}(t) = \begin{cases} \frac{b_{22}}{\mu_2} t & \text{if } \alpha_2 = 0 \\ \frac{b_{22}}{\alpha_2 \mu_2} e^{\alpha_2 t} (e^{\alpha_2 t} - 1) & \text{if } \alpha_2 \neq 0. \end{cases}$$

Finally, we use the equation $\frac{\partial^3 \varphi_1}{\partial t \partial s_2^2} = \frac{\partial^2 f_2}{\partial s_1^2} \left(\frac{\partial \varphi_1}{\partial s_2} \right)^2 + \frac{\partial f_1}{\partial s_1} \frac{\partial^2 \varphi_1}{\partial s_2^2} + \frac{\partial f_1}{\partial s_2} \frac{\partial^2 \varphi_2}{\partial s_2^2}$. Setting $s_1 = s_2 = 1$ yields the linear equation:

$$\frac{d}{dt} A_{22}(t) = \alpha_1 A_{22}(t) + \frac{a_{11}}{\mu_1} A_2^2(t) + \frac{a_2}{\mu_1} B_{22}(t).$$

Since the initial condition is $A_{22}(0) = 0$, the solution to this equation is

$$A_{22}(t) = e^{\alpha_1 t} \int_0^t e^{-\alpha_1 x} \phi(x) dx,$$

where $\phi(x) = \frac{a_{11}}{\mu_1} A_2^2(x) + \frac{a_2}{\mu_1} B_{22}(x)$. To obtain the expression for $A_{22}(t)$ we consider the following four cases:

Case 1. If $\alpha_1 = \alpha_2 = 0$, then $A_2(t) = a_2 t / \mu_1$, $B_{22}(t) = t / \mu_2$, hence $\phi(x) = \frac{a_2^2}{\mu_1^3} x^2 + \frac{a_2}{\mu_1 \mu_2} x$, and

$$A_{22}(t) = \int_0^t \phi(x) dx = \frac{a_2^2}{3\mu_1^3} t^3 + \frac{a_2}{2\mu_1 \mu_2} t^2.$$

Case 2. If $\alpha_1 = \alpha_2 = 0$, then $A_2(t) = a_2 t e^{\alpha_1 t} / \mu_1$, $B_{22}(t) = \frac{b_{22}}{\alpha_1 \mu_2} e^{\alpha_1 t} (e^{\alpha_1 t} - 1)$, and

$$A_{22}(t) = e^{\alpha_1 t} \left\{ \frac{a_{11} a_2^2}{\alpha_1^3 \mu_1^3} \left[\alpha_1^2 t^2 e^{\alpha_1 t} - 2 \alpha_1 t e^{\alpha_1 t} + 2 (e^{\alpha_1 t} - 1) \right] + \frac{a_2 b_{22}}{\alpha_1^2 \mu_1 \mu_2} (e^{\alpha_1 t} - \alpha_1 t - 1) \right\}.$$

Case 3. If $\alpha_1 = \alpha_2 = 0$, then $A_2(t) = \frac{a_2}{\mu_1 \alpha_1} (e^{\alpha_1 t} - 1)$, $B_{22}(t) = t / \mu_2$, and

$$A_{22}(t) = \frac{a_{11} a_2^2}{\alpha_1^3 \mu_1^3} \left[e^{2\alpha_1 t} - 2 \alpha_1 t e^{\alpha_1 t} - 1 \right] + \frac{a_2}{\alpha_1^2 \mu_1 \mu_2} \left[e^{\alpha_1 t} - \alpha_1 t - 1 \right].$$

Case 4. If $\alpha_1 = \alpha_2, \alpha_2 = 0$, then $A_2(t) = \frac{a_2}{\mu_1 (\alpha_1 - \alpha_2)} (e^{\alpha_1 t} - e^{\alpha_2 t})$,

$B_{22}(t) = \frac{b_{22}}{\alpha_2 \mu_2} e^{\alpha_2 t} (e^{\alpha_2 t} - 1)$, and

$$\begin{aligned} A_{22}(t) = & \frac{a_{11} a_2^2}{\mu_1^3 (\alpha_1 - \alpha_2)^2} \left[e^{\alpha_1 t} (e^{\alpha_1 t} - 1) / \alpha_1 - 2 (e^{(\alpha_1 + \alpha_2)t} - e^{\alpha_1 t}) / \alpha_2 \right] \\ & + \left(\frac{a_{11} a_2^2}{\mu_1^3 (\alpha_1 - \alpha_2)^2} + \frac{b_{22}}{\alpha_2 \mu_1 \mu_2} \right) \frac{(e^{2\alpha_2 t} - e^{\alpha_1 t})}{(2\alpha_2 - 1)} \\ & - \frac{b_{22} (e^{\alpha_2 t} - e^{\alpha_1 t})}{\alpha_2 \mu_1 \mu_2 (\alpha_2 - \alpha_1)}. \end{aligned}$$

Next, expressions for the variances can be deduced from the above expressions and the following formulas:

$$\begin{aligned} D_1^+(t) &= \text{Var} \{X_1(t) | \mathbf{X}(t) = (1, 0)\} = A_{11}(t) + A_1(t) - A_1^2(t), \\ D_2^+(t) &= \text{Var} \{X_2(t) | \mathbf{X}(t) = (1, 0)\} = A_{22}(t) + A_2(t) - A_2^2(t), \\ D_3^+(t) &= \text{Var} \{X_2(t) | \mathbf{X}(t) = (0, 1)\} = B_{22}(t) + B_2(t) - B_2^2(t). \end{aligned}$$

Finally, the variances of the process with immigration can be derived using eqns. (8). Specifically,

$$V_1^+(t) = \begin{cases} rt \left(\frac{a_{11}}{2\mu_1} t + 1 \right) & \text{if } \alpha_1 = 0 \\ \frac{ra_{11}}{2\alpha_1^2\mu_1} e^{\alpha_1 t} \left[e^{\alpha_1 t} + 2 \left(\frac{\alpha_1\mu_1}{a_{11}} - 1 \right) \right] + \frac{r}{\alpha_1} \left(\frac{a_{11}}{\mu_1} - 1 \right) & \text{if } \alpha_1 \neq 0, \end{cases}$$

