Multistage hematopoietic stem cell regulation in the mouse: A combined biological and mathematical approach

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Highlights

- We describe a new 6-compartment time-dependent regulated model of hematopoiesis
- Biological data under steady state and stress and cell dynamics were used
- Modeling is able to recapitulate effects from chemotherapy, bleeding, or HSC depletion

6-compartment time-dependent regulated model of hematopoiesis

PHZ Experiments

Model-guided optimisation

Validation

Chemotherapy effect

Bleeding effect

HSC depletion effect
Multistage hematopoietic stem cell regulation in the mouse: A combined biological and mathematical approach

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SUMMARY

We have reconciled steady-state and stress hematopoiesis in a single mathematical model based on murine in vivo experiments and with a focus on hematopoietic stem and progenitor cells. A phenylhydrazine stress was first applied to mice. A reduced cell number in each progenitor compartment was evidenced during the next 7 days through a drastic level of differentiation without proliferation, followed by a huge proliferative response in all compartments including long-term hematopoietic stem cells, before a return to normal levels. Data analysis led to the addition to the 6-compartment model, of time-dependent regulation that depended indirectly on the compartment sizes. The resulting model was finely calibrated using a stochastic optimization algorithm and could reproduce biological data in silico when applied to different stress conditions (bleeding, chemotherapy, HSC depletion). In conclusion, our multi-step and time-dependent model of immature hematopoiesis provides new avenues to a better understanding of both normal and pathological hematopoiesis.

INTRODUCTION

Hematopoiesis provides the lifelong supply of mature blood cells derived from a rare population of bone marrow (BM) multipotent hematopoietic stem cells (HSCs) (Orkin and Zon, 2008; Till and McCulloch, 1961). The lineage relationship between HSCs and the mature blood cells was first proposed as a multistep process in which generations of diverse blood cells are coupled with movement through consecutive hematopoietic stem and progenitor cell (HSPC) compartments. HSPC division and differentiation are controlled by extracellular signals and by intracellular networks. In the classical model, successive HSPC compartments along hematopoietic differentiation processes are defined at an immunophenotypic level, typically by the expression of cell surface marker combinations (Eich et al., 2019; Notta et al., 2016). In this context, since the 1960s, HSCs have typically been functionally defined by their ability to sustain multi-lineage engraftment for an extended period of time upon serial transplantation into irradiated recipient mice (Till and McCulloch, 1961). Recently, however, a new generation of experimental tools allowing in situ analysis of HSC output has challenged the classical model by pointing out fundamental differences between physiological unperturbed and stress hematopoiesis (Busch et al., 2015; Carrelha et al., 2018; Chapple et al., 2018; Pei et al., 2017, 2020; Rodriguez-Fraticelli et al., 2018; Säwen et al., 2018; Sun et al., 2014). In native hematopoiesis in particular, progenitors directly downstream of long-term hematopoietic stem cells (LT-HSCs) such as short-term hematopoietic stem cells (ST-HSCs) and multipotent Progenitors (MPPs), serve as a major and nearly self-renewing source of cells for day-to-day hematopoiesis. This renders the blood and immune system less dependent on LT-HSCs, which is consistent with the quiescence of adult LT-HSCs (Busch and Rodewald, 2016; Busch et al., 2015; Foudi et al., 2009; Schoedel et al., 2016; Wilson et al., 2008). In addition, recent single-cell profiling analysis has unveiled the tremendous heterogeneity of each previously defined compartment leading to a continuum in the hematopoiesis process (Giladi et al., 2018; Laurenti and Göttgens, 2018; Paul et al., 2015; Velten et al., 2017). However, punctuated transitions across this continuous gene expression landscape may still exist and represent functionally distinct groups of cells (Liggett and Sankaran, 2020).

Mathematical modeling that integrates both unperturbed and stress hematopoiesis is a powerful approach to addressing key questions in hematopoiesis and can provide both qualitative and quantitative insights...
into stem cell dynamics and fate commitment. Previous mathematical models based on in vivo analyses of immature compartments have been previously reported. They have mostly described immature hematopoiesis dynamics in a steady state. Notably also, these studies have relied upon parameters such as cell division rates or probabilities of self-renewal (Busch et al., 2015; Foudi et al., 2009; Mackey, 2001; Sawai et al., 2016; Wilson et al., 2008). Among these reports, Rodewald and colleagues described a major breakthrough by modeling hematopoiesis based on stem compartments from LT-HSCs to MPPs in unperturbed hematopoiesis (Busch et al., 2015). Nevertheless, these researchers noticed that their steady state model could not fully recapitulate stress hematopoiesis such as 5-fluorouracil (5-FU) treatment, suggesting that unperturbed and stress hematopoiesis do not follow the same biological rules.

Stress hematopoiesis has been also modeled but the in vivo data used for this purpose have often focused only on mature cells and not on immature compartments (Angulo et al., 2018; Crauste et al., 2008, 2010; Fuertinger et al., 2013; Klose et al., 2019; Knauer et al., 2020; Loeffler et al., 1989; Mahadik et al., 2019; Manesso et al., 2013; Marciniak-Czochra et al., 2009; Mon Père et al., 2021; Østby et al., 2003, 2004; Rogg et al., 2019; Rubinow and Lebowitz, 1975; Stehl et al., 2018). In our present study, we have recapitulated steady-state and stress hematopoiesis in a single mathematical model based on in vivo experimental data and focusing on HSC and progenitor compartments. We focused in particular on the erythroid responses since these cells represent ~95% of the total blood cells. As the stress condition, we first chose in vivo “peripheral stress” through hemolytic anemia (phenylhydrazine [PHZ] administration known to destroy mature red blood cells [RBCs]) to avoid direct alterations of the stem cell compartments (Hara and Ogawa, 1976; Klinken et al., 1987). Most studies to date related to PHZ administration have focused on committed progenitors, whereas we targeted immature compartments in our current analysis.

We demonstrate from our present data that several stress conditions and unperturbed hematopoiesis can be combined into a single system based on a six-immature compartment model. We integrated time-dependent regulations that depended indirectly on compartment sizes. Self-renewal and differentiation dynamics were modulated through two regulators. The model was finely calibrated using a stochastic optimization algorithm (Covariance Matrix Adaptation in Evolution Strategy, CMA-ES) (Hansen and Ostermeier, 2001) based on our experimental observations. Its robustness was assessed numerically, and it was finally validated using various in silico stresses such as bleeding, chemotherapy (S-FU administration) or destruction of the most immature LT-HSC compartment as it successfully reproduced biological data in silico that had previously been reported in the literature.

RESULTS
General form of a novel regulated compartmental mathematical model
See also STAR Methods, method details.

We developed a mathematical model that could recapitulate both steady-state and stress hematopoiesis from LT-HSC to RBC production. Modeling the kinetics of proliferation/differentiation processes requires a theoretical number of compartments between LT-HSCs and RBCs. We focused on the behavior of the immature LT-HSC, ST-HSC, MPP, common myeloid progenitor (CMP), and megakaryocytic-erythroid progenitor (MEP) cell compartments and that of mature RBCs. Hence, we considered a system of six equations to describe the dynamics on these compartment sizes, comparable with the dynamics introduced previously by Mon Père and colleagues (2021). An additional and formal explanation for the 6-compartment model was developed (STAR Methods, explanation of 6 compartments choice) based on an optimal argument that explains the data under homeostatic conditions, as previously described (Dingli et al., 2007).

