A structured population model of clonal selection in acute leukemias with multiple maturation stages

Tommaso Lorenzi · Anna Marciniak-Czochra · Thomas Stiehl

Abstract  Recent progress in genetic techniques has shed light on the complex coevolution of malignant cell clones in leukemias. However, several aspects of clonal selection still remain unclear. In this paper, we present a multicompartamental continuously structured population model of selection dynamics in acute leukemias, which consists of a system of coupled integrodifferential equations. Compared to classical models formulated in terms of ordinary differential equations, our model can be handled analytically in a more efficient way. Exploiting the analytical tractability of our model, we investigate how clonal selection is shaped by the self-renewal fraction and the proliferation rate of leukemic cells at different maturation stages. We integrate the results of our analyses with numerical solutions of a calibrated version of the model based on real patient data. In summary, our mathematical results formalise the biological notion that clonal selection is driven by the self-renewal fraction of leukemic stem cells and the clones that possess the highest value of this parameter will ultimately be selected. Moreover, we demonstrate that the self-renewal fraction and the proliferation rate of non-stem cells do not have a substantial impact on clonal selection. Taken together, our results indicate that interclonal variability in the self-renewal fraction of leukemic stem cells provides the necessary substrate for clonal selection to act on.

Tommaso Lorenzi
School of Mathematics and Statistics, University of St Andrews, North Haugh, St Andrews, Fife, KY16 9SS, United Kingdom
E-mail: tl47@st-andrews.ac.uk

Anna Marciniak-Czochra
Institute of Applied Mathematics, BIOQUANT and IWR, Im Neuenheimer Feld 205, Heidelberg University, 69120 Heidelberg, Germany
E-mail: Anna.Marciniak@iwr.uni-heidelberg.de

Thomas Stiehl
Institute of Applied Mathematics, Im Neuenheimer Feld 205, Heidelberg University, 69120 Heidelberg, Germany
E-mail: thomas.stiehl@iwr.uni-heidelberg.de
Keywords  Acute leukemia · Clonal selection · Continuously structured population models · Integrodifferential equations · Asymptotic analysis

1 Introduction

Statement of the biological problem. Leukemias are malignant diseases of the hematopoietic system. Similar as the healthy hematopoietic system, the leukemic cell bulk is organised as a hierarchy which is comprised of multiple maturation stages (i.e. maturation compartments) – from stem cells through a number of increasingly mature progenitor cells to the most mature cells [6, 27]. Red blood cells, white blood cells and platelets constitute the most mature compartment of healthy cells, whereas non-functional leukemic blasts represent the most mature leukemic cells. The extensive growth of non-functional leukemic blasts leads to impaired hematopoiesis and causes a shortage of healthy blood cells.

In contrast to the most mature cells, which do not divide and die at constant rates, stem and progenitor cells can proliferate and give rise to progeny cells which are either at the same maturation stage as their parent (self-renewal) or at a successive maturation stage (differentiation). These processes can be quantitatively characterised in terms of two parameters: the cell proliferation rate (i.e. cell divisions per unit of time) and the cell self-renewal fraction (i.e. the probability that a progeny adopts the same cell fate as its parent) [42, 63]. There is theoretical [57] and experimental [29, 43, 64] evidence suggesting that the proliferation rate and the self-renewal fraction of leukemic cells have a significant impact on disease dynamics and patient prognosis.

The collection of all stem, progenitor and most mature cells which carry the same set of genetic alterations defines a leukemic clone. Recent experimental evidence indicates that the leukemic cell bulk of an individual patient is composed of multiple clones carrying different mutations [2, 17, 35] and having different functional properties, amongst which different proliferation rates and self-renewal fractions [20, 24]. Such clonal heterogeneity poses a major obstacle to successful therapy and management of disease relapse [11, 13, 38, 39]. In fact, it has been reported that in many cases of acute lymphoblastic leukemias (ALL) and acute myeloid leukemias (AML) relapse is triggered by the selection of clones that have been present as minor (small) clones at the time of diagnosis rather than by newly acquired mutations [11, 13, 17, 28].

It is becoming increasingly apparent that clonal heterogeneity results from a selection process at the cellular level which triggers the expansion of some clones and the out-competition of others [5, 11, 26, 47, 56, 67]. However, several aspects of clonal selection are so far not well understood and a number of important questions remain open. In this paper, we focus on clonal selection processes taking place before diagnosis or relapse of acute leukemias. We use a mathematical modelling approach to address the following questions:

Q1 What is the role of the proliferation rate and the self-renewal fraction of leukemic cells in clonal selection?
Q2. Can we observe clonal selection among clones that have identical stem-cell properties (in terms of proliferation rate and self-renewal fraction) and differ in their progenitor-cell properties?  

Q3. What are the necessary conditions (in terms of proliferation rate and self-renewal fraction of cells) for long-term coexistence of clones?