and

$$V_2^+(t) = \begin{cases} \frac{ra_2^2}{12\mu_1^3} t^4 + \frac{ra_2}{6\mu_1\mu_2} t^3 + \frac{ra_2}{2\mu_1} t^2 & \text{if } \alpha_1 = \alpha_2 = 0; \\ \frac{ra_{11}\alpha_2^2}{4\mu_1^3} (2\alpha_1^2 t^2 e^{2\alpha_1 t} - 6\alpha_1 t e^{2\alpha_1 t} + 6\alpha_1 e^{2\alpha_1 t} - 7e^{\alpha_1 t} + 1) \\ \quad + \frac{ra_2 b_{22}}{\alpha_1^2 \mu_1 \mu_2} (\mu_1 e^{\alpha_1 t} - 2\alpha_1 t^2 - \alpha_1 \mu_1 t - \mu_1) \\ \quad + \frac{ra_2}{\alpha_1^2 \mu_1} (\alpha_1 t e^{\alpha_1 t} - e^{\alpha_1 t} + 1) & \text{if } \alpha_1 = \alpha_2 \neq 0; \\ \frac{ra_{11}\alpha_2^2}{\mu_1^3(\alpha_1 - \alpha_2)^2} \left[\frac{(e^{\alpha_1 t} - 1)^2}{2\alpha_1^2} - \frac{2}{\alpha_2} \left(\frac{e^{(\alpha_1 + \alpha_2)t} - 1}{\alpha_1 + \alpha_2} - \frac{e^{\alpha_1 t} - 1}{\alpha_1} \right) \right] \\ \quad + r \left(\frac{a_{11}\alpha_2^2}{\mu_1^3(\alpha_1 - \alpha_2)^2} + \frac{b_{22}}{\alpha_2 \mu_1 \mu_2} \right) \times \dots & \text{if } \alpha_1 \neq \alpha_2, \alpha_2 \neq 0, \alpha_2 \neq 0; \\ \frac{\alpha_1(e^{2\alpha_2 t} - 1) - 2\alpha_2(e^{\alpha_1 t} - 1)}{2\alpha_1\alpha_2(2\alpha_2 - 1)} \\ \quad - \frac{rb_{22}[\alpha_1(e^{\alpha_2 t} - 1) - \alpha_2(e^{\alpha_1 t} - 1)]}{\alpha_1\alpha_2^2\mu_1\mu_2(\alpha_2 - \alpha_1)} \\ \quad + \frac{ra_2}{(\alpha_1 - \alpha_2)\mu_1} \left(\frac{e^{\alpha_1 t} - 1}{\alpha_1} - \frac{e^{\alpha_2 t}}{\alpha_2} \right) & \text{if } \alpha_1 = 0, \alpha_2 \neq 0; \\ \frac{ra_2^2}{2\mu_1^3\alpha_2^3} [\alpha_2 t^2 - 4(e^{\alpha_2 t} - \alpha_2 t - 1)] \\ \quad + \frac{r}{\alpha_2\mu_1} \left(\frac{a_2^2}{\mu_1^2\alpha_2} + \frac{b_{22}}{\mu_2} \right) \frac{e^{2\alpha_2 t} - \alpha_2 t - 1}{2\alpha_2(2\alpha_2 - 1)} \\ \quad + \frac{r}{\alpha_2^2\mu_1} \left(a_2 - \frac{b_{22}}{a_2\mu_2} \right) (e^{\alpha_2 t} - \alpha_2 t - 1) & \text{if } \alpha_1 = 0, \alpha_2 = 0. \\ \frac{ra_{11}\alpha_2^2}{2\alpha_1^4\mu_1^3} (e^{2\alpha_1 t} - 4\alpha_1 t e^{\alpha_1 t} + 4e^{\alpha_1 t} - 2\alpha_1 t - 5) \\ \quad + \frac{ra_2}{2\alpha_1^2\mu_1\mu_2} (2e^{\alpha_1 t} - \alpha_1^2 t^2 - 2\alpha_1 t - 2) \\ \quad + \frac{ra_2}{\alpha_1^2\mu_1} (e^{\alpha_1 t} - 1 - \alpha_1 t) & \text{if } \alpha_1 \neq 0, \alpha_2 = 0. \end{cases}$$

Proposition 1. Suppose that $\alpha_2 > \alpha_1$ and $r > 0$. Then $M_2^+(t)$ is asymptotically a decreasing function of the probability of division of BFU-E (p_1) over the interval $[0, 1]$.

Proof When $\alpha_1 < \alpha_2$, as t gets large, we have

$$M_2^+(t) \sim \frac{ra_2}{\alpha_2(\alpha_2 - \alpha_1)\mu_1} e^{\alpha_2 t} = \frac{r(1 - p_1)}{\alpha_2(\alpha_2\mu_1 - 2p_1 - 1)} e^{\alpha_2 t}.$$

Taking the first order partial derivative of the right-hand side of the last identity with respect to p_1 , we obtain

$$\frac{\partial M_2^+(t)}{\partial p_1} \sim \frac{r(1 - \alpha_2 \mu_1)}{\alpha_2^2 (\alpha_2 \mu_1 - 2p_1 - 1)^2} e^{\alpha_2 t}. \quad (9)$$

Next, we remark that the inequality $\alpha_1 < \alpha_2$ is equivalent to $1 + \alpha_2 \mu_1 > 2p_1$. Hence the numerator of the right-hand side of identity (9), which can be rewritten as $r(1 - \alpha_2 \mu_1) = r(2 - (1 + \alpha_2 \mu_1))$, satisfies the inequality $r(1 - \alpha_2 \mu_1) < r(2 - 2p_1)$, from which we deduce that $r(1 - \alpha_2 \mu_1) < 0$, hence $\partial M_2^+(t) / \partial p_1 < 0$, for every $p_1 \in [0, 1]$.

4.5 Asymptotic behavior of first and second order moments

Closed-form expressions for the moments are generally unattainable for age-dependent (non-Markovian) processes. Accurate approximations may be constructed in a number of ways, including using simulations or saddlepoint approximations (Hyrien et al, 2005, 2010). We do not pursue such approximations here because they will not be suitable for our estimation procedure which will require that we simplify the parametric structure of the model to avoid nonidentifiability issues. Instead, we construct approximations to the moments using their asymptotic behavior as t gets large.

Let α_1 and α_2 denote the roots to the equations $a_1 \int_0^\infty e^{-\alpha_1 x} dG_1(x) = 1$ and $b_2 \int_0^\infty e^{-\alpha_2 x} dG_2(x) = 1$. These parameters will characterize the rates at which the numbers of cells generated by a single BFU-E or a single CFU-E are expected to increase or decrease over time in the absence of immigration. They are referred to as the *Malthusian parameters*. The process $Z_k(t)$, started with a single type- k cell, is subcritical if $\alpha_k < 0$ ($\alpha_1 < 1$ or $\alpha_2 < 1$), critical if $\alpha_k = 0$ ($\alpha_1 = 1$ or $\alpha_2 = 1$) and supercritical if $\alpha_k > 0$ ($\alpha_1 > 1$ or $\alpha_2 > 1$) (Athreya and Ney, 1972). In the critical case it is well-known that $A_1(t) = 1$ and $B_2(t) = 1$, $t \geq 0$. It is also well-known that the expectations increase or decrease exponentially fast:

$$A_1(t) \sim C_1 e^{\alpha_1 t} \quad \text{and} \quad B_2(t) \sim C_2 e^{\alpha_2 t}, \quad (10)$$

where the constants C_1 and C_2 are given by

$$C_1 = \begin{cases} (a_1 - 1) / \alpha_1 a_1 \tilde{\mu}_1 & \text{if } \alpha_1 \neq 0 \\ 1 & \text{if } \alpha_1 = 0 \end{cases}$$

and

$$C_2 = \begin{cases} (b_2 - 1) / \alpha_2 b_2 \tilde{\mu}_2 & \text{if } \alpha_2 \neq 0 \\ 1 & \text{if } \alpha_2 = 0, \end{cases}$$

where $\tilde{\mu}_1 = a_1 \int_0^\infty x e^{-\alpha_1 x} dG_1(x)$ and $\tilde{\mu}_2 = b_2 \int_0^\infty x e^{-\alpha_2 x} dG_2(x) = \int_0^\infty x d\tilde{G}_2(x)$ (Athreya and Ney, 1972). The constants $\tilde{\mu}_1$ and $\tilde{\mu}_2$ are also the expectations of the distribution functions

$\tilde{G}_1(t) = a_1 \int_0^t e^{-\alpha_1 x} dG_1(x)$ and $\tilde{G}_2(t) = b_2 \int_0^t e^{-\alpha_2 x} dG_2(x)$. They are assumed finite: $\tilde{\mu}_1 < \infty$ and $\tilde{\mu}_2 < \infty$.