Our model describes a hierarchy of 6-cell compartments evolving over time. In brief, a cell of a given compartment can self-renew or differentiate independently from the others and is characterized by its self-renewal and differentiation rates (and for the last compartment corresponding to RBCs, its death rate). Each division is considered symmetric (it was not possible, based on our data, to distinguish symmetrical and asymmetrical divisions). The HSPC death rate (apoptosis) was low (Domen et al., 2000 and our data) and then neglected in our model.

The entire dynamics can be summarized as follows:
Moreover, for any i, \( \alpha \) cell “differentiates” at rate \( d_{5i}(t) > 0 \) and gives rise to 2 \( n(t) \) 6-type cells. These dynamics summarize the \( n(t) \) be simplified as 2 \( n(t) \) RBC issued from 1 MEP since terminal erythroid differentiation has been largely mitosis observed at the end of erythroid differentiation (between MEPs and RBCs). This amplification could initiates to a non-erythroid lineage at rate \( d_{6i} \). The net proliferation rate is given by \( r_{i} = r_{i+1} \) at rate \( d_{i}(t) > 0 \) (erythroid differentiation). The net proliferation rate is given by \( r_{i} = R_{i}(t) - d_{i}(t) x_{i}(t) \) for \( i \in \{2, \ldots, 5\} \),

\[
\frac{dx_{i}}{dt}(t) = (r_{i}(t) - d_{i}(t))x_{i}(t)
\]

\[
\frac{dx_{6}}{dt}(t) = 2^{i(i)} d_{6}(t)x_{6}(t)
\]

For any \( i = 1, \ldots, 4 \), a cell belonging to the \( i \) compartment self-renews at time \( t \) at rate \( R_{i}(t) > 0 \) or differentiates to a non-erythroid lineage at rate \( \mu_{i} \) or to an erythroid lineage giving rise to two cells in compartment \( i+1 \) at rate \( d_{3i}(t) > 0 \) (erythroid differentiation). The net proliferation rate is given by \( r_{i}(t) = R_{i}(t) - \mu_{i} \). A 5-type cell “differentiates” at rate \( d_{4i}(t) > 0 \) and gives rise to 2 \( n(t) \) 6-type cells. These dynamics summarize the \( n(t) \) mitosis observed at the end of erythroid differentiation (between MEPs and RBCs). This amplification could be simplified as 2 \( n(t) \) RBC issued from 1 MEP since terminal erythroid differentiation has been largely modeled before and was not our concern. A 6-type cell corresponds to a mature RBC that is dying at the constant rate \( d_{6} > 0 \).

### Parameter calibration of the mathematical model under homeostatic conditions

See also STAR Methods, method details.

Our model system was first calibrated under homeostatic conditions. We evaluated the compartment cell numbers in vivo and the proliferation rates at a steady state using BrdU analysis (Table 1). The cell compartment sizes are denoted by \( x_{i}^{*} \). In the steady state, the net proliferation and differentiation rates are the positive constant values \( r_{i}^{*} \) and \( d_{i}^{*} \). Furthermore, presuming that the LT-HSC compartment is at equilibrium, the LT-HSC net proliferation, \( r_{1}^{*} \), is equal to the differentiation LT-HSC rate, \( d_{1}^{*} \). Moreover, for any \( i \), \( i \in \{2, ..., 5\} \): \( d_{i}^{*} \geq 0 \) to ensure the stability of the system (STAR Methods, explanation for the 6-compartments choice). The lifespan of mature RBCs was based on that reported in the literature: \( d_{6} = 1/40 \) (day).

We estimated the values of \( d_{i}^{*} \) and later of \( r_{i}^{*} \) from our BrdU experiments (BrdU being injected 20 h prior to the analysis) and from the erythro-myeloid commitment rates values \( \mu_{i} \) (based on Busch et al., 2015; STAR Methods, calibration of the parameters). The BrdU-labeled cell proportion was estimated by:

\[
p_{i}^{BrdU} = \frac{x_{i}^{D}(20h)}{x_{i}^{*}}
\]

where \( x_{i}^{D}(20h) \) is the number of cells having made a division at least once in a time interval of 20 h. The dynamics of \( x_{i}^{D} \) is given by the following equation system:

\[
\frac{dx_{i}^{D}}{dt}(t) = r_{i}^{D} x_{i}^{D}(t) + 2(r_{i}^{*} + \mu_{i})(x_{i}^{*} - x_{i}^{D}(t)) - d_{i}^{D} x_{i}^{D}(t) \]

\[
\frac{dx_{6}^{D}}{dt}(t) = r_{6}^{D} x_{6}^{D}(t) + 2(r_{6}^{*} + \mu_{6})(x_{6}^{*} - x_{6}^{D}(t)) + 2d_{5i} x_{5i-1}^{*} - d_{6}^{D} x_{6}^{D}(t).
\]

This led us to estimate the differentiation and net proliferation rate values at equilibrium as follows:

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### Table 1. Determination of the cell number in each compartment per mouse and the percentage of BrdU-positive cells in steady-state hematopoiesis

| Cell type | n (10^6/mouse) | BrdU (%) |
|-----------|----------------|----------|
| LT-HSC    | 0.011 ± 0.070  | 1.0 ± 2.71 |
| ST-HSC    | 0.022 ± 0.013  | 1.4 ± 0.75 |
| MPP       | 0.067 ± 0.045  | 6.32 ± 3.58 |
| CMP       | 0.196 ± 0.111  | 12.42 ± 2.31 |
| MEP       | 0.359 ± 0.189  | 31.75 ± 1.15 |
| GMP       | 0.677 ± 0.430  | 19.50 ± 7.50 |

Data represent the mean values from at least six mice.
The n* number of mitoses (between MEPs and RBCs) at equilibrium was deduced by log 2 

We then computed all appropriate parameters. We took into consideration the population size (Table 1), net proliferation and differentiation rates, mi erythro-myeloid commitment rates (obtained from Busch et al., 2015), and the n* value. These parameters (Table 2) allowed us to characterize unperturbed native hematopoiesis for the normal lifespan of the laboratory mouse.

As a stress condition, we induced an acute hemolytic anemia using PHZ administration (Figure 3A). After a 40% hemolysis level (observed on day 3), a rapid recovery of RBCs was evidenced after 10–16 days (Figure 1A). Our 6-step model was applied to recapitulate this RBC recovery but, as expected, the recovery time exceeded 1 month, which was totally discrepant from the observed biological data (Figure 1A). Indeed, different regulatory processes have to be considered for each compartment’s cell dynamics. To determine these factors, biological data were requested.