Mathematical framework. A well-established mathematical approach to describing the dynamics of multiple leukemic clones is to use multicompartmental models formulated in terms of ordinary differential equations (ODEs) [56, 66]. In these models, every cell is characterised by a pair of discrete indexes $i = 1, \ldots, M$ and $j = 0, \ldots, J$. The index $i$ corresponds to the maturation stage of the cell (i.e., the compartment to which the cell belongs). The index $j = 1, \ldots, J$ indicates what leukemic clone the cell belongs to, whilst the index $j = 0$ is conventionally associated with healthy cells. In this setting, a collection of parameters $p^i_j$ and $a^i_j$ is introduced to model, respectively, the proliferation rate and the self-renewal fraction of cells of clone $j = 1, \ldots, J$ at the maturation stage $i = 1, \ldots, M - 1$. Clonal heterogeneity is incorporated into the model by allowing the values of these parameters to change from one clone to the other. The dynamics of every clone are described by a system of $M$ coupled ODEs which track the time evolution of the density of cells of the clone at each maturation stage. An additional system of $M$ coupled ODEs is introduced to model the dynamics of healthy cells. In accordance with biological findings [32, 34, 55], all equations of the model are coupled through a feedback signal that regulates cell properties and depends on the total density of cells at the maturation stage $M$ (i.e, all the most mature healthy and leukemic cells). A prototype version of such models can be found in Section 2 of this paper.  

These models consist of systems of $(J + 1)M$ coupled ODEs. Due to the high degree of clonal heterogeneity usually observed in leukemia patients, the biologically realistic values of $J$ can be very high. As a result, such models become hard (if not impossible) to be handled analytically in scenarios which are clinically relevant. This impinges on the robustness of the biological conclusions that can be achieved. To overcome this problem, we present here a modelling framework in which the discrete index $j$ is replaced by a continuous structuring variable $x$. This variable can be seen as a parametrisation of the self-renewal fraction and proliferation rate of the different clones. As a consequence, in our modelling framework the parameters $p^i_j$ and $a^i_j$ of the ODE model are replaced by some functions $p_i(x)$ and $a_i(x)$ which represent, respectively, the proliferation rate and the self-renewal fraction of cells that are at the maturation stage $i$ and belong to the clone identified by the variable $x$. Our multicompartmental continuously structured population model consists of a system of $M$ coupled integrodifferential equations (IDEs) which can be handled analytically in a more efficient way than its ODE counterpart.

Results of this paper. Exploiting the analytical tractability of our model, we address questions Q1-Q3 listed above by elucidating how clonal selection
is shaped by the self-renewal fraction and the proliferation rate of leukemic cells at different maturation stages. We integrate the results of our analysis with numerical solutions of a calibrated version of the model which is based on patient data from the existing literature. In summary, our mathematical results formalise the idea that clonal selection is controlled by the self-renewal fraction of leukemic stem cells and the clones with the highest value of this parameter will ultimately be selected. This implies that only the clones whose stem cells have the highest self-renewal fraction can stably coexist. Finally, our results suggest that interclonal variability in the self-renewal fraction of leukemic stem cells provides the necessary substrate for clonal selection to act on.

Our analytical work follows earlier papers on the asymptotic analysis of IDEs that arise in the mathematical modelling of selection dynamics in populations structured by physiological traits [4,7,8,10,14–16,36,37,48,50]. In particular, Busse et al. [7] have studied a basic version of the model, in which only two maturation stages are considered (i.e. \( M = 2 \)) and the cell proliferation rate does not depend on the clones, i.e. the functions \( p_1(x), \ldots, p_{M-1}(x) \) are constant. The main novelty of our work is that we let the number of maturation stages \( M \) be arbitrarily large and we allow the cell proliferation rate to vary from clone to clone, i.e. the functions \( p_1(x), \ldots, p_{M-1}(x) \) are not necessarily constant. This makes the application domain of our results significantly wider and strengthens the robustness of our biological conclusions. Due to these additional aspects, our analysis builds on a method of proof which is different from that proposed by Busse et al. in [7] and is based on asymptotic arguments analogous to those presented by Desvillettes et al. in [15]. Our analytical results rely on general assumptions and are applicable to different cohorts of leukemia patients.

2 Model description

We present here a multicompartmental continuously structured population model for the dynamics of healthy cells and cells of multiple leukemic clones at different maturation stages. The model is defined as a continuous version of the multicompartmental ODE model presented in [56]. For the sake of completeness, we provide a short description of such an ODE model in Appendix A. A continuous version of the model with a two-compartment maturation structure was investigated in [7].