The asymptotic behavior of $A_2(t)$ depends on both $X_1(t)$ and X_2 . Put $\delta = \alpha_2 - \alpha_1$ and write $\hat{G}_1(\alpha_2) = \int_0^\infty e^{-\alpha_2 x} dG_1(x)$. Jagers (1969) showed that, as t gets large,

$$A_2(t) \sim \begin{cases} K_1 t e^{\alpha_1 t} & \text{if } \delta = 0 \\ K_2 e^{\alpha_1 t} & \text{if } \delta < 0 \\ K_3 e^{\alpha_2 t} & \text{if } \delta > 0, \end{cases} \quad (11)$$

where $K_1 = a_2 C_2 / a_1 \tilde{\mu}_1$, $K_2 = a_2 (a_1 \tilde{\mu}_1)^{-1} \int_0^\infty e^{-\alpha_1 x} B_2(x) dx$, and $K_3 = a_2 C_2 \hat{G}_1(\alpha_2) / \{1 - a_1 \hat{G}_1(\alpha_2)\}$. Let $\alpha_{\max} = \max\{\alpha_1, \alpha_2\}$, and define the constant

$$K_{\alpha_{\max}} = \begin{cases} K_1 & \text{if } \alpha_{\max} = \alpha_1 = \alpha_2 \\ K_2 & \text{if } \alpha_{\max} = \alpha_1 > \alpha_2 \\ K_3 & \text{if } \alpha_{\max} = \alpha_2 > \alpha_1. \end{cases}$$

The asymptotic behavior of the expectations of the process with immigration is finally deduced from eqns. (7) and (10). Specifically, we find, as $t \rightarrow \infty$,

$$M_1^+(t) \sim \begin{cases} D_1 = r \int_0^\infty A_1(u) du & \text{if } \alpha_1 < 0 \\ rt & \text{if } \alpha_1 = 0 \\ r C_1 e^{\alpha_1 t} / \alpha_1 & \text{if } \alpha_1 > 0. \end{cases} \quad (12)$$

Remark 1. When $Z_1(t)$ is subcritical ($\alpha_1 < 0$), the expected number of type-1 cells converges to a constant D_1 as $t \rightarrow \infty$. When the process is critical ($\alpha_1 = 0$), $M_1^+(t)$ increases linearly with time. When the process is supercritical ($\alpha_1 > 0$), the population grows on average exponentially fast. Interestingly, the exponential growth rates of the process with immigration is identical to that of the embedded Bellman-Harris process. Intuitively, as the size of the population increases, the contribution of the immigration process to the dynamics of the population becomes negligible relative to the contribution of the cells that are already present in the population. Thus, the impact of immigration is important when the population size is small (for example, it prevents extinction of the population), but it becomes otherwise negligible.

The asymptotic behavior of $M_2^+(t)$ follows from eqns. (7) and (11):

$$M_2^+(t) \sim \begin{cases} -r K_{\alpha_{\max}} / \alpha_{\max} & \text{if } \alpha_1 \neq \alpha_2 \text{ and } \alpha_{\max} < 0 \\ r K_{\alpha_{\max}} t & \text{if } \alpha_1 \neq \alpha_2 \text{ and } \alpha_{\max} = 0 \\ r K_{\alpha_{\max}} e^{\alpha_{\max} t} / \alpha_{\max} & \text{if } \alpha_1 \neq \alpha_2 \text{ and } \alpha_{\max} > 0 \\ -r K_1 / \alpha_{\max} & \text{if } \alpha_1 = \alpha_2 = \alpha_{\max} \text{ and } \alpha_{\max} < 0 \\ r a_2 t^2 / 2 \mu_1 & \text{if } \alpha_1 = \alpha_2 = \alpha_{\max} \text{ and } \alpha_{\max} = 0 \\ r K_1 t e^{\alpha_{\max} t} / \alpha_{\max} & \text{if } \alpha_1 = \alpha_2 = \alpha_{\max} \text{ and } \alpha_{\max} > 0. \end{cases} \quad (13)$$

The asymptotic behavior of $V_1^+(t)$ and of $V_2^+(t)$ is presented in Theorems 1 and 2 and proven in the Appendix.

Theorem 1. Assume that the constants $r, a_1, a_2, b_2, a_{11}, a_{12}, a_{22}, b_{22}, \mu_1, \mu_2$, and $\hat{G}_1(2\alpha_1)$ are all finite. Then, as t gets large, we have

$$V_1^+(t) \sim \begin{cases} \frac{ra_{11}C_1^2\hat{G}_1(2\alpha_1)}{2\alpha_1(1-a_1\hat{G}_1(2\alpha_1))}e^{2\alpha_1 t} & \text{if } \alpha_1 > 0 \\ \frac{r}{2\mu_1}t^2 & \text{if } \alpha_1 = 0 \\ D_{11} = r \int_0^\infty A_{11}(u) du + D_1 & \text{if } \alpha_1 < 0, \end{cases}$$

where $0 < D_{11} < \infty$.

Theorem 2. Assume that the first and second individual moments are finite. Then, as $t \rightarrow \infty$,

$$V_2^+(t) \sim \begin{cases} \frac{ra_2C_2}{a_1\mu_2^2[1-a_1\hat{G}_1(2\alpha_1)]2\alpha_1}t^2e^{2\alpha_1 t} & \text{if } \alpha_1 = \alpha_2 > 0 \\ \frac{ra_{11}K_2^2\hat{G}_1(2\alpha_1)}{[1-a_1\hat{G}_1(2\alpha_1)]2\alpha_1}e^{2\alpha_1 t} & \text{if } \alpha_1 > \alpha_2 \text{ and } \alpha_1 > 0 \\ \frac{rC(\alpha_2)\hat{G}_1(2\alpha_2)}{1-a_1\hat{G}_1(2\alpha_2)}e^{2\alpha_2 t} & \text{if } \alpha_2 > \alpha_1 \text{ and } \alpha_2 > 0 \\ \frac{ra_2^2}{12\mu_1^3}t^4 & \text{if } \alpha_1 = \alpha_2 = 0 \\ \frac{ra_2^2}{2a_1\mu_1^3}\left\{\int_0^\infty B_2(x) dx\right\}^2t^2 & \text{if } \alpha_1 = 0 > \alpha_2 \text{ and } \hat{G}_1(\alpha_2) < \infty \\ \frac{ra_2}{2\mu_2(1-a_1)}t^2 & \text{if } \alpha_2 = 0 > \alpha_1 \\ & \text{if } \{\alpha_1 < \alpha_2 < 0\} \\ r \int_0^\infty [A_{22}(x) + A_2(x)] dx & \text{or } \left\{\alpha_1 = \alpha_2 < 0, \hat{G}_1(2\alpha_1) < \infty\right\} \\ & \text{or } \left\{\alpha_2 < \alpha_1 < 0, \hat{G}_1(\alpha_2) < \infty, \hat{G}_1(2\alpha_1) < \infty\right\}. \end{cases}$$

Remark 2. Steady state is expected under normal conditions. It is attained only if $\alpha_1 < 0$ and $\alpha_2 < 0$; that is, when both embedded Bellman-Harris processes are subcritical, reflecting the fact that the probabilities of self-renewing division of both cell types are smaller than 0.5.

From the asymptotic behavior of $M_1^+(t)$ and $M_2^+(t)$ as $t \rightarrow \infty$, it is easily seen that the points of convergence are

$$\lim_{t \rightarrow \infty} M_{11}(t) = -\frac{rC_1}{\alpha_1}, \quad (14)$$

and

$$\lim_{t \rightarrow \infty} M_{12}(t) = -\frac{rK_{\alpha_{\max}}}{\alpha_{\max}}. \quad (15)$$

Since the number of CFU-E is generally larger than the number of BFU-E at steady state, we deduce that the model parameters should satisfy the inequality $K_{\alpha_{\max}}/\alpha_{\max} < C_1/a_1$. Although the clone generated by any cell of a subcritical process will eventually become extinct with probability one, the size of the population is maintained over time at steady

state level by the immigration process. In all other cases, the size of at least one compartment will diverge. We notice that $M_2^+(t)$ increases exponentially fast if either $\alpha_1 > 0$ or $\alpha_2 > 0$, with its growth rate being determined by the largest of these two Malthusian parameters. When both Bellman-Harris processes are critical, the growth rate is linear for $M_1^+(t)$ and quadratic for $M_2^+(t)$.

4.6 Markovian compensation of the asymptotic approximations

In the previous section we have constructed asymptotic approximations to $M_k^+(t)$, $k = 1, 2$, under general lifespan distributions. These approximations do not always satisfy the initial conditions $M_k^+(0) = 0$. For example, when $\alpha_1 < \alpha_2 < 0$, we have $M_1^+(0) \sim r \int_0^\infty A_1(u) du$ and $M_2^+(0) \sim -rK_3/\alpha_2$. We propose to correct these approximations via a multiplicative factor constructed using the explicit expressions of the moments obtained when the process is Markovian.