PHZ treatment impacts immature compartments from LT-HSCs to MEPs in vivo

To further elucidate the biological mechanisms responsible for the fast RBC recovery at the level of the HSPC compartments, we analyzed immature compartment modifications after PHZ injections into the blood, BM, and spleen. Briefly, during the first 3 days after injection, we observed a drastic RBC fall, and an increase in platelet and leukocyte counts (Figures 1A–1C), suggesting an inflammatory process associated with hemolytic anemia. Multi-cytokine concentration follow-ups in the blood revealed huge increases in IL-1α, IL-2, IL-6, IL-13, G-CSF, MIP-1α and β, and TNFα at 5 days after PHZ injection (Figure 1D), confirming an inflammatory process. The total number of BM cells was slightly reduced at day 3 (~25%) and enhanced at day 10 (~33%) (Figure 1G). The spleen weight strongly increased 24 h after PHZ injection (Figures 1E and 1F), becoming 3 times larger than normal at day 3, as previously observed after erythroid (Hara and Ogawa, 1976; Sanchez et al., 2006) or social stress in mice (McKim et al., 2018). The three hematopoietic tissues were analyzed to assess the proportion and absolute cell numbers in the different compartments (LT-HSC, ST-HSC, MPP, CMP, and MEP) as well as their proliferation rates (BrdU incorporation), and apoptosis (AnnexinV labeling). In the BM, decreases in all compartment sizes (from LT-HSC to CMP) were evident (Figure 2), whereas the BM MEP population remained relatively stable during this phase. No differences in BrdU incorporation were observed at day 3 in the different BM

### Table 2. Determination of the net proliferation and differentiation rates, number of the last mitosis, and size of each compartment in the steady state

| Type  1 | Type 2 | Type 3 | Type 4 | Type 5 | Type 6 |
|--------|--------|--------|--------|--------|--------|
| LT-HSC | ST-HSC | MPP    | CMP    | MEP    | RBC    |
| Net proliferation rate (/day)  |
| r*1 = 0.006 | r*2 = 0.004 | r*3 = 0.014 | r*4 = 0.032 | r*5 = 0.2 |
| Differentiation rate (/day)  |
| d*1 = 0.006 | d*2 = 0.01 | d*3 = 0.02 | d*4 = 0.046 | d*5 = 0.254 |
| Size of the compartment (10⁶ cell/mouse)  |
| x*1 = 0.011 | x*2 = 0.022 | x*3 = 0.067 | x*4 = 0.196 | x*5 = 0.359 | x*6 = 5.17 x 10⁴ |
| RBC death rate (/day)  |
| d*6 = 0.25 |
| Last mitosis number  |
| n* = 13.79 |
| Non-erythroid differentiation rate (/day)  |
| μ1 = 0 | μ2 = 0 | μ3 = 0.022 | μ4 = 0.04 | μ5 = 0 |
compartments except for MEP, which displayed a 2-fold increased cell proliferation compared with day 0 (Figure 3). In contrast, the number of splenic MEPs, negligible at day 0 compared with the number of BM MEPs (10 times less abundant), displayed a huge increase to reach values comparable with those for
BM MEPs at 5 days after PHZ injection (Figure 1G). The splenic MEP compartment showed a return to normal values at day 10.

To gain further insights into the mechanisms underlying the drastic decrease in the BM compartment size, we assessed hypothetical mechanisms such as apoptosis, egress from the BM to the blood or to the spleen, or phenotypic changes due to PHZ treatment. No increase in the proportion of AnnexinV-labeled cells was observed for any BM progenitor compartment examined, allowing us to exclude any excess cell death. Blood cell analysis performed at days 3 and 5 after PHZ injection showed a less than 1% proportion of Lin- cells, thus precluding an egress of Lin- or LSK cells from the BM to the blood (Figure S2A). In the spleen, the number of mature cells increased by 3-fold (Figure 1F), whereas the LSK and CMP compartments displayed no significant variations, ruling out any role of splenic immature progenitors on day 3 (data not shown). Lastly, to confirm the functional stemness properties of LT-HSCs exposed to PHZ, competitive BM transplantation with 50% of CD45.1 BM cells from untreated animals and 50% of total BM cells from day 3-PHZ-treated mice was performed in CD45.2-9.5 Gy-irradiated recipients. Blood chimerism analysis 3 months post transplantation showed a decreased proportion of PHZ-treated cells, confirming the decreased number of LT-HSCs in the BM in day 3-PHZ-treated mice (Figure S2C). These data collectively suggest that the decreased BM progenitor compartment sizes (but not involving increased proliferation, apoptosis, or egress to the spleen or blood), and the stable number of MEPs displaying a 2-fold increased proliferation in the BM and spleen, is related to a rapid differentiation process, in order to quickly compensate for the loss of RBCs through a versatile mechanism.

**Figure 2. Evolution of experimental and model parameters during PHZ-mediated chemical hemolysis**

Red blood cell (RBC) and bone marrow cell numbers per mouse (x10⁶/mouse) were assessed from the in vivo experiments (red points, RBCs; blue points, total BM cells; green points, BM MEPs; gray points, BM CMPs; orange points, BM MPPs; dark blue points, BM ST-HSCs; brown points, BM LT-HSCs); Mann-Whitney test, **p < 0.05. Values and standard errors were generated from the experimental data for each time point. Evolution curves were deduced from the mathematical modeling.
The dynamics of progenitor compartments (determined using the BrdU data and compartment size) during the recovery from the PHZ treatment were analyzed at days 3, 5, 7, 10, 16, and 28 post injection. Recovery was almost effective in all compartments from day 7 to day 10 (Figure 1A). On day 10, all compartments displayed a 2- to 3-fold increase in cell numbers before returning to normal values on day 28. BrdU analysis revealed that all BM compartments exhibited a drastic increase in cell proliferation at day 10 (the hematocrit normalization time point). Thereafter, BrdU incorporation returned to normal values at days 16 and 28 (Figure 3), confirming previous observations (Mackey, 2001).
Adding compartment regulations allows the modeling of steady-state and stress-response hematopoiesis

See also STAR Methods, Method details, Mathematical model.

To model both unperturbed and stress-related hematopoiesis with our dynamical system, we introduced regulatory processes by assuming that previously defined parameters depend indirectly upon the compartment size through dynamics of two regulators (ur and ud) that modulate net proliferation and differentiation, thereby exerting positive and negative effects, respectively, on compartment sizes. More precisely, we assumed that ri(t) and di(t) are given by the following equations:

\[
\begin{align*}
\dot{r}_i(t) &= r_i^* \left( 25 m_r u_r(t) \right)^{a_i}; \\
\dot{d}_i(t) &= d_i^* \left( \frac{m_d}{25} u_d(t) \right)^{b_i}.
\end{align*}
\]

ri* and di* have been previously defined. ai and bi are non-negative real numbers. The two functions ur and ud were considered to integrate all of the various biological factors implicated in immature cell compartment dynamics, such as cytokines, chemokines, ROS, and cell-cell interactions (Batsivari et al., 2020). Their dynamics follow a double system of production and clearance described by the following equations:

\[
\begin{align*}
\frac{du_r}{dt}(t) &= \frac{1}{y(t)^2} - m_r u_r(t); \\
\frac{du_d}{dt}(t) &= y(t)^2 - m_d u_d(t).
\end{align*}
\]

Production of the regulators is given by functions that depend on BM cell numbers. The y(t) quantity denotes the total number of cells at time t in the five immature compartments, renormalized by their size at equilibrium. The clearance of the regulators after their production is denoted by linear degradation terms described by the m_r and m_d positive parameters. Note that regulatory processes in cellular dynamics are typically considered to be in a quasi-steady state (Knauer et al., 2020; Mon Père et al., 2021; Stiehl et al., 2014). However, such regulation cannot explain the damped oscillations around the equilibrium we observed in vivo. However, the two dynamic parameters ur(t) and ud(t) in our model allowed us to explain such oscillations by inducing delays between regulator modifications and their effects on compartment sizes. Assuming that the net proliferation rate during late erythropoiesis is regulated in a specific and different manner (erythropoietin, stem cell factor, etc.), RBC production dynamics from MEPs can be considered to be controlled via a different type of regulation. MEP differentiation gives birth to 2n(t) RBCs that summarize the n(t) last mitosis with a c-logistic type function of the RBC number as follows:

\[
n(t) = n^* + \left( 33 - n^* \right) \left( 1 - \left( \frac{y(t)}{y^*_c} \right)^{c} \right).
\]

We hypothesized, based on the existing literature (Hayflick, 1965), that the maximum number of mitoses between an MEP and mature RBC was 33, which gave us an upper-bound for n* in our model. The c parameter is a positive number.