The key ideas underlying the IDE model (2) are summarised by the schematic diagram presented in Fig. 1(A). In order to capture the high degree of clonal heterogeneity usually observed in leukemia patients, we replace the discrete set of compartments described by ODEs by a continuous structuring variable \( x \in [0, 1] \), vid. Fig. 1(B). We assume that \( x = 0 \) corresponds to healthy cells whereas different leukemic clones are characterised by different values of \( x \in (0, 1] \). In this framework, the population densities of stem cells (compartment...
A continuously structured model of clonal selection in acute leukemias

Fig. 1: Schematic overview of the model. Panel (A): Processes at the cellular level and their mathematical description Panel (B): Multicompartment continuously structured population model. For each maturation compartment \( i \), different leukemic clones are characterised by different values of the continuous variable \( x \in [0, 1] \), whereas \( x = 0 \) corresponds to healthy cells. All compartments are coupled through the feedback signal \( s(t) \). Proliferation, self-renewal and differentiation of cells are modelled as schematised in Panel (A).

\( i = 1 \), increasingly mature progenitor cells (compartments \( i = 2, \ldots, M - 1 \)) and mature cells/leukemic blasts (compartment \( i = M \)) at time \( t \geq 0 \) are represented by the functions \( n_i(t, x) \geq 0 \). At every time instant \( t \), the total density of cells at the \( i \)-th maturation stage is computed as

\[
\rho_i(t) = \int_0^1 n_i(t, x) \, dx \quad \text{with} \quad i = 1, \ldots, M. \tag{1}
\]

The evolution of the cell population density functions is governed by the following system of coupled IDEs

\[
\begin{align*}
\frac{\partial}{\partial t} n_1(t, x) &= \left( \frac{2 a_1(x)}{1 + K \rho_M(t)} - 1 \right) \rho_1(x) n_1(t, x), \\
\frac{\partial}{\partial t} n_i(t, x) &= 2 \left( 1 - \frac{a_{i-1}(x)}{1 + K \rho_M(t)} \right) \rho_{i-1}(x) n_{i-1}(t, x) \\
&\quad + \left( \frac{2 a_i(x)}{1 + K \rho_M(t)} - 1 \right) \rho_i(x) n_i(t, x), \quad i = 2, \ldots, M - 1, \\
\frac{\partial}{\partial t} n_M(t, x) &= 2 \left( 1 - \frac{a_{M-1}(x)}{1 + K \rho_M(t)} \right) \rho_{M-1}(x) n_{M-1}(t, x) - d n_M(t, x).
\end{align*}
\]  

The functions \( p_i(x) > 0 \) and \( a_i(x) > 0 \) model, respectively, the proliferation rate and the self-renewal fraction of cells of clone \( x \) at the maturation
The population densities of stem cells (compartment \( i = 1 \)), increasingly mature progenitor cells (compartments \( i = 2, \ldots, M-1 \)) and mature cells/leukemic blasts (compartment \( i = M \)) at time \( t \geq 0 \) are represented by the functions \( n_i(t, x) \geq 0 \). The flux to mitosis of cells of clone \( x \) at the maturation stage \( i \) at time \( t \) is given by \( n_i(t, x) p_i(x) \). These cells divide into 2 cells \( x \rightarrow 2 \) and the fraction \( a_i(x) \) remains at the maturation stage \( i \) (self-renewal) and the fraction \( 1-a_i(x) \) is at the maturation stage \( i+1 \) (differentiation). The factor \( s(t) = \frac{1}{1+K \rho_M(t)} \) models the concentration of feedback signal which promotes the self-renewal of dividing cells and is absorbed by mature cells and leukemic blasts at a rate that depends on the total density of these cells \( \rho_M(t) \). It can be derived from an explicit model of signalling factor dynamics using a quasi-stationary approximation [22, 41]. The parameter \( K > 0 \) depends on the degradation rate of the feedback signal. Mature cells and leukemic blasts do not divide and die at a constant rate \( d \). We close the system of IDEs (2) with the following initial conditions for \( i = 1, \ldots, M \):

\[
n_i(t=0, x) = n_i^0(x) \in L^1 \cap L^{\infty}([0, 1]) \text{ with } n_i^0(x) > 0 \text{ a.e. on } [0, 1].
\]  

We let the assumptions below hold throughout the paper:

\[
a_i \in W^{1, \infty}([0, 1]) \text{ with } a_i : [0, 1] \rightarrow \left( \frac{1}{2}, 1 \right) \text{ for all } i = 1, \ldots, M-1,
\]

\[
p_i \in W^{1, \infty}([0, 1]) \text{ with } p_i : [0, 1] \rightarrow (0, 1) \text{ for all } i = 1, \ldots, M-1,
\]

\[
\frac{1}{2} < a_i(x) < a_1(x) < 1 \text{ for all } x \in [0, 1], \text{ for all } i = 2, \ldots, M-1.
\]

These assumptions are justified by the following biological considerations. In summary, it has been shown that the self-renewal fraction of stem cells has to be larger than \( 1/2 \) to allow for cell expansion [62, 63]. This justifies assumptions (4) for \( i = 1 \). Moreover, we justify assumptions (4) for \( i = 2, \ldots, M-1 \) on the basis of biological evidence indicating that progenitor cells at different maturation stages are able to expand with little or no influx from the stem-cell compartment [52, 68]. Previous experimental and theoretical studies [27, 51, 62] have shown that stem cells have higher self-renewal fractions than the progenitor cells to which they give rise. This justifies assumption (6). Furthermore, cellular proliferation rates are bounded from above due to the time needed for genome replication. A reasonable upper bound is between 1 and 2 divisions per day [44], from which one can deduce assumptions (5).