To construct these approximations, we introduce the following notation: $\tilde{M}_k^+(t)$ denotes the asymptotic approximation to $M_k^+(t)$ that we constructed in Section 3.5; $M_k^{+,M}(t)$ denotes the expression for $M_k^+(t)$ under the assumption of the Markovian process defined in Section 3.4; and $\tilde{M}_k^{+,M}(t)$ stands for the asymptotic approximation to $M_k^{+,M}(t)$. Thus, when the model is Markovian, $M_k^+(t) = M_k^{+,M}(t)$ and $\tilde{M}_k^+(t) = \tilde{M}_k^{+,M}(t)$. We propose to approximate $M_k^+(t)$ by $\hat{M}_k^+(t) = \Delta_k(t) M_k^{+,M}(t)$, where the correction factor $\Delta_k(t)$ is $\Delta_k(t) = \tilde{M}_k^{+,M}(t) / \tilde{M}_k^+(t)$. For example, when $\alpha_1 < 0$ and $\alpha_2 < 0$, we have $\tilde{M}_1^+(t) = r \int_0^\infty A_1(u) du$ and $M_1^{+,M}(t) = r(e^{\alpha_1 t} - 1)/\alpha_1$, hence $\tilde{M}_1^{+,M}(t) = r/\alpha_1$ and $\Delta_1(t) = 1 - e^{\alpha_1 t}$. We do not attempt to establish properties of these approximations and acknowledge that other approximations, sometimes better, could be constructed. However, we remark that $\lim_{t \rightarrow \infty} \Delta_k(t) = 1$, $\hat{M}_k^+(0) = 0$, and $\hat{M}_k^+(t) = M_k^{+,M}(t)$ when the model is Markovian. Thus, these approximations exhibit several interesting features. For example, they are exact when the process is Markovian, and almost exact when departure from the Markovian assumptions are moderate. They do not involve any additional parameters compared to what the asymptotic approximations $\tilde{M}_k^+(t)$ require; this parsimony is statistically attractive. Finally, we have empirical evidence from numerical studies that these approximations work well for non-Markovian processes (Hyrien and Yanev, 2012).

5 Application to the kinetics of erythroid progenitors

5.1 Experimental data and parameter estimation

In our experiments, we estimated the frequencies of BFU-E and CFU-E at discrete time points $(t_i, i = 1, \dots, n)$ in n cohorts of mice using colony assays. An average of 3 cohorts of mice were observed every 24 hours post-radiation exposure. Each cohort included two mice: one control (unexposed to radiation; $g = 1$) and one that was exposed to a radiation dose of 4 Gy ($g = 2$). Let Z_{igk} denote the true, unobservable number of type- k cells ($k = 1, 2$) at time t_i in the i th mouse of the g th treatment group, and let the random variables Y_{igk} , $k = 1, 2$,

denote an estimate of Z_{igk} obtained using these burst- and colony-forming unit assays t_i units of time post-irradiation.

We introduce notation to distinguish expectations of cell frequencies in each treatment group: $M_{gk}(t)$ denotes the expected number of type- k cells at time $t = 0$ in any mouse of the g th treatment group, $g = 1, 2$. Likewise, the vector $\tau^{(g)}$, $g = 1, 2$, denotes the value of the vector θ for mice of treatment group g . In particular, $\alpha_k^{(g)}$ stands for the k th Malthusian parameter of treatment group g .

Under normal conditions, the system is in steady state and we must have $\alpha_1^{(1)} < 0$ and $\alpha_2^{(1)} < 0$. It follows from eqns. (14) and (15) that the expected numbers of cells at any time $t = 0$ in unexposed mice are given by $M_{11}(t) = \eta_{01}^{(1)} = -rC_1^{(1)}/\alpha_1^{(1)}$, and

$$M_{12}(t) = \eta_{02}^{(1)} = -rK_{\alpha_{max}^{(1)}}/\alpha_{max}^{(1)}.$$

To reflect the fact that the colony assays underestimate the actual number of BFU-E and CFU-E, we assume that there exists a multiplicative constant $A_{igk} < 1$, which represents the fraction of cells (either BFU-E or CFU-E) that were successfully grown into colonies, such that $Y_{igk} = A_{igk}Z_{igk}$, $i = 1, \dots, m$, $k = 1, 2$, $g = 1, 2$. Using image analyses, we found that approximately 35-40% of imaged (phenotypic) CFU-E were detected by colony formation (Peslak, unpublished data). We looked at this percentage on several occasions and found that the percentage fell consistently in this range. The constants A_{igk} may be subject to experimental variability (such as day-to-day variation) and are assumed to be random. The plating efficiency does not vary according to the starting cell number, and it is reasonable to suppose that they do not depend on the number of cells in the population (Z_{igk}). In particular, they are identically distributed between treatment groups. Thus, for each cell type ($k = 1, 2$), there exists a constant $\bar{a}_k$ (0, 1) identical across treatment groups and cohorts of mice such that $E(A_{igk}|Z_{igk}) = \bar{a}_k$. Under this assumption, we can write $E(Y_{igk}) = \bar{a}_k M_{gk}(t_i)$.

Define the relative frequencies of BFU-E and CFU-E in mice exposed to 4 Gy compared to control mice: $R_{ik} = Y_{i2k}/Y_{i1k}$. A bivariate first-order Taylor expansion gives

$$R_{ik} = \frac{M_{2k}(t_i)}{M_{1k}(t_i)} + \frac{Y_{i2k} - \bar{a}_k M_{2k}(t_i)}{\bar{a}_k M_{1k}(t_i)} - \frac{M_{2k}(t_i) \{Y_{i1k} - \bar{a}_k M_{1k}(t_i)\}}{\bar{a}_k M_{1k}(t_i)^2} + O_p \left[\left\| \begin{pmatrix} Y_{i1k} - \bar{a}_k M_{1k}(t_i) \\ Y_{i2k} - \bar{a}_k M_{2k}(t_i) \end{pmatrix} \right\|^2 \right].$$

Ignoring the remainder term in the expansion yields the approximate statistical model $R_{ik} = r_k(t_i) + \varepsilon_{ik}$, where $r_k(t_i) = M_{2k}(t_i)/M_{1k}(t_i)$ and where $\varepsilon_i = (\varepsilon_{i1}, \varepsilon_{i2})'$ is a random vector assumed centered about $\mathbf{0} = (0, 0)$ and with variance-covariance matrix $\Sigma(t_i) = \text{Var}(\varepsilon_i)$.

In all analyses we assumed that the disintegration times of BFU-E and CFU-E exposed to radiation are gamma distributed:

$$F_k(t) = F_k(t; \nu_{0k}, \beta_{0k}) := \int_0^t \frac{\beta_{0k}^{\nu_{0k}}}{\Gamma(\nu_{0k})} x^{\nu_{0k}-1} e^{-\beta_{0k}x} dx \quad t \geq 0, k=1, 2.$$

We write $\mu_{0k} = \nu_{0k} / \beta_{0k}$ and $\sigma_{0k}^2 = \nu_{0k} / \beta_{0k}^2$ for the mean and variance of the distribution. We note that the shift parameter introduced in Assumption (A4) is set to 0 here. This choice is motivated by the observation that BFU-E and CFU-E depleted quickly after radiation exposure (Fig. 3).

The parameters entering the expression for the expectation and variance functions of the process are not all estimable based on our experimental data. Visual inspection of the data (Fig. 2) indicates that the dynamics of both cell populations were close to the asymptotic behavior predicted by the branching process. For example, the number of BFU-E increased almost linearly between day 3 and day 7 (suggesting that the population of BFU-E met the assumptions of a critical or near-critical branching process), whereas the number of CFU-E increased either quadratically or exponentially fast between day 3 and day 6 (suggesting that the population of CFU-E might be governed by a critical or super-critical branching process). Since convergence of the moments to their asymptotic approximations occurs generally quickly, we propose to approximate the regression functions $r_k(t)$, $k = 1, 2$, via either the asymptotic approximations of the expectations $M_k^+(t)$ or their Markov-compensated version, as appropriate.