Parameter calibration based on experimental data with the CMA-ES algorithm

The parameters in our regulated model were calibrated using a stochastic optimization algorithm based on a CMA-ES method (CMA-ES; Hansen and Ostermeier, 2001), which minimizes discrepancy between simulated and experimental data (STAR Methods, calibration of feedback parameters). The optimal parameters obtained by this method are presented in Table 3, and the corresponding cell dynamics are shown in Figure 1. Sensitivity analysis was also performed (STAR Methods, sensitivity analysis) and matched the experimental data in all compartments after PHZ-induced stress (including the damped oscillation around the equilibrium) as well as in the steady state.

In silico model validation through various hematopoietic stress conditions

To assess the robustness of our model under different stress conditions, we developed three different in silico hematopoietic stresses and compared the predictive values obtained using our model with biological data reported in the literature. At first, we assessed a 15% loss of RBCs (comparable with a hemorrhage) as studied previously in the mouse in vivo (Raabe et al., 2011). Our model predicted a normalization of the red cell mass within 3–4 days, as expected (Figure 4A). Moreover, no major effect on the most immature compartments was predicted by our model in accordance with previously reported biological data (data not shown). We next modeled the effects of the commonly used 5-FU treatment, a chemotherapeutic compound that can force quiescent LT-HSCs to enter the cell cycle at 4–10 days after injection without perturbation of blood parameters. 5-FU specifically kills cycling cells at 24–48 h post injection, and consistently,
our model indicated the drastic and direct death of all proliferating hematopoietic cells for 2 days. We further observed in silico that immature compartments entered the cell cycle at 4–10 days after 5-FU treatment without any dramatic effect on mature circulating cells (Figure 4B), consistent with previous report (D’Hondt et al., 2001). Finally, we applied a third type of stress based on a specific eradication of the LT-HSC compartment as reported by Schoedel and co-workers (Schoedel et al., 2016). We found that our in silico mouse model maintained mature hematopoiesis and also immature compartment (MEP, CMP) sizes with a reduced number of ST-HSCs as observed in the Schoedel study for the lifespan of the mice (Figure 4 C). Taken together, these three aforementioned in silico predictions confirmed the robustness of our model.

DISCUSSION

Our present interdisciplinary study marries mathematical modeling and experimental work to provide a mathematical model that can reconcile steady-state and stress hematopoiesis under various stress conditions. We focused on erythropoietic differentiation since this represents more than 90% of all blood cells, thus excluding other differentiation lineages that have previously been modeled quite extensively (Craig et al., 2016; Knauer et al., 2020). We also highlighted a mechanism in our current analyses by which transient stress exposure leads to a protracted influence on all progenitor compartments, including LT-HSCs, resulting in a fast repopulation of mature compartments.

In order to study stress-response hematopoiesis, the PHZ “stress model” was initially considered. PHZ is a chemical compound that can induce hemolysis. Nevertheless, this process also induced a huge inflammatory response leading to changes in all blood cell types and a very large cytokine storm in the few days following PHZ administration. Regardless of the nature of the alterations to hematological parameters (such as anemia or inflammation), we took advantage of the direct in vivo modifications of immature compartments to model the normalization of the compartment sizes upon exposure to stress. Our model could faithfully reproduce the return to baseline of the hematopoietic compartments. We next applied it to three other types of “classical” murine stress conditions, such as bleeding, 5-FU chemotherapy, and LT-HSC attrition. The in silico predictions in each case accorded with the biological data reported in the literature, further reinforcing the robustness of our model.

The consequences of hematopoietic stress on stem cell compartments such as LT-HSC, ST-HSC, MPP, CMP, and MEP can be drastic. Our present experiments demonstrated that, after an acute stress event that caused massive RBC loss, associated with a cytokine storm, the first compensatory mechanism entails all compartments, from LT-HSC to CMP, and, surprisingly, induces differentiation without proliferation as a first step in this recovery process. The following recovery phase not only replenishes the different “flushed” compartments but exceeds steady-state values. Normalization of all compartment sizes occurs in a third phase similar to dampened oscillations around the standard values. Importantly,

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**Table 3. Compartment proliferation and erythroid differentiation rate constants, clearance of proliferation and differentiation regulators, and mitotic number in the transition from MEPs to mature RBCs after a stress event**

| Type 1 LT-HSC | Type 2 ST-HSC | Type 3 MPP | Type 4 CMP | Type 5 MEP |
|---------------|---------------|------------|------------|------------|
| Regulation sensitivity parameter for the net proliferation rate | $\alpha_1 = 4.56$ | $\alpha_2 = 4.53$ | $\alpha_3 = 4.0$ | $\alpha_4 = 2.2$ | $\alpha_5 = 0.0$ |
| Regulation sensitivity parameter for the differentiation rate | $\beta_1 = 9.17$ | $\beta_2 = 8.95$ | $\beta_3 = 8.24$ | $\beta_4 = 6.2$ | $\beta_5 = 4.9$ |
| Proliferation regulator clearance (1/day) | $m_r = 0.1$ |
| Differentiation regulator clearance (1/day) | $m_d = 0.057$ |
| Last mitosis sensitivity parameter | $c = 0.79$ |

All parameters have been defined in the results section.
these cell number oscillations targeting each compartment revealed by our model accord with the 
in vivo data previously reported for post-chemotherapy stressed hematopoiesis (Busch et al., 2015). In 
contrast to the above responses, the MEP compartment was found not to be subjected to such a strong 
regulatory step in the BM, probably because of the well-known spleen stress erythropoiesis process 
that is mediated by a different kind of regulation, as documented here and in previous reports 
(Perry et al., 2009).

Our current study focused on immature cell compartments unlike most prior studies that have focused on 
late erythropoiesis. We considered late erythropoiesis to be one single compartment regulated by specific 
factors (such as erythropoietin [EPO] and stem cell factor) that are capable of modulating the mitotic
number between MEPs and RBCs. Conversely, immature compartments are regulated by numerous factors. Cytokines/chemokines (e.g., IFNs, TNF, TGFβ, BMP), interactions with niche cells, and neuronal influxes play major roles in the regulation of stem cell compartments (Baldridge et al., 2011; Batsivari et al., 2020; Mataall et al., 2016; Renders et al., 2021). Although biological processes regulating stem compartments in the BM niche seem to be very complex, a surprising finding was that a “proliferation” and a “differentiation” regulator were sufficient to integrate this complexity.

Modeling of the regulation of immature compartments (based on human data) using a mathematical approach similar to ours was recently reported (Mon Père et al., 2021). However, that prior human modeling approach focused on erythroid mature stress conditions related to the loss of mature cells (due to blood loss) or chronic hemolysis (paroxysmal hemolytic anemia). By contrast, we validated our current model using a larger spectrum of stress events targeting not only erythroid mature compartments but also more immature ones via inflammation and acute hemolysis, the destruction of proliferative cells, or LT-HSCs.