3 Analysis of clonal selection

In this section, we prove a general asymptotic result (vide Subsection 3.1) that provides some fresh insights into the way in which the self-renewal fraction and the proliferation rate of cells at different maturation stages affect clonal selection in acute leukemias (vide Subsection 3.2).
3.1 A general asymptotic result

In line with previous studies on the long-time behaviour of the solutions to continuous phenotype-structured models [4, 10, 15, 16, 37, 48], we introduce a small parameter $\varepsilon > 0$ and we make the change of variable $\tau = \varepsilon t$. Under this scaling of the time line, considering the asymptotic regime $\varepsilon \to 0$ is equivalent to studying the dynamics of the model equations over many cell generations.

After having renamed the rescaled time variable $\tau$ to $t$, the Cauchy problem for the rescaled cell population densities $n_i \left( \frac{\tau}{\varepsilon}, x \right) = n_{i\varepsilon}(t, x)$ with $i = 1, \ldots, M$ reads as

\[
\begin{align*}
\varepsilon \frac{\partial}{\partial t} n_{i\varepsilon}(t, x) &= P_i(\rho_{M\varepsilon}(t), x) n_{i\varepsilon}(t, x), \\
\varepsilon \frac{\partial}{\partial t} n_{(i-1)\varepsilon}(t, x) &= Q_{i-1}(\rho_{M\varepsilon}(t), x) n_{(i-1)\varepsilon}(t, x) \\
&\quad + P_i(\rho_{M\varepsilon}(t), x) n_{i\varepsilon}(t, x), \quad i = 2, \ldots, M - 1, \\
\varepsilon \frac{\partial}{\partial t} n_{M\varepsilon}(t, x) &= Q_{M-1}(\rho_{M\varepsilon}(t), x) n_{M-1\varepsilon}(t, x) - d n_{M\varepsilon}(t, x), \\
n_{i\varepsilon}(t = 0, x) &= n^0_i(x), \quad i = 1, \ldots, M,
\end{align*}
\]  

(7)

with the initial data $n^0_i(x)$ satisfying the assumptions given by equation (3),

\[
\rho_{i\varepsilon}(t) = \int_0^1 n_{i\varepsilon}(t, x) \, dx \quad \text{for} \quad i = 1, \ldots, M,
\]

\[
P_i(\rho_{M\varepsilon}(t), x) = \left( \frac{2 a_i(x)}{1 + K \rho_{M\varepsilon}(t)} - 1 \right) p_i(x) \quad \text{for} \quad i = 1, \ldots, M - 1
\]

and

\[
Q_i(\rho_{M\varepsilon}(t), x) = 2 \left( 1 - \frac{a_i(x)}{1 + K \rho_{M\varepsilon}(t)} \right) p_i(x) \quad \text{for} \quad i = 1, \ldots, M - 1.
\]

Moreover, we introduce the following notation, which will be used in the remainder of this section:

\[
R_{i\varepsilon}(t, x) = \int_0^t P_i(\rho_{M\varepsilon}(s), x) \, ds \quad \text{for} \quad i = 1, \ldots, M - 1.
\]  

(8)

We let $t \in [0, T]$, where $T > 0$ is an arbitrary final time, and we make use of the following technical assumptions on the initial data $n^0_i(x)$ for all $i = 2, \ldots, M - 1$

\[
\|n^0_i\|_{L^1([0,1])} \geq \frac{2 \left( 1 - \|a_{i-1}\|_{L^\infty([0,1])} \right) \inf_{p_{i-1}} p_{i-1}}{\|p_i\|_{L^\infty([0,1])}} \|n^0_{i-1}\|_{L^1([0,1])},
\]  

(9)
and
\[
\|n_i^0\|_{L^1([0,1])} \leq 2 \left( \frac{p_{i-1}}{\inf p_i} \right) \|n_{i-1}^0\|_{L^1([0,1])} + \frac{\|a_i\|_{L^\infty([0,1])} \|p_i\|_{L^\infty([0,1])}}{B_i \inf p_i}
\]
with
\[
B_i = \begin{cases} 
K \frac{\inf p_{M-1}}{d} & (1 - \|a_{M-1}\|_{L^\infty([0,1])}) \\
\times \prod_{k=M-2}^{1} \frac{2}{\|p_{k+1}\|_{L^\infty([0,1])}} \inf p_k, & \text{for } i = 2, \ldots, M-2, \\
K \frac{\inf p_{M-1}}{d} & (1 - \|a_{M-1}\|_{L^\infty([0,1])}), & \text{for } i = M-1.
\end{cases}
\]