It is also worth noting that these approximations simplify appreciably the parametric structure of the model. The original parameterization included parameters for the distributions of the lifespans, for the probabilities of division, differentiation and death of both cell types, and the immigration rate. These parameters are not all identifiable from the data, and attempting to estimate all of them would reduce our ability to interpret the data. By using the asymptotic approximations to the expectation and variance, the main features of the dynamics of the process are still captured via the Malthusian parameters ($\alpha_1^{(2)}$ and $\alpha_2^{(2)}$), which are both estimable when $\alpha_1^{(2)} < \alpha_2^{(2)}$, which is what the experimental data suggest. These parameters have also a clear biological interpretation.

We estimated the vector $\alpha = (\theta^{(1)'}, \theta^{(2)'})'$ by minimizing the weighted least squares criterion:

$$C(\theta) = \sum_{k=1}^2 \sum_{i=1}^n \frac{\left\{ R_{ik} - r_k(t_i, \theta^{(k)}) \right\}^2}{\hat{s}_{ik}^2},$$

where $\hat{s}_{ik}^2$ denotes an estimate of the variance of R_{ik} computed using the sample variance of all the ratios R_{ik} observed t_i units of time post-irradiation. Standard errors of the parameter estimates were constructed by using a bootstrap procedure (Efron and Tibshirani, 1993), where we resampled vectors $\mathbf{R}_i = (R_{i1}, R_{i2})$ within the set of ratios observed at the same time post-irradiation. The fit of the model was assessed graphically (e.g., by visually inspecting the model residuals).

We performed three analyses on our experimental data. In the first analysis, we fitted the model under the assumption that the cell kinetics parameters were identical before and after radiation exposure; in particular, $\alpha_k^{(1)} = \alpha_k^{(2)}$, $k = 1, 2$. Under this assumption, the expressions

for the ratios $M_{2k}^+(t)/M_{1k}^+(t)$, $k = 1, 2$, and their asymptotic approximations simplify. For example, when $\alpha_1^{(1)} < 0$ and $\alpha_2^{(1)} < 0$, the bias-corrected approximations yields

$\hat{M}_{2k}^+(t)/\hat{M}_{1k}^+(t) \sim 1 - e^{\alpha_k^{(1)}t}$, $k = 1, 2$. Hence, we set

$r_k(t) = 1 - F_k(t) + \left(1 - e^{\alpha_k^{(1)}(t-T_0)}\right) \mathbf{1}_{\{t \geq T_0\}}$. The goal of this first analysis was to confirm that the dynamics of erythroid progenitors changed in response to the damages induced by radiation exposure, as one would expect.

In the next two analyses, the previously made assumption that cell kinetics parameters did not change after radiation exposure was relaxed. We used two models that differed in the constraints that they imposed on the distributions of the time to disintegration of BFU-E and of CFU-E exposed to radiation exposure: in *Analysis 2*, we assumed that the distributions for the time to disintegration of BFU-E and of CFU-E that were born before radiation exposure were identical: $F_1(\cdot) \equiv F_2(\cdot)$; in *Analysis 3*, we did not impose this constraint in order to make the model more flexible, thereby allowing the disintegration process to operate differently between the two cell types, if needed. In these analyses, the regression functions $r_k(t)$, $k = 1, 2$, differed from those used in *Analysis 1*. For example, when $\alpha_1^{(2)} \neq \alpha_2^{(2)}$, $\alpha_1^{(2)} \neq 0$ and $\alpha_2^{(2)} \neq 0$ we set

$$r_1(t) = 1 - F_1(t) + \psi_1 \left\{ e^{\alpha_1^{(2)}(t-T_0)} - 1 \right\} \mathbf{1}_{\{t \geq T_0\}},$$

and

$$r_2(t) = 1 - F_2(t) + \psi_2 \left\{ \frac{e^{\alpha_1^{(2)}(t-T_0)} - 1}{\alpha_1^{(2)}} - \frac{e^{\alpha_2^{(2)}(t-T_0)} - 1}{\alpha_2^{(2)}} \right\} \mathbf{1}_{\{t \geq T_0\}},$$

where ϕ_1 and ϕ_2 are unknown coefficients that depend on $\alpha^{(1)}$ and $\theta^{(2)}$.

5.2 Results from model-based analyses

The parameter estimates obtained in these three analyses are reported in Table 1 with their standard errors (se). The model fitted in the first analysis is plotted in Figure 5. It is able to capture properly the linear expansion of the BFU-E compartment, but it clearly underestimates the number of CFU-E. We take this lack-of-fit as evidence that some cell kinetics parameters changed in response to radiation exposure. Since the model underestimates the number of CFU-E observed during the experiment, likely changes in parameter values include an increase in the probability of self-renewal of CFU-E, a decrease of their probabilities of death and of differentiation, and a perturbation of the mitotic cycle duration, or all of the above together.

The fit obtained in the other analyses were all equally good and provided estimates that were in close agreement with each other. Figure 6 displays the fitted model obtained from *Analysis 2*. Unless otherwise stated, our discussion below will be based on results from this

analysis because it used the most parsimonious model that was able to fit the data properly. The main conclusions are as follows:

- We estimated that the BFU-E and CFU-E that were exposed to radiation disappeared on average after about 0.44 day ± 0.45 day for both cell types.
- The delay parameter T_0 was estimated as 2.18 days ($\pm se = 0.37$ day). Its value represents the duration that was needed for upstream hematopoietic progenitors to start differentiating again into BFU-E following radiation exposure. Thus, newly differentiated BFU-E started to appear in the bone marrow about 2.18 days ($\simeq 52$ hours) after radiation exposure, thereby indicating that radiation, as expected, disrupted the kinetics of the upstream hematopoietic progenitors.

- The Malthusian parameter for the population of BFU-E was estimated as

$\hat{\alpha}_1^{(2)} = 0.06$ ($\pm se = 0.12$), indicating that this population of cells behaved as a (near) critical branching process during the recovery phase. This means that every BFU-E would either divide with probability close to 0.5, or, with probability 0.5, exit the mitotic cycle to either die or differentiate into a CFU-E. We remark that the approximate linear expansion of the BFUE between day 2 and day 6 is typical of the assumptions of a constant immigration rate r and a probability of division (p_1) equal to 0.5; thus, the BFU-E compartment appears to be described by a critical branching process.

- The Malthusian parameter for the population of CFU-E was estimated as

$\hat{\alpha}_2^{(2)} = 1.02$ ($\pm se = 0.28$), suggesting that the population would behave as a super-critical branching process between $\sim T_0$ and day 6 post-exposure; thus, CFU-E would divide with a probability strictly greater than 0.5, hence also greater than that of BFU-E. We fitted also the model under the assumption that both probabilities of division (p_1 and q_1) are equal to 0.5, in which case the function $M_2(t)$ is asymptotically quadratic in t . The fit provided by the model was not as good as the one obtained under the assumption that $q_1 = 0.5$, suggesting that the Malthusian parameter of the CFU-E compartment was strictly larger than 0 (see Figure 7).

- If we assume that the life-span of CFU-E follows a (central) gamma distribution with shape and scale parameters (ψ_2, β_2), the Malthusian parameters of the CFU-E

compartment takes the form $\alpha_2 = \beta_2 (b_2^{1/\nu_2} - 1)$. The mean and variance of the lifespan, parameterized as a function of α_2, b_2 and the shape parameter ν_2 , are given by

$\mu_2(\alpha_2, b_2, \nu_2) = \nu_2 (b_2^{1/\nu_2} - 1) / \alpha_2$ and $\sigma_2^2(\alpha_2, b_2, \nu_2) = \nu_2 (b_2^{1/\nu_2} - 1)^2 / \alpha_2^2$. The expectation $\mu_2(\alpha_2, b_2, \nu_2)$ is a decreasing function of ν_2 , and, taking the limit as $\nu_2 \rightarrow \infty$, we find that $\mu_2(\alpha_2, b_2, \nu_2) \rightarrow \log(b_2)/\alpha_2$ for all $\nu_2 > 0$ and $b_2 > 0$. Replacing α_2 by the estimate $\hat{\alpha}_2^{(2)} = 1.02$ yields an upper bound for the expected lifespan of $\log(b_2)/1.02 = \log(2q_1)/1.02$. The probability of division of CFU-E during the recovery phase is likely strictly greater than 0.5 because $\hat{\alpha}_2^{(2)} > 0$. Thus, with a probability of division of $q_1 = 0.85$ (resp., $q_1 = 0.65, 0.75, 0.95$), the minimum expected lifespan of CFU-E is ~ 12.5

hours (resp., 6.2, 9.5, 15.1 hours). These calculations indicate that the estimate of the Malthusian parameter $\hat{\alpha}_2^{(2)}=1.02$ is plausible.