Our post-stress experimental data revealed dampened oscillations that were not explained when considering the regulators in a quasi-steady state. We then considered their production/clearance dynamics, which implicitly created a delay in the dampening phenomenon and better matching to our data. Many models to date have included the regulation of the more immature HSC compartments following a brief stress exposure and are based on in vitro cell counts (Mahadik et al., 2019), in vivo immature (Klose et al., 2019) or mature (Marciniak-Czochra et al., 2009) cell counts, or in vivo recovery times (Manesso et al., 2013). To our knowledge, none of these systems displayed the dampening of oscillations within stem cell compartments that we observed previously in vivo (we do not consider this to include sustained periodic oscillations that can emerge in chronic infections) (Knauer et al., 2020) Similar oscillations within the late progenitor compartment following EPO administration (Schirm et al., 2013) or RBC suppression (Crauste et al., 2008, 2010; Wulff et al., 1989) have been exhibited by several prior models. However, as the so-called late progenitors in those studies are downstream of the MEP compartment, their proliferation rate is regulated by an EPO-dependent mechanism in contrast to the immature stem cell compartments that we focused on here.

In conclusion, we have successfully modeled the plasticity of hematopoiesis that recapitulates steady-state as well as stress hematopoiesis impacting all immature compartments. Our mathematical model integrates, as finely as possible, the reactions to the principal stress events in immature and RBC compartments and may thus be used to forecast stem cell compartment behaviors in silico to the hematopoietic toxicity of drugs such as chemotherapeutics or environmental toxins when the mechanisms of action of these agents are known. Our model also opens avenues to the analysis of malignant clonal development and invasion, noting that stem cell disorders, just like leukemias and cancers in general, could arise from successive stress events if pathological clonal cells react differently from normal cells in response to these conditions.

Limitations of the study
The biological limitations of our present study mainly arise from the use of mouse models in the place of human data. This is a particularly important consideration as hematopoietic stress involves a splenic response in the mouse but not in humans. Another notable limitation was our use of BrdU to analyze the proliferation of immature compartments as this type of analysis has been extensively criticized for its lack of precision. The mathematical limitations of our study include a simplified regulation of the erythroid terminal maturation process (from MEPs to RBCs) that has been extensively described and modeled previously. Modeling of the dynamics between these cell types is summarized by the instant production of 2" RBCs from MEPs in our model. A second mathematical limitation of note was our reduction of the complex stem cell dynamics to two simple regulators. This reduction was related to the system complexity. However, identifying real and measurable regulators that target these compartments has thus far proved to be very difficult through current biological approaches.

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Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103399.

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AUTHOR CONTRIBUTIONS
C.B., S. Girel, V.B., and S.M. conducted the mathematical modeling. P.G., C.L., K.B., M.-H.S., E.L., and S. Giraudier conducted the in vivo experiments. E.L. and S. Giraudier performed the data analysis. C.B., S. Girel, E.L., S.M., and S. Giraudier wrote the paper.

DECLARATION OF INTERESTS
The authors declare no competing financial or other interests in relation to this study.

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Biotin anti-mouse Lin Panel | Biolegend | 133307 |
| Ter-119, M1/70, RB6-8C5, 145-2C11, RA3-6B2 |        |            |
| APC/Cy7 Streptavidin | Biolegend | 405208 |
| BV 510™ anti-mouse Ly-6A/E (Sca-1-) D7 | Biolegend | 108129 |
| BV421 anti-mouse CD135 A2F10 | Biolegend | 135315 |
| BV711 anti-mouse CD48 HM48-1 | Biolegend | 103439 |
| PE/Cy7 anti-mouse CD150 TC15-12F12.2 | Biolegend | 115914 |
| BB700 Anti-Mouse CD117 (PerCP) 2B8 | BD Pharmingen | 566414 |
| BV786 Anti-Mouse CD16/CD32 2.4G2 | BD Pharmingen | 740851 |
| Alexa Fluor® 647 anti-Mouse CD34 Ram34 | BD Pharmingen | 560230 |
| Alexa Fluor 488 anti-BrdU Antibody | Biolegend | 364105 |
| FITC anti-mouse CD45.1 A20 | Biolegend | 110705 |
| PE anti mouse CD45.2 104 | Biolegend | 109808 |
| **Chemicals, peptides, and recombinant proteins** |        |            |
| PHZ (Phenylhydrazine) | Sigma-Aldrich | P26252 |
| **Critical commercial assays** |        |            |
| Zombie UV fixable viability kit | Ozyme | BLE423107 |
| BD Horizon Brilliant staining buffer | BD Pharmingen | 563794 |
| Ultracompe beads | Life Technologies | 01-2222-42 |
| Rainbow Calibration Particles (8 - PEaks), 3.0-3.4 mm SPHERO Particles | BD Pharmingen | 559123 |
| BD Pharmingen BrdU Flow Kit | BD Pharmingen | 557892 |
| FITC Annexin V Apoptosis Detection Kit | BD Pharmingen | 559763 |
| ACK Lysing Buffer, Quality Biological | VWR | 10128-802 |
| ProcartaPlex TM Multiplex Immunoassay | Thermofisher (custom multiplex assay have been designed by the authors) | |
| **Experimental models: Organism/strain** |        |            |
| C57BL/6JJanvier (Le Genest, France) |        |            |
| B6 SJL-Pptcr©Pepcl©BoyCrl Congenic (Ly5.1 mice) Janvier (Le Genest, France) |        |            |
| **Software and algorithms** |        |            |
| Diva software | Beckman |        |
| Kaluza software | Beckman |        |
| Prism 7 | https://www.graphpad.com/scientificsoftware/prism/ |        |
| **Covariance matrix adaptative in Evolution algorithm (CMAES) Python language** |        |            |
| **Others** |        |            |
| Tubes MS EDTA | #3MSR0506 Melet Schoesing Lab |        |
| MS9 coulter | Schloessing Melet, Cergy-Pontoise, France |        |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to Professor S. Giraudier (stephane.giraudier@aphp.fr).

Materials availability
All reagents used in this study are commercially available.

Data and code availability
- Data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice
Adult 6-12 week old C57BL/6 (CD45.2) and C57BL/6.SJL (CD45.1) mice were used in the experiments and were obtained from Janvier (Le Genest, France). These animals were bred and maintained in pathogen-free conditions in our bespoke animal facilities at the University of Paris. All animal procedures were approved by both the local Committee on the Use and Care of Animals and by our Institutional Animal Care and Use Committee.

Hematologic evaluations
Orbital plexus blood was collected in EDTA tubes from anesthetized mice. Blood cell counts were determined using an automated device (MS9; Schloessing Melet, Cergy-Pontoise, France). Blood samples were also used for cytometry analysis. All mouse sera were collected and stored at 20°C before assessing the cytokine levels.

Bone marrow and spleen analysis
For BrdU analysis, the mice received an intraperitoneal injection of 0.2 ml of BrdU (0.25 mg/ml) at 16 h before sacrifice. The mice were then killed by cervical dislocation and their bones (femurs, tibias, pelvis and humerus) and spleen were harvested. The muscle and tendon tissue were removed using a scalpel and Kim wipes. BM and spleen tissues were used for cell counting and cytometry. The BM fraction was flushed out using a syringe containing 1 x PBS supplemented with 2% fetal bovine serum (FBS). The resulting cell suspensions were filtered through a 40μM cell strainer (Corning, NY) and pelleted by centrifugation. For mechanical grinding, spleens were smashed and ground between the rough sides of frosted glass slides, and the cells were then collected in DMEM containing 2% FBS. After subsequent incubation in a 24-well plate for 30 min at 37°C in a humidified incubator, cell suspensions were passed through a 40μm cell strainer and re-suspended in DMEM containing 10% FBS. Suspensions were subjected to cytometry analysis after RBC lysis.