Furthermore, we assume
\[
\|n_M^0\|_{L^1([0,1])} \geq \frac{2 \inf p_{M-1}}{d} (1 - \|a_{M-1}\|_{L^\infty([0,1])}) \|n_{M-1}^0\|_{L^1([0,1])}
\]

and
\[
\|n_M^0\|_{L^1([0,1])} \leq \frac{2 \|p_{M-1}\|_{L^\infty([0,1])}}{d} \|n_{M-1}^0\|_{L^1([0,1])}.
\]

A general result on the asymptotic behaviour of the cell population density functions \(n_{i\varepsilon}(t, x)\) for \(\varepsilon \to 0\) (i.e., in the limit of many cell generations) is established by the following theorem:

**Theorem 1 (A general asymptotic result)** Under assumptions (4), (5), (9), (10), (12) and (13), the solutions to the Cauchy problem (7) are such that, upon extraction of subsequences,
\[
n_{i\varepsilon} \overset{\varepsilon \to 0}{\longrightarrow} n_i \text{ on } \mathbb{R}^+ - L^\infty((0, T), M^1([0,1])) \text{ for all } i = 1, \ldots, M
\]
and
\[
R_{i\varepsilon} \overset{\varepsilon \to 0}{\longrightarrow} R_i \text{ uniformly in } [0, T] \times [0,1] \text{ for all } i = 1, \ldots, M-1.
\]
Moreover, under the additional assumption (6), the limits \(R_i\) are such that
\[
\max_{x \in [0,1]} R_1(t, x) = 0 \text{ for any } t \in [0, T]
\]
and
\[
\max_{x \in [0,1]} R_i(t, x) < 0 \text{ for any } t \in [0, T], \text{ for all } i = 2, \ldots, M-1.
\]
Finally, the limits \(n_i\) are such that for a.e. \(t \in [0, T]\) and for all \(i = 1, \ldots, M\)
\[
\text{supp}(n_i(t, \cdot)) \neq \emptyset \text{ with } \text{supp}(n_i(t, \cdot)) \subseteq \arg \max_{x \in [0,1]} a_i(x).
\]
Remark 1 Under the additional assumptions
\[ n_i^0(x) \in C([0,1]) \quad \text{for all } i = 1, \ldots, M \]
and
\[ n_i^0(x) > 0 \quad \text{for any } x \in \arg \max_{x \in [0,1]} a_1(x), \quad \text{for all } i = 1, \ldots, M, \]
the result given by equation (18) becomes
\[ \text{supp}(n_i(t, \cdot)) = \arg \max_{x \in [0,1]} a_1(x) \quad \text{for a.e. } t \in [0,T], \quad \text{for all } i = 1, \ldots, M. \] (19)

Proof We divide the proof of Theorem 1 into five parts.

Part 1: Non-negativity of \( n_{i\varepsilon}(t, x) \) and continuity of \( \rho_{i\varepsilon}(t) \). For all \( \varepsilon > 0 \), standard arguments based on the Banach fixed point theorem allow one to prove that, in the framework of the assumptions under consideration, the Cauchy problem (7) admits a unique solution of non-negative components \( n_{i\varepsilon}(t, x) \) with \( \rho_{i\varepsilon}(t) \equiv \|n_{i\varepsilon}(t, \cdot)\|_{L^1([0,1])} \) continuous for all \( i = 1, \ldots, M \).