6 Discussion

We used a two-type age-dependent branching process with immigration to model the kinetics of erythroid progenitor populations (BFU-E and CFU-E) and their recovery following radiation exposure. The model allows both cell types to either divide, die or differentiate. It assumes that cell life-span varies between cells and is age-dependent, and it accounts for a stochastic influx of cells into the BFU-E compartment via an immigration process. We characterized the range of long-term behaviors of the model, and proposed estimates of the Malthusian parameters of the process.

The main results suggested by the analysis of our experimental data is that, during the recovery phase of the erythron, BFU-E self-renewed with probability 0.5, whereas CFU-E self-renewed with a probability strictly greater than 0.5. In both cases, these probabilities were higher than expected under steady state normal conditions, indicating that both BFU-E and CFU-E increase their ability to self-renew during stress erythropoiesis, as suggested by Crauste et al (2008). Another important finding is that it took about 2.18 days for the BFU-E compartment to start replenishing from the time of radiation exposure, and thus cells from which BFU-E arise were likely damaged by the exposure as well.

The analysis of our experimental data suggested that the Malthusian parameter of the CFU-E compartment was larger than that of the BFU-E compartment during the expansion phase:

$\alpha_2^{(2)} > \alpha_1^{(2)}$. We showed, when this inequality holds and when the model obeys the assumptions of the Markovian process (Section 3.4), that $M_2^+(t)$ decreases asymptotically with the probability of division of BFU-E, p_1 . Thus, everything else being held constant, the smaller the probability of division p_1 , the larger (on average) the number of CFU-E produced by the system. The average number of CFU-E in the population is therefore maximized if the BFU-E neither die nor divide but instead differentiate into CFU-E with probability one. We remark, when $p_1 < 0.5$, that the number of BFU-E increases over time until reaching a steady state level that may be different from that of the BFU-E compartment prior to radiation exposure. In order for the BFU-E compartment to continue expanding until it reaches at least its pre-exposure level before negative feedback mechanisms come into play to modulate the recovery process, we must have $p_1 \geq 0.5$, with the population size increasing linearly when $p_1 = 0.5$ and exponentially when $p_1 > 0.5$. Taken together, these findings suggest that the strategy that seems to be adopted by the erythron in response to the damage caused to the erythroid progenitor compartments by radiation exposure ensures that the BFU-E compartment will replenish, while optimizing the rate at which the CFU-E compartment will recover.

We did not study the third phase of the kinetics of erythroid progenitors where the population of CFU-E stabilizes. Instead, we focused on the dynamics of these cells before negative feedback mechanisms slowed down the expansion of the CFU-E. It is unclear at this point which aspects of the kinetics of these cells were altered by such feedbacks. It is

likely that the distribution of cell fate was modified as CFU-E entered the stabilization phase. For example negative feedbacks might decrease the probability of self-renewal of CFU-E (q_1). While we could not estimate the probabilities of differentiation and of death, the generation of robust erythroblast precursors provide evidence that differentiation is increased (Peslak, 2012).

We computed the expected relative frequency of BFU-E predicted by the model that we fitted in *Analysis 2*, and plotted these predictions together with our experimental data from day 0 to day 14. Although the model was only fitted to the first half of the data points, it was able to capture the overall kinetics of the BFU-E compartment without having to change any of the parameter values. However a closer look at the graph reveals patterns in the recovery of the BFU-E compartment that suggest that the expansion of the BFU-E compartment might have undergone several phases which yielded the two “steps” that are visible between day 6 and day 14. This finding suggests that either the rate of self-renewal or the rate at which newly formed BFU-E immigrate into the BFU-E compartment, or both of them, might increase and decrease over time. Thus, negative feedback mechanisms would also play a role in the recovery of the BFU-E compartment.

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7 Appendix

We notice first that the renewal-type equations (3) and (4)-(5) have all the general form:

$$U(t) = \kappa \int_0^t U(t-x) dG(x) + f(t), \quad (16)$$

for some constant $\kappa > 0$, where $G(\cdot)$ denote the c.d.f. of a non-negative r.v. with expectation $\mu = \int_0^\infty x dG(x)$ assumed finite, and where $f(t)$ is a real-valued function. For every $\lambda \geq 0$, let $\hat{G}(\lambda) := \int_0^\infty e^{-\lambda x} dG(x)$ denote the Laplace transform of the equation $G(\cdot)$. The solution a to the equation $\kappa \hat{G}(a) = 1$, referred to as *Malthusian parameter*, plays a major role in the asymptotic behavior of $U(t)$. It always exists if $\kappa \geq 1$ (in which case $a = 0$, with $a = 0$ iff $\kappa = 1$), but that need not be the case when $\kappa < 1$. The existence of a is assumed throughout. Define the c.d.f. $\tilde{G}(t) := \kappa \int_0^t e^{-ax} dG(x)$. Its expectation is $\tilde{\mu} := \int_0^\infty x d\tilde{G}(x)$, assumed finite. To prove Theorems 1 and 2, we will use repeatedly the following result established by Hyrien and Yanev (2012);

$$U(t) \sim \begin{cases} e^{\alpha t} \int_0^\infty f(x) e^{-\alpha x} dx / \tilde{\mu} & \text{if } \alpha > \beta, \\ C t^{\rho+1} e^{\alpha t} / \tilde{\mu} (\rho+1) & \text{if } \alpha = \beta, \\ C t^\rho e^{\beta t} / (1 - \kappa \hat{G}(\beta)) & \text{if } \alpha < \beta. \end{cases}$$

Proof of Theorem 1. We first remark that eqns. (16) coincide with eqn. (4) if $G(\cdot) = G_1(\cdot)$, $\kappa = a_1$ and

$$f(t) = a_{11} \int_0^t A_1^2(t-u) dG_1(u).$$

If $\alpha_1 > 0$, we deduce from eqn. (10) that

$$f(t) \sim a_{11} C_1^2 \hat{G}_1(2\alpha_1) e^{2\alpha_1 t}, \quad t \rightarrow \infty.$$

Therefore, applying Theorem H-Y with $\rho = 0$ and $\beta = 2\alpha_1 > \alpha_1$ gives

$$A_{11}(t) \sim \frac{a_{11} C_1^2 \hat{G}_1(2\alpha_1)}{1 - a_1 \hat{G}_1(2\alpha_1)} e^{2\alpha_1 t}, \quad t \rightarrow \infty,$$

and we deduce from eqns. (8) and (12) that

$$V_1^+(t) \sim \frac{ra_{11} C_1^2 \hat{G}_1(2\alpha_1)}{2\alpha_1 [1 - a_1 \hat{G}_1(2\alpha_1)]} e^{2\alpha_1 t}, \quad t \rightarrow \infty.$$

If $\alpha_1 = 0$, then $a_1 = a_{11} = 1$ and $A_1(\cdot) \equiv 1$. Hence $f(t) = a_{11} G(t)$, and, applying Theorem H-Y with $\rho = 0 = \beta$, and with $\tilde{\mu}_1 = \mu_1$, we find that $A_{11}(t) \sim t/\mu_1, t \rightarrow \infty$. We deduce from eqns. (8) and (12) that

$$V_1^+(t) \sim rt^2/2\mu_1, \quad t \rightarrow \infty.$$

Finally, assume that $\alpha_1 < 0$. Write $A_{11}(t) = e^{-\alpha_1 t} \bar{A}_{11}(t)$. It follows from eqn. (4) that

$$\bar{A}_{11}(t) = \int_0^t \bar{A}_{11}(t-x) d\tilde{G}_1(x) + \bar{f}(t),$$

where $\bar{f}(t) = a_{11} e^{-\alpha_1 t} \int_0^t A_1^2(t-u) dG_1(u)$. Put $C_{11} = \int_0^\infty \bar{f}(t) dt$. Then

$$C_{11} = \int_0^\infty e^{-\alpha_1 x} A_1^2(x) dx < \infty.$$

The Key Renewal Theorem entails that $\bar{A}_{11}(t) \rightarrow C_{11}/\tilde{\mu}_1$, hence

$A_{11}(t) \sim C_{11} e^{\alpha_1 t} / \tilde{\mu}_1, t \rightarrow \infty$. Finally we deduce from eqns. (8) and (12) that $V_1^+(t) \rightarrow D_{11}$ as $t \rightarrow \infty$, where $D_{11} = r \{ \int_0^\infty A_{11}(x) dx + \int_0^\infty A_1(x) dx \} < \infty$.