Cytometry analysis
Mouse erythrocytes were lysed using ACK lysing buffer (Lonza, Basel, Switzerland) prior to flow cytometry staining. Total BM and splenic cell populations were stained using a Zombie Viability kit for 15 min at room temperature and thereafter stained with biotinylated anti-mouse lineage cocktail antibodies for 30 min (Biolegend). After washing, the cells were stained for 30 min using the following monoclonal antibodies in the BD Horizon Brilliant staining buffer: anti-CD117/c-Kit (2B8)-BB700 and anti-CD34 (RAM34)-AF647.
(BD Pharmingen); anti-Ly6a/Sca-1 (D7)-BV510; anti-CD150 (TC15-12F12.2)-PE-Cy7; anti-CD48 (HM48-1)-
BV711; streptavidin APC-Cy7 (Biolegend).

For proliferation analysis, surface staining was performed as described above, and cell growth was 
analyzed using a commercially available kit in accordance with the manufacturer’s instructions (BD Phar-
migen BrdU Flow kits) with BrdU-AF488 (BD Biosciences, Franklin Lakes, NJ). Data acquisition and/or 
analysis was performed on the Cochin Cytometry and Immunobiology Facility Cytometry analyses using 
a Fortessa cytometer (Becton Dickinson), and Kaluza software. To assess cell viability, surface labelled cells 
were resuspended in AnnexinV binding buffer, and AnnexinV (FITC) was added 15 min before analysis. The 
cells were then evaluated using a FITC-conjugated AnnexinV labeling detection kit (BD Pharmingen). The 
list of antibodies used for flow cytometry is provided in the key resources table above.

Reconstitution assays
Donor cells were isolated from 6-12-week-old B6.SJL-Ptprc<sup>+</sup>Pepc<sup>b</sup>/BoyCrl conegenic mice (Ly5.1) and Ly5.2 mice at 3 days after a phenylhydrazine (PHZ) (60 mg/Kg, IP) injection or not. The hind limbs were extracted from these animals after sacrifice and cleaned. The total BM was flushed and then passed through a 40
μM cell strainer to obtain a single cell suspension. A mix of cells from PHZ-treated and untreated mice was pre-
pared in a 1:1 ratio. A mix of 5x10<sup>6</sup> of these cells was then resuspended in a total volume of 0.2 mL and trans-
planted into 5 irradiated (9Gy) CD45.1 C57/BL6 recipient mice. Chimerism analysis was performed three 
months post-reconstitution using cytometry.

Cytokine bead array
Sera were separated from blood cells within 2 hours of blood collection by 2 successive centrifugations, and 
were immediately frozen at -80°C. Twenty cytokines (G-CSF (CSF-3), GM-CSF, IFNalpha, IFNgamma, IL-
1alpha, IL-1beta, IL-2, IL-2R, IL-3, IL-4, IL-6, IL-10, IL-12p70, IL-13, IL-17A (CTLA-8), IP-10 (CXCL10), 
MCP-1(CCL2), MIP-1 alpha (CCL3), MIP-1 beta (CCL4), TNF alpha) were measured simultaneously using 
a multiplex magnetic Luminex Assay (ProcartaPlex TM; ThermoFisher Scientific) with the data read on a 
Bioplex MAGPIX Multiplex Reader (Biorad). All assays were performed in accordance with the manufac-
turer’s instructions. Results were expressed in pg/mL and if levels were not detectable, as 50% of the lowest 
standard value.

**METHOD DETAILS**

**Mathematical model**

Our Mathematical model focuses on the time behaviors of the immature compartment sizes of the 
erthyroid lineages including different regulation effects initiated by different stresses.

The model considers six hematopoietic cell types, i.e. five immature cell types, LT-HSC, ST-HSC, MPP, 
CMP, MEP and the mature RBC. It describes the dynamics of the compartment sizes, denoted by (x<sub>i</sub>(t)), 
i=1,...,6. Under homeostatic conditions, the system is stable and stays at its equilibrium x<sub>i</sub>*, i=1,...,6. These 
steady-state values are biologically observable and are considered as given data in our construction (Table 
2). Let us now precisely describe our model.

For any i=1,...,5, we denote by r<sub>i</sub>(t) and d<sub>i</sub>(t) respectively the net proliferation rate and the erythroid differ-
entiation rate at time t and we denote by d<sub>6</sub> the death rate of the RBCs which is a fixed positive real number. 
We assume that, for the five immature compartments, the net proliferation and erythroid differentiation 
rates depend on the dynamics of different regulators modelled by the functions u<sub>i</sub>(t) and u<sub>6</sub>(t). The dy-
namics of these functions will depend on the state of the five immature compartments. The dynamics of 
the RBC will depend on a steady-state regulation through the function n(t). The compartment sizes 
(x<sub>i</sub>(t)), i=1,...,6, satisfy the following system of ordinary differential equations:

\[
\frac{dx_i(t)}{dt} = (r_i(t) - d_i(t))x_i(t) 
\]  

(Equation 1)

Where

\[
r_i(t) = r_i^* \left( 25 m \right) m_u(t)^{m_o}; \quad d_i(t) = d_i^* \left( \frac{mu(t)}{25} \right)^{d_i}.
\]  

(Equation 2)
The parameters \( r_i^* \), \( d_i^* \) are positive numbers and the parameters \( \alpha, \beta \) are non-negative numbers. The dynamics of the regulators \( u_r \) and \( u_d \) are given by the equations

\[
\frac{du_r}{dt}(t) = \frac{1}{y(t)^2} - m_r u_r(t); \quad \frac{du_d}{dt}(t) = y(t)^2 - m_d u_d(t).
\]  

(Equation 3)

with \( y(t) \) the total number of cells in the five immature compartments renormalized by their size at equilibrium, \( m_r \) and \( m_d \) the positive parameters of the linear degradation terms. More precisely, we have \( y(t) = \sum_{i=1}^{5} \frac{x_i(t)}{x_i^*} \) with \( x_i^* \) the equilibrium value of \( x_i(t) \) corresponding to the cell size of type \( i \) biologically observed (Table 2).

Further, the term \( 2^{10} \) represents the RBC produced following one MEP differentiation. We assume that the regulation of \( n(t) \) occurs (essentially through EPO) at a short time-scale and directly depends on the RBC number (Knaupp et al., 1985). Following this phenomenological approach and based on the data, we chose \( n(t) \) as a \(-\logistic\) type function

\[
n(t) = n^* + \left(33 - n^*\right) \left(1 - \frac{x_i(t)}{x_i^*}\right)^c.
\]  

(Equation 4)

The parameter \( n^* \) is the value of \( n(t) \) at the equilibrium of the system and 33 is an upperbound of this quantity (Hayflick, 1965). The parameter \( c \) is a positive number which will be calibrated by the algorithm.

The regulation shapes have been chosen in such a way that \( 25 m_r u_r(t), 25 m_d u_d(t) \) and \( x_i^* \) are equal to 1 when the system is at its given biological equilibrium \( x_i^* \), \( i = 1, \ldots, 6 \), under homeostatic conditions.

We can prove by a straightforward computation (assuming that \( a_1 \) or \( b_1 \) is non zero), the uniqueness of the positive steady-state of the regulated system (1), which is also given by \( (x_i^*), i = 1, \ldots, 6 \). The system is nonlinear and it is very hard to rigorously prove that it converges effectively to \( (x_i^*), i = 1, \ldots, 6 \) when \( t \) tends to infinity. Nevertheless a numerical analysis based on a large number of simulations shows this convergence.