Part 2: Estimates for \( \rho_{i\varepsilon}(t) \). Throughout the proof of the theorem, we will make use of the following estimates (uniform in \( \varepsilon \)) for \( \rho_{i\varepsilon}(t) \). The related proofs can be found in Appendix B. For all \( t \in [0,T] \) and for any \( \varepsilon > 0 \):
\[
\rho_{1\varepsilon}(t) \leq \max \left\{ \|n_1^0\|_{L^1([0,1])}, \frac{2 \|a_1\|_{L^\infty([0,1])}\|p_1\|_{L^\infty([0,1])} - \inf p_1}{G \inf p_1} \right\} \quad (20)
\]
with
\[ G = 2K \frac{\inf p_{M-1}}{d} (1 - \|a_{M-1}\|_{L^\infty([0,1])}) \prod_{k=1}^{M-2} \frac{2 (1 - \|a_k\|_{L^\infty([0,1])}) \inf p_k}{\|p_{k+1}\|_{L^\infty([0,1])}}. \]
Moreover, for all \( t \in [0,T] \), for any \( \varepsilon > 0 \) and for all \( i = 2, \ldots, M - 1 \):
\[
\rho_{i\varepsilon}(t) \geq \rho_{1\varepsilon}(t) \prod_{k=2}^{i} \frac{2 (1 - \|a_{k-1}\|_{L^\infty([0,1])}) \inf p_{k-1}}{\|p_k\|_{L^\infty([0,1])}} \quad (21)
\]
and
\[
\rho_{i\varepsilon}(t) \leq \frac{\|a_i\|_{L^\infty([0,1])} \|p_i\|_{L^\infty([0,1])}}{B_i \inf p_i} + \frac{2 \|p_{i-1}\|_{L^\infty([0,1])}}{\inf p_{i}} F_i (\rho_{i\varepsilon}(t)) \quad (22)
\]
where the constant \( B_i \) is defined by equation (11) and \( F_i(\cdot) \) is a positive linear functional of \( \rho_{i\varepsilon}(t) \). Finally, for all \( t \in [0,T] \) and for any \( \varepsilon > 0 \):
\[
\rho_{M\varepsilon}(t) \geq \rho_{1\varepsilon}(t) \frac{2 \inf p_{M-1}}{d} (1 - \|a_{M-1}\|_{L^\infty([0,1])}) \prod_{k=1}^{M-2} \frac{2 (1 - \|a_k\|_{L^\infty([0,1])}) \inf p_k}{\|p_{k+1}\|_{L^\infty([0,1])}} \quad (23)
\]
and
\[
\rho_{M \varepsilon}(t) \leq \frac{2 \|p_{M-1}\|_{L^\infty([0,1])}}{d} \left[ \frac{\|a_{M-1}\|_{L^\infty([0,1])} \|p_{M-1}\|_{L^\infty([0,1])}}{B_{M-1} \inf p_{M-1}} \right] + \frac{2 \|p_{M-2}\|_{L^\infty([0,1])}}{\inf p_{M-1}} F_{M-1}(\rho_{1 \varepsilon}(t)),
\]
where the constant \(B_{M-1}\) is defined by equation (11) and \(F_{M-1}(\cdot)\) is a positive linear functional of \(\rho_{1 \varepsilon}(t)\).

The upper bounds given by equations (20), (22) and (24) guarantee that \(\rho_{i \varepsilon}(t)\) is uniformly bounded from above for any \(\varepsilon > 0\) and for all \(i = 1, \ldots, M\).

**Part 3: Proof of** (14). By virtue of the upper bounds given by equations (20), (22) and (24), the Banach-Alaoglu theorem allows one to conclude that, up to extraction,
\[
n_{i _{\varepsilon}} \underset{\varepsilon \to 0}{\longrightarrow} n_i \quad \text{on} \quad w^* - L^\infty ([0,T], M^1 ([0,1])) \quad \text{for all} \quad i = 1, \ldots, M.
\]

**Part 4: Proof of** (15). Under assumptions (4) and (5), the upper bound given by equation (24) allows us to conclude that there exists a subsequence of \(R_{i \varepsilon}\), that we denote again as \(R_{i \varepsilon}\), such that
\[
R_{i \varepsilon} \underset{\varepsilon \to 0}{\longrightarrow} R_i \quad \text{pointwise in} \quad [0,T] \times [0,1] \quad \text{for all} \quad i = 1, \ldots, M - 1
\]
where
\[
R_i(t, x) = \int_0^t P_i(p_M(s), x) \, ds = \int_0^t \left( \frac{2 a_i(x)}{1 + K p_M(s)} - 1 \right) p_i(x) \, ds.
\]
Furthermore, assumptions (4) and (5) together with the boundedness of \(\rho_{M \varepsilon}(t)\) imply that, for any \(\varepsilon > 0\), the function \(R_{i \varepsilon}\) and its first derivatives with respect to \(t\) and \(x\) are bounded in \(L^\infty ([0,T] \times [0,1])\). Therefore, \(R_{i \varepsilon}\) belongs to \(W^{1,\infty} ([0,T] \times [0,1])\). Using the fact that \(W^{1,\infty} ([0,T] \times [0,1])\) is compactly embedded in \(C([0,T] \times [0,1])\) we achieve the result established by equation (15).

**Part 5: Proof of** (16)-(18). We prove the results given by equations (16)-(18) in four steps.

**Part 5 – Step 1.** We prove that
\[
R_1(t, x) \leq 0 \quad \text{for all} \quad (t, x) \in (0,T) \times [0,1]
\]
and
\[
R_i(t, x) < 0 \quad \text{for all} \quad (t, x) \in (0,T) \times [0,1], \quad \text{for all} \quad i = 2, \ldots, M - 1.
\]
By contradiction, assume that there exists \((\hat{t}, \hat{x}) \in [0,T] \times (0,1)\) such that \(R_1(\hat{t}, \hat{x}) > 0\). The convergence results given by equation (15) imply that
$R_{1c}(t, x) \geq \sigma$ for some $\sigma > 0$, as long as $|t - \hat{t}| \leq \sigma$, $|x - \hat{x}| \leq \sigma$ and $\sigma \geq \varepsilon > 0$. Since $n_1^0(x) > 0$ for a.e. $x \in [0, 1]$ we can conclude that