Proof of Theorem 2. Here the renewal equation (5) is identical to eqn. (16) if $\kappa = a_1$, $G(\cdot) = G_1(\cdot)$ and

$$f(t) = a_2 \int_0^t B_{22}(t-u) dG_1(u) + a_{11} \int_0^t A_2^2(t-u) dG_1(u), \quad (17)$$

where $B_{22}(t)$ satisfies the renewal-type equation

$$B_{22}(t) = b_2 \int_0^t B_{22}(t-u) dG_2(u) + b_{22} \int_0^t B_2^2(t-u) dG_2(u).$$

The similarity with eqn. (4) allows to conclude, using the proof of Theorem 1, that

$$B_{22}(t) \sim \begin{cases} \frac{b_{22} C_2^2 \hat{G}_2(2\alpha_2)}{1 - b_2 \hat{G}_2(2\alpha_2)} e^{2\alpha_2 t} & \text{if } \alpha_2 > 0, \\ t/\mu_2 & \text{if } \alpha_2 = 0, \\ \frac{C_{22}}{\mu_2} e^{\alpha_2 t} & \text{if } \alpha_2 < 0, \end{cases} \quad (18)$$

where $C_{22} = \int_0^\infty e^{-\alpha_2 x} B_2^2(x) dx < \infty$.

Case $\alpha_1 > \alpha_2$ and $\alpha_1 > 0$. Then

$$f(t) \sim a_{11} \int_0^t A_2^2(t-u) dG_1(u),$$

and eqn. (11) implies that

$$f(t) \sim a_{11} K_2^2 \hat{G}_1(2\alpha_1) e^{2\alpha_1 t}.$$

Applying Theorem H-Y with $\rho = 0$ and $\beta = 2\alpha_1 > \alpha_1$, we obtain

$$A_{22}(t) \sim \frac{a_{11} K_2^2 \hat{G}_1(2\alpha_1)}{1 - a_1 \hat{G}_1(2\alpha_1)} e^{2\alpha_1 t}, \quad t \rightarrow \infty.$$

Finally, we deduce from eqns. (8) and (13) that

$$V_2^+(t) \sim \frac{ra_{11} K_2^2 \hat{G}_1(2\alpha_1)}{[1 - a_1 \hat{G}_1(2\alpha_1)] 2\alpha_1} e^{2\alpha_1 t}, \quad t \rightarrow \infty.$$

Case $\alpha_1 = 0$ and $\alpha_2 < 0$. Eqn. (11) implies that $A_2(t) \rightarrow \infty$. K_2 If $G_1(a) < \infty$, then

$f(t) \rightarrow a_{11} K_2^2$ as $t \rightarrow \infty$. Applying Theorem H-Y with $\rho = 0 = \beta$ yields

$A_{22}(t) \sim a_{11} K_2^2 t / \mu_1$. Hence

$$V_2^+(t) \sim \frac{ra_{11} K_2^2}{2\mu_1} t^2.$$

Case $0 > \alpha_1 > \alpha_2$. Suppose that $\hat{G}_1(\alpha_2) < \infty$ and $\hat{G}_1(2\alpha_1) < \infty$. Firstly, the asymptotic behavior of $f(t)$ depends on the values of $2\alpha_1 - \alpha_1$:

i. if $2\alpha_1 < \alpha_2$ then

$$f(t) \sim a_2 \int_0^t B_{22}(t-u) dG_1(u) \sim \frac{a_2 C_{22} \hat{G}_1(\alpha_2)}{\tilde{\mu}_2} e^{\alpha_2 t};$$

ii. if $2\alpha_1 > \alpha_2$ then

$$f(t) \sim a_{11} \int_0^t A_2^2(t-u) dG_1(u) \sim a_{11} K_2^2 \hat{G}_1(2\alpha_1) e^{2\alpha_1 t};$$

iii. if $2\alpha_1 = \alpha_2$ then

$$f(t) \sim \frac{a_2 C_{22} \hat{G}_1(2\alpha_1)}{\tilde{\mu}_2} e^{2\alpha_1 t} + a_{11} K_2^2 \hat{G}_1(2\alpha_1) e^{2\alpha_1 t}.$$

Applying Theorem H-Y with either $\beta = 2\alpha_1$ or $\beta = \alpha_2$, and noticing that $\beta < \alpha_1$ in either case, and $\rho = 0$, yields

$$A_{22}(t) \sim e^{\alpha_1 t} \int_0^\infty f(x) e^{-\alpha_1 x} dx / \tilde{\mu}_1, \quad t \rightarrow \infty.$$

We deduce from eqns. (10) and (15), as $t \rightarrow \infty$, that

$$V_2^+(t) \rightarrow \bar{V}_2^+ = r \int_0^\infty [A_{22}(x) + A_2(x)] dx < \infty.$$

Case $\alpha_2 > \alpha_1$ and $\alpha_2 > 0$. Eqn. (18) and (20) entails that

$$A_2(t) \sim K_3 e^{\alpha_2 t} = \frac{a_2 C_2 \hat{G}_1(\alpha_2)}{1 - a_1 \hat{G}_1(\alpha_2)} e^{\alpha_2 t}, \quad t \rightarrow \infty.$$

This identity, together with eqns. (17) and (18), implies that

$$f(t) \sim C(\alpha_2) e^{2\alpha_2 t}, \quad t \rightarrow \infty,$$

where

$$C(\alpha_2) = a_2 C_2^2 \hat{G}_1(2\alpha_2) \left\{ \frac{a_2 a_{11} \hat{G}_1^2(\alpha_2)}{[1 - a_1 \hat{G}_1(\alpha_2)]^2} + \frac{b_{22} \hat{G}_2(2\alpha_2)}{2 - b_2 \hat{G}_2(2\alpha_2)} \right\}.$$

Applying Theorem H-Y with $\rho = 0$ and $\beta = 2\alpha_2 > \alpha_1$ gives

$$A_{22}(t) \sim \frac{C(\alpha_2)}{1 - a_1 \hat{G}_1(2\alpha_2)} e^{2\alpha_2 t}, \quad t \rightarrow \infty.$$

Eqns. (15) and (22) yield finally:

$$V_2^+(t) \sim \frac{rC(\alpha_2)}{1 - a_1 \hat{G}_1(2\alpha_2)} \frac{1}{2\alpha_2} e^{2\alpha_2 t}, \quad t \rightarrow \infty.$$

Case $\alpha_2 = 0 > \alpha_1$. Eqn. (20) implies that

$$A_2(t) \rightarrow a_2 / (1 - a_1), \quad t \rightarrow \infty.$$

It follows from eqn. (18) that

$$f(t) \sim a_2 \int_0^t B_{22}(t-u) dG_1(u) \sim a_2 t / \mu_2, \quad t \rightarrow \infty.$$