**Explanation for the 6-compartments choice**

In the previous section, the number of compartments has been chosen equal to 6 since we focused on the immature cell compartments LT-HSC, ST-HSC, MPP, CMP, MEP plus the RBC for which we have data.

In this paragraph (independent of the other ones), we wished to formally justify the number of 5 compartments as the minimal number of immature compartments (including MEP) explaining the following biological informations 1 to 4,

**Information 1**: The RBC number \( x_k^* \) and the RBC death rate \( d_k \) are known, approximatively equal respectively to \( 5.17 \times 10^{10} \) RBC per mouse and a 40-day life expectancy.

**Information 2**: The LT-HSC number \( x_1^* \) was evaluated from the proportion of LT-HSC (Lin-Sca-1+ c-kit+, LSK CD150+ CD48-) estimated as described in Figure S1. Knowing that a C57B16 mouse displays \( 250 \times 10^6 \) BM cells. (Boggs, 1984; Chervenick et al., 1968; Mahajan et al., 2015), the LT-HSC number was around 15248 +/- 2200/mouse.

**Information 3**: By calibration \( \tau_1 = 0.012 \) (per day), \( D_{\text{min}} = 0.0845 \).

**Information 4**: The \( n^* \) number of mitosis between the two last compartments was assessed as follows: we assume that the \((k-1)\)-compartment is upstream of BFU-E (Burst-Forming-Unit Erythroid)-enriched cells known to perform \( \approx 11 \) cell divisions in vitro to generate mature RBC (Li et al., 2019). Therefore, we assume that \( n^* \approx 10 \).

Let us consider a similar system as (1) but with \( k \) compartments, always assuming that the first and the last compartments correspond respectively to the LT-HSC and the RBC. Let us assume that the
system is at equilibrium. The model has been constructed so that the net proliferation and erythroid differentiation rates at equilibrium are given respectively by \( r_i^* \) and \( d_i^* \). The net proliferation rate \( r_i^* \) is defined as \( r_i^* = R_i^* - \mu_i \), \( R_i^* \) being the renewal rate in compartment \( i \) and \( \mu_i \) the rate of differentiation to non-erythroid lineage. The cell death is assumed negligible (cf. Domen et al., 2000). We assume that \( r_i^* > 0 \) and \( d_i^* > 0 \). (cf. Busch et al., 2015).

We denote by \( \tau_i = d_i^* + R_i^* + \mu_i \), the total jump rate for a type-\( i \) cell. When a jump event for such a cell occurs, with probability \( p_i^d = d_i^*/\tau_i \), it is considered as differentiation event, with probability \( p_i^r = R_i^*/\tau_i \), as renewal event and with probability \( p_i^n = \mu_i/\tau_i \) as death event.

The two first compartments are the LT-HSC and ST-HSC compartments. We assume that at equilibrium, the number of LT-HSC is constant and then \( r_1^* = d_1^* \). We also assume that there is a unique differentiation lineage at this step. Then, \( \mu_1 = \mu_2 = 0 \). In particular, \( \tau_1 = 2r_1^* = 2d_1^* \) and \( \tau_2 = d_2^* + r_2^* \).

We easily obtain that the stationary point \( x^* = (x_1^*, ..., x_k^*) \) satisfies the following system

\[
\begin{align*}
x_1^* &= \frac{2d_1^*}{d_2^* - r_2^*} x_1^*, \\
x_2^* &= \frac{2d_2^* - r_2^*}{d_1^* - r_1^*} x_1^* + \frac{2m}{d_2^*}, \quad \text{for } i = 3, 4, ..., k - 1, \\
x_k^* &= \frac{2m}{d_k^*} x_{k-1}^*.
\end{align*}
\]

Note that if there exists an integer \( i \) between 2 and \( k-1 \) such that \( d_i^* \leq r_i^* \), there is no stationary point such that \( x_i^* > 0 \) and \( x_i^* \geq 0 \). Indeed, if \( d_i^* < r_i^* \) then from (Equation 5), we obtain \( x_i^* < 0 \) and assuming that there exists a stationary point \( x^* = (x_1^*, ..., x_k^*) \) such that \( d_i^* = r_i^* \) then \( x_i^* < 0 \) implies \( x_{i+1}^* = 0 \) and recursively we get \( x_1^* = 0 \).

From now and for any \( i = 2, ..., k-1 \), we assume that \( D_i = (d_i^* - r_i^*) / \tau_i > 0 \). Note that \( D_i = p_i^d + p_i^n - p_i^r \). The factors \( D_i \), for \( i \in (2, ..., k-1) \), play a main role in the amplification mechanism. The stationary compartment sizes can be given in an explicit form in terms of the \( D_i \) and \( p_i^n \). Indeed, computation using that \( p_i^a = p_i^n + p_i^r > 0 \) and that \( D_i = p_i^d + p_i^n - p_i^r \), implies that

\[
x_2^* = \frac{\tau_1 \tau_2}{D_2} x_1^*, ..., x_k^* = \frac{(1 + D_{k-1} - 2p_k^n)(1 + D_k)\tau_k}{D_{k-1} D_k} x_1^*, ..., x_k^* = \frac{2^{m-1} (1 + D_{k-1} - 2p_k^n)\tau_k}{D_{k-1} \cdots D_2 d_k} x_1^*.
\]

Since \( p_i^n \geq 0 \) and \( D_i \geq D_{\text{min}} \), where \( D_{\text{min}} = \min_{i \geq 1} D_i \), it comes that

\[
x_k^* \leq \frac{2^{m-1} \tau_k}{D_{\text{min}}} \left(1 + \frac{1}{D_{\text{min}}} \right)^{k-2}.
\]

That is,

\[
k \geq \frac{\log \left( \frac{2^{m-1} \tau_k}{D_{\text{min}}} \right)}{\log \left(1 + \frac{1}{D_{\text{min}}} \right)} + 2. \quad \text{(Equation 6)}
\]

Then adding altogether Informations 1 to 4, we obtain from (Equation 6) that \( k \geq 5.32 \), hence a minimal of 6 compartments (i.e. 5 immature compartments) is needed to explain the biological data.

**Calibration of the parameters**

*Calibration of rest cell dynamics parameters.* We have seen that the global system (1) admits the unique equilibrium \( x^* \) and that near equilibrium, the regulation functions are all close to 1. Then if the initial conditions are close to the equilibrium \( x^* \), the dynamical system approximatively is written as
\[
\frac{dx_i}{dt}(t) = (r_i - d_i) x_i(t)
\]
\[
\frac{dx_i}{dt}(t) = 2d_{i-1} x_{i-1}(t) + (r_i - d_i) x_i(t); \quad \text{for } i = 2, \ldots, 5,
\]
\[
\frac{dx_i}{dt}(t) = 2d_i x_i(t) + d_6 x_6(t).
\]

The aim of this section is to determine the values of the parameters \((x_i^*, r_i^*, d_i^*, n_i^*)\) from the biological data of the rest cell system. Recall that \(r_i^*\) is the net proliferation rate, equal to the difference between the prolif-
eration rate \(R_i^*\) and the death rate \(d_i\) due to the differentiation of cells to non-erythroid lineages.