$$
\int_0^1 n_{1c}(t, x) \, dx = \int_0^1 n_1^0(x) e^{\frac{R_{1c}(t, x)}{\varepsilon}} \, dx \geq C \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} n_1^0(x) \, dx \, \varepsilon \to 0 \to \infty
$$

for all $t \in [\hat{t} - \sigma, \hat{t} + \sigma]$. This contradicts the upper bound for $\mu_{1c}(t)$ given by equation (20) and thus concludes the proof of the result given by equation (27). Moreover, such a result on $R_1$ guarantees that

$$
\int_0^t p_1(\rho_M(s), x) \, ds = p_1(x) \int_0^t \left( \frac{2a_1(x)}{1 + K\rho_M(s)} - 1 \right) \, ds \leq 0
$$

for all $(t, x) \in (0, T) \times [0, 1]$ and, since $p_1(\cdot) > 0$ [cf. assumption (5)], the above inequality implies that

$$
\int_0^t \left( \frac{2a_1(x)}{1 + K\rho_M(s)} - 1 \right) \, ds \leq 0 \text{ for all } (t, x) \in (0, T) \times [0, 1].
$$

Since $a_1(\cdot) > a_i(\cdot)$ for all $i = 2, \ldots, M - 1$ [cf. assumption (6)], the latter inequality allows one to conclude that for all $i = 2, \ldots, M - 1$

$$
\int_0^t \left( \frac{2a_i(x)}{1 + K\rho_M(s)} - 1 \right) \, ds < 0 \text{ for all } (t, x) \in (0, T) \times [0, 1]
$$

and, therefore, using the fact that $p_i(\cdot) > 0$ [cf. assumption (5)] one obtains

$$
p_i(x) \int_0^t \left( \frac{2a_i(x)}{1 + K\rho_M(s)} - 1 \right) \, ds < 0 \text{ for all } (t, x) \in (0, T) \times [0, 1]
$$

for all $i = 2, \ldots, M - 1$. This concludes the proof of the results given by equation (28) and proves the results given by equation (17).

**Part 5 – Step 2.** We prove that

$$
\text{if } R_1(t, \cdot) < 0 \text{ on } [0, 1] \text{ then } n_i(t, \cdot) = 0 \text{ a.e. on } [0, 1] \quad (29)
$$

for all $i = 1, \ldots, M$.

**Proof that if $R_1(t, \cdot) < 0 \text{ on } [0, 1] \text{ then } n_i(t, \cdot) = 0 \text{ a.e. on } [0, 1].** Let $(\hat{t}, \hat{x}) \in [0, T] \times (0, 1)$ be such that $R_1(t, \hat{x}) < 0$. The uniform convergence results given by equation (15) guarantee that there exists some $\sigma > 0$ such that $R_{1c}(t, x) \leq -\sigma$ for $|t - \hat{t}| \leq \sigma$, $|x - \hat{x}| \leq \sigma$ and $\sigma \geq \varepsilon > 0$. This allows us to conclude that

$$
\lim_{\varepsilon \to 0} \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} \int_{\hat{x} - \sigma}^{\hat{x} + \sigma} n_{1c}(t, x) \, dx \, dt = \lim_{\varepsilon \to 0} \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} \int_{\hat{x} - \sigma}^{\hat{x} + \sigma} n_1^0(x) e^{\frac{R_{1c}(t, x)}{\varepsilon}} \, dx \, dt
\leq 2\sigma \lim_{\varepsilon \to 0} e^{-\frac{C}{\varepsilon}} \int_{\hat{x} - \sigma}^{\hat{x} + \sigma} n_1^0(x) \, dx,
$$
that is,
\[
\lim_{\varepsilon \to 0} \int_{t-\varepsilon}^{\hat{t} + \sigma} \int_{\hat{x} - \sigma}^{\hat{x} + \sigma} n_{1\varepsilon}(t, x) \, dx \, dt = 0. \tag{30}
\]