Applying Theorem H-Y with $\rho = 1$ and $\beta = 0 > \alpha_1$, we obtain

$$A_{22}(t) \sim \frac{a_2}{\mu_2(1 - a_1)} t, \quad t \rightarrow \infty,$$

and, using eqns. (15) and (22), we finally deduce that

$$V_2^+(t) \sim \frac{ra_2}{2\mu_2(1 - a_1)} t^2, \quad t \rightarrow \infty.$$

Case $0 > \alpha_2 > \alpha_1$. Eqn. (20) gives

$$A_2(t) \sim K_3 e^{\alpha_2 t},$$

where $K_3 = a_2 C_2 \hat{G}_1(\alpha_2) / \{1 - a_1 \hat{G}_1(\alpha_2)\}$. Therefore, as $t \rightarrow \infty$

$$f(t) \sim a_2 \int_0^t B_{22}(t-u) dG_1(u) \sim \frac{\alpha_2 C_{22} \hat{G}_1(\alpha_2)}{\tilde{\mu}_2} e^{\alpha_2 t}.$$

Applying Theorem H-Y with $g=r=1$ and $\beta = \alpha_2 > \alpha_1$, we find that

$$A_{22}(t) \sim \frac{a_2 C_{22} \hat{G}_1(\alpha_2)}{\tilde{\mu}_2 [1 - a_1 \hat{G}_1(\alpha_2)]} e^{\alpha_2 t}, \quad t \rightarrow \infty.$$

Finally, eqns. (10) and (15) entail that

$$V_2^+(t) \rightarrow \bar{V}_2^+ = r \int_0^\infty [A_{22}(x) + A_2(x)] dx < \infty.$$

Case $\alpha_1 = \alpha_2 > 0$. Eqn. (20) yields

$$A_2(t) \sim \frac{a_2 C_2}{a_1 \tilde{\mu}_2} t e^{\alpha_1 t}, \quad t \rightarrow \infty.$$

Therefore, as $t \rightarrow \infty$,

$$f(t) \sim a_{11} \int_0^t A_2^2(t-u) dG_1(u) \sim \frac{a_2^2 C_2^2 \hat{G}_1(2\alpha_1)}{a_1 \tilde{\mu}_2^2} t^2 e^{2\alpha_1 t}.$$

Then, applying Theorem H-Y with $\rho = 2$ and $\beta = 2\alpha_1 > \alpha_1$ we find that

$$A_{22}(t) \sim \frac{a_2^2 C_2^2 \hat{G}_1(2\alpha_1)}{a_1 \tilde{\mu}_2^2 [1 - a_1 \hat{G}_1(2\alpha_1)]} t^2 e^{2\alpha_1 t}.$$

Hence, using eqns. (10) and (15), we deduce that

$$V_2^+(t) \sim r \int_0^t A_{22}(x) dx \sim \frac{r a_2^2 C_2^2 \hat{G}_1(2\alpha_1)}{a_1 \tilde{\mu}_2^2 [1 - a_1 \hat{G}_1(2\alpha_1)] 2\alpha_1} t^2 e^{2\alpha_1 t}.$$

Case $a_1 = a_2 = 0$. We remark that $a_1 = C_1 = C_2 = 1$ here. Then, we obtain from eqn. (20) that $A_2(t) \sim a_2 t / \mu_1$, as $t \rightarrow \infty$. Hence

$$f(t) \sim \int_0^t A_2^2(t-u) dG_1(u) \sim \frac{a_2^2}{\mu_1^2} t^2.$$

Applying Theorem H-Y with $\rho = 2$ and $\beta = 0 = \alpha_1$ gives

$$A_{22}(t) \sim \frac{a_2^2}{3\mu_1^3} t^3, \quad t \rightarrow \infty.$$

Then, eqns. (10) and (15) imply that

$$V_2^+(t) \sim r \int_0^t A_{22}(x) dx \sim \frac{r a_2^2}{12\mu_1^3} t^4.$$

Case $a_1 = a_2 < 0$. Eqn. (20) gives $A_2(t) \sim [a_2 C_2 / a_1 \tilde{\mu}_2] t e^{\alpha_1 t}$, $t \rightarrow \infty$. Therefore, assuming that $\hat{G}_1(2\alpha_1) < \infty$, we find that

$$f(t) \sim a_2 \int_0^t B_{22}(t-u) dG_1(u) \sim \frac{a_2 C_{22} \hat{G}_1(\alpha_1)}{\tilde{\mu}_2} e^{\alpha_1 t}.$$

Applying Theorem H-Y with $\rho = 0$ and $\beta = \alpha_1$ gives

$$A_{22}(t) \sim \frac{a_2 C_{22} \hat{G}_1(\alpha_1)}{\tilde{\mu}_1 \tilde{\mu}_2} t e^{\alpha_1 t}.$$

We deduce from eqns. (10) and (15) that

$$V_2^+(t) \rightarrow \bar{V}_2^+ = r \int_0^\infty [A_{22}(x) + A_2(x)] dx < \infty.$$

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Fig. 1.

Flow diagram representing the sequential maturation of cells during erythropoiesis; HSC: hematopoietic stem cells; CLP: common lymphoid progenitors; CMP: common myeloid progenitors; GMP: granulocyte macrophage progenitors; MEP: megakaryocyte erythroid progenitors; Mega: megakaryocytes; BFU-E: burst forming unit-erythroid; CFU-E: colony forming unit-erythroid; Pro-E: pro-erythroblasts, Baso-E: basophilic erythroblasts; Poly-E: polychromatic erythroblasts; Ortho-E: orthochromatic erythroblasts; Ret: reticulocytes; RBC: red blood cells.

Fig. 2. Schematic representation of the model structure (see section 4.1 for detail).

Fig. 3.

Frequency of BFU-E and CFU-E in mice following total body irradiation (dose of 4 Gy) relative to control (sham-irradiated) mice over time. The recovery of the CFU-E compartment exhibits an expansion phase between day 2 and 6 following radiation exposure. The objective of this paper is to studying this phase. Following the expansion phase, the size of the compartment oscillated over time as it returns to normal, steady-state levels.

Fig. 4.
Results from simulations (see Section 3.2 for detail).

Fig. 5.
Fitted model from *Analysis 1*. Error bars indicate one standard error for the mean.

Fig. 6.

Fitted model from *Analysis 2*. Error bars indicate one standard error for the mean.

Fig. 7.

Fitted model obtained during *Analysis 2* when both embedded Bellman-Harris processes are assumed to be critical ($\alpha_1^{(2)} = \alpha_2^{(2)} = 0$). This model did not capture the expansion of the CFU-E compartment as well as the model assuming non-criticality of the CFU-E compartment ($\alpha_2^{(2)} \neq 0$; see Figure 6). Error bars indicate one standard error for the mean.

Fig. 8.

Relative frequency of BFU-E predicted for the entire duration of the experiment by the model that we fitted in *Analysis 2* (error bars indicate one standard error for the mean). Although fitted to data from day 1 to 6 only, the model captures the kinetics of the BFU-E compartment up to day 14 quite well. Starting from day 8, the data appear to exhibit non-random oscillations about the fitted model that suggest that the recovery of the BFU-E compartment may be controlled by feedback mechanisms that modulate the rate of recovery.

Table 1
Parameter estimates and their standard errors computed using $B = 1000$ bootstrap samples.

Analysis	parameters:		T_0	$\alpha_{(2)}^1$	ψ_1	$\mu_{(2)}^{01}$	$\sigma_{(2)}^{01}$	$\alpha_{(2)}^2$	ψ_2	$\mu_{(2)}^{02}$	σ_{02}
	(unit)	(day)	-	-	-	(day)	(day)	-	-	(day)	(day)
1*	estimate:	2.64	-0.07	-	-	0.58	0.44	-0.15	-	0.39	0.45
2	estimate:	2.18	0.06	79.3	0.44	0.45	0.45	1.02	4.71	(= μ_1)	(= σ_1)
	(s.e.)	(0.37)	(0.12)	(1.1×10^3)	(0.15)	(0.38)	(0.28)	(84.8)	-	-	-
3	estimate:	2.25	0.014	386.4	0.59	0.44	1.00	5.62	0.38	0.45	
	(s.e.)	(0.42)	(0.06)	(699.0)	(0.16)	(1.4)	(0.25)	(62.9)	(0.12)	(1.40)	

* Standard errors are not provided for Analysis 1 because the model did not fit well the data.