Let us recall that \(d_1^* = r_1^*\) and that the equilibrium satisfies (Equation 5) with \(k = 6\). Let us also recall that \(d_6 = 1/40\) \(d^{-1}\). In what follows, we will estimate from the biological BrdU data under homeostatic conditions and from the values of \(d_i\) found in (Busch et al., 2015), the value of the differentiation rates \(d_i^*\) and then of the proliferation rates \(R_i^*\).

Our biological data allow to estimate the values of the population sizes at equilibrium (under homeostatic conditions) \((x_1^*, \ldots, x_6^*)\), and the cell proportion that are BrdU positive after 20 hours for the 5 first types: \(p_{1}^{\text{BrdU}}, \ldots, p_{5}^{\text{BrdU}}\).

Let us now explain how we use these BrdU positive cell proportions, that are the proportions of cells that have made a division at least once in 20h. An estimation is given by

\[
p_{i}^{\text{BrdU}} = \frac{x_i^D(20h)}{x_i^*}
\]

with \(x_i^D(t)\) the number of cells having made a division at least once in a time interval of length \(t\). Indeed, the type-\(i\) cell number is assumed (almost) constant equal to \(x_i^*\). The dynamics of \(x_i^D\) are then described by the following equations

\[
\frac{dx_i^D}{dt}(t) = r_i x_i^D(t) + 2\left(r_i + \mu_i\right)\left(x_i^* - x_i^D(t)\right) - d_i x_i^D(t),
\]

(Equation 7)

\[
\frac{dx_i^D}{dt}(t) = r_i x_i^D(t) + 2\left(r_i + \mu_i\right)\left(x_i^* - x_i^D(t)\right) + 2d_{i-1} x_{i-1}^D - d_i x_i^D(t), \quad \text{for } i = 2, \ldots, 5
\]

with \(x_i^D(0) = 0\). Indeed, each dividing type-\(i\) cell becomes a type-\(i\) cell that has made a division and each type-
(\(i-1\)) cell differentiating in the erythroid lineage gives two cells of type \(i\) that have made a division at least once. In addition, each type-\(i\) cell that has made at least one division gives cells of type \(i+1\) by differentiation.

By resolving (Equation 7), we obtain that

\[
x_i^D(20h) = \frac{2R_i x_i^*}{R_i + \mu_i + d_i} \left(1 - e^{-\left(r_i + \mu_i + d_i\right)24}\right)
\]

and for \(i = 2, \ldots, 5\),

\[
x_i^D(20h) = \frac{2R_i x_i^* + 2d_{i-1} x_{i-1}^*}{R_i + \mu_i + d_i} \left(1 - e^{-\left(r_i + \mu_i + d_i\right)24}\right)
\]

We finally deduce that

\[
p_{i}^{\text{BrdU}} = \frac{2\left(r_i + \mu_i\right) x_i^* + 2d_{i-1} x_{i-1}^*}{R_i + 2\mu_i + d_i} \left(1 - e^{-\left(r_i + 2\mu_i + d_i\right)24}\right)
\]

And then \(r_i^* = d_i^* = -\frac{24}{24}\log(1 - p_{i}^{\text{BrdU}}) - \mu_i\).

We also obtain that for \(i = 2, \ldots, 5\),

\[
p_{i}^{\text{BrdU}} = \frac{2\left(r_i + \mu_i\right) x_i^* + 2d_{i-1} x_{i-1}^*}{\left(r_i + 2\mu_i + d_i\right) x_i^*} \left(1 - e^{-\left(r_i + 2\mu_i + d_i\right)24}\right)
\]

But, since by (Equation 5), for \(i = 2, \ldots, 5\), \(d_i^* = r_i^* + \frac{2d_{i-1} x_{i-1}^*}{x_i^*}\), we deduce that for \(i = 2, \ldots, 5\),
\[
p_{i}^{\text{BrdU}} = 1 - e^{-\left(\frac{\mu_{i} + 2\mu_{i}^{*} + 2m_{i}X_{i}}{m_{i}}\right)}.
\]

That implies
\[
t_{i}^{*} = -\frac{24}{40} \log\left(1 - p_{i}^{\text{BrdU}}\right) - \mu_{i} - \frac{d_{i} - x_{i-1}^{*}}{x_{i}^{*}}.
\]

These formulae allow us to compute from one to the next the values of the differentiation rates and then the net proliferation rates, using our biological data of BrdU positive proportions at equilibrium and the values \(\mu_{i}\) given in (Busch et al., 2015).

**Calibration of feedback parameters**

Values of the 13 parameters associated with the regulation functions (namely \(c_{i}, m_{r}, m_{d}, a_{i}\), and \(b_{i}\)) were estimated from in vivo cell counts in the 6 observed cell compartments following PHZ administration (see Figure 2). Note that our model has not been designed to encompass the effect of PHZ and the corresponding loss of erythropoietic cells observed during the first three days. Then we considered only data from day 3 and later. We added two artificial data points at days 70 and 90, accounting for a return to equilibrium after two months.

Formally, parameters values have been chosen to minimize the residual sum of squares
\[
\text{RSS} = \sum_{i,j} \left(\frac{x_{i}(t_{j}) - X_{i}(t_{j})}{x_{i}(t_{j})}\right)^{2}
\]

where \(x_{i}\) and \(X_{i}\) denote respectively the in silico and in vivo cell numbers in compartment \(i\) and \(t_{j}\) are the days when the cell numbers in the \(i\)-th compartment has been measured.

Hence, the initial conditions at day 3 have been chosen as
\[
\begin{align*}
x_{1}(3) &= 0.006 \times 10^{6} \text{ cells/mouse} \\
x_{2}(3) &= 0.0152 \times 10^{6} \text{ cells/mouse} \\
x_{3}(3) &= 0.02 \times 10^{6} \text{ cells/mouse} \\
x_{4}(3) &= 0.093 \times 10^{6} \text{ cells/mouse} \\
x_{5}(3) &= 0.395 \times 10^{6} \text{ cells/mouse} \\
x_{6}(3) &= 0.94 \times 10^{6} \text{ cells/mouse} \\
\end{align*}
\]
\[
\begin{align*}
u_{r}(3) &= \frac{1}{m_{r} y(3)} \\
u_{d}(3) &= \frac{y(3)^{2}}{m_{d}}
\end{align*}
\]

Minimization has been achieved with the Covariance Matrix Adaptation in Evolution Strategy (CMA-ES) from Python package cma. Optimal parameters obtained by this method are presented in Table 3 and the corresponding cell dynamics in Figure 2.

While we did not formally prove the existence of a unique optimal set of parameters, the same set, within \(10^{-3}\), has been obtained with ten independent runs of the stochastic CMA-ES algorithm, implying numerical identifiability.

The identifiability of the system (1), (Equation 2), (Equation 3), and (Equation 4) has been assessed on synthetic data produced from random set of parameters. The model was efficient to retrieve the parameter set behind the synthetic data.

**Sensitivity analysis**

The sensitivity of the cost function \(\text{RSS} (\text{Equation 8})\) to the parameters estimated in the previous subsection has been assessed by plotting its values with respect to variation of each parameter.

We also deduced from this figure that the variation of each parameter in the following intervals induces a maximal variation of 15% of the cost function \(\text{RSS}\) above its minimal value:
Note that parameter $c$ (Equation 4) only affects the number of RBCs and weakly influences the value of the cost function RSS. Then this parameter is poorly indicative but remains numerically identifiable (as seen in the previous section).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

The data shown in all of the figures are the mean values +/- SD. Statistical details relevant to each experiment including the sample numbers and experimental repeats are listed in the Figure legends. Data were statistically analyzed using the Mann-Whitney test.