The weak convergence result for \( n_{1\varepsilon}(t, x) \) given by equation (14) and proven in Part 3 guarantees that
\[
\int_{t-\varepsilon}^{\hat{t} + \sigma} \int_{0}^{1} \varphi(x) n_{1}(t, x) \, dx \, dt = \lim_{\varepsilon \to 0} \int_{t-\varepsilon}^{\hat{t} + \sigma} \int_{0}^{1} \varphi(x) n_{1\varepsilon}(t, x) \, dx \, dt \tag{31}
\]
for every smooth test function \( \varphi : [0, 1] \to \mathbb{R} \). Therefore, choosing a test function \( \varphi \) that satisfies the following conditions
\[
1_{[\hat{t} - \sigma, \hat{t} + \sigma]} \leq \varphi \leq 1_{[\hat{t} - \sigma, \hat{t} + \sigma]} \tag{32}
\]
and using the fact that the function \( n_{1\varepsilon}(t, x) \) is non-negative we find
\[
\int_{t-\varepsilon}^{\hat{t} + \sigma} \int_{0}^{1} \varphi(x) n_{1}(t, x) \, dx \, dt \leq \lim_{\varepsilon \to 0} \int_{t-\varepsilon}^{\hat{t} + \sigma} \int_{0}^{1} \varphi(x) n_{1\varepsilon}(t, x) \, dx \, dt \tag{33}
\]
Substituting the result given by equation (30) into the latter integral inequality we can conclude that
\[
\int_{t-\varepsilon}^{\hat{t} + \sigma} \int_{0}^{1} \varphi(x) n_{1}(t, x) \, dx \, dt = 0
\]
for every smooth test function that satisfies conditions (32). Hence, the result for \( n_{1} \) given by equation (29) is verified.

**Proof that if** \( R_{1}(t, \cdot) < 0 \) \( \text{on} \ [0, 1] \) \( \text{then} \ n_{2}(t, \cdot) = 0 \ a.e. \ [0, 1] \). Multiplying both sides of equation (7) for \( n_{2\varepsilon}(t, x) \) by a smooth test function \( \varphi : [0, 1] \to \mathbb{R} \) and integrating over the set \( [t - \sigma, t + \sigma] \times [0, 1] \) we find
\[
\varepsilon \left[ \int_{0}^{1} \varphi(x) n_{2\varepsilon}(t + \sigma, x) \, dx - \int_{0}^{1} \varphi(x) n_{2\varepsilon}(t - \sigma, x) \, dx \right] = \\
= \int_{t-\varepsilon}^{\hat{t} + \sigma} \int_{0}^{1} Q_{1}(\rho_{M\varepsilon}(t), x) \varphi(x) n_{1\varepsilon}(t, x) \, dx \, dt \\
+ \int_{t-\varepsilon}^{\hat{t} + \sigma} \int_{0}^{1} P_{2}(\rho_{M\varepsilon}(t), x) \varphi(x) n_{2\varepsilon}(t, x) \, dx \, dt.
\]
Since the uniform upper bound given by equation (22) guarantees that
\[
\lim_{\varepsilon \to 0} \varepsilon \left[ \int_{0}^{1} \varphi(x) n_{2\varepsilon}(t + \sigma, x) \, dx - \int_{0}^{1} \varphi(x) n_{2\varepsilon}(t - \sigma, x) \, dx \right] = 0,
\]
we can conclude that
\[
\lim_{\varepsilon \to 0} \int_{t-\sigma}^{t+\sigma} \int_0^1 Q_1(\rho_M \varepsilon(t), x) \varphi(x) n_{1c}(t, x) \, dx \, dt \\
+ \lim_{\varepsilon \to 0} \int_{t-\sigma}^{t+\sigma} \int_0^1 P_2(\rho_M \varepsilon(t), x) \varphi(x) n_{2c}(t, x) \, dx \, dt = 0,
\]
for every smooth test function \( \varphi \). Hence, choosing a test function that satisfies conditions (32) and using the result given by equation (30) together with the fact that \( Q_1(\rho_M \varepsilon(t), x) > 0 \) on \([0, T] \times [0, 1]\) we achieve
\[
\lim_{\varepsilon \to 0} \int_{t-\sigma}^{t+\sigma} \int_{x-\sigma}^{x+\sigma} P_2(\rho_M \varepsilon(t), x) n_{2c}(t, x) \, dx \, dt = 0. \tag{34}
\]
Since \( P_2(\rho_M \varepsilon(t), \cdot) < 0 \) for a.e. \( t \in (0, T) \) [vid. the result on the function \( R_2 \) given by equation (28)], equation (34) allows us to conclude that
\[
\lim_{\varepsilon \to 0} \int_{t-\sigma}^{t+\sigma} \int_{x-\sigma}^{x+\sigma} n_{2c}(t, x) \, dx \, dt = 0. \tag{35}
\]
The weak convergence result given by equation (14) for \( n_2c(t, x) \) guarantees that
\[
\int_{t-\sigma}^{t+\sigma} \int_0^1 \varphi(x) n_{2c}(t, x) \, dx \, dt = \lim_{\varepsilon \to 0} \int_{t-\sigma}^{t+\sigma} \int_0^1 \varphi(x) n_{2c}(t, x) \, dx \, dt \tag{36}
\]
for every smooth test function \( \varphi : [0, 1] \to \mathbb{R} \). Therefore, choosing a test function \( \varphi \) that satisfies conditions (32) and using the fact that the function \( n_{2c}(t, x) \) is non-negative we find
\[
\int_{t-\sigma}^{t+\sigma} \int_0^1 \varphi(x) n_{2c}(t, x) \, dx \, dt \leq \lim_{\varepsilon \to 0} \int_{t-\sigma}^{t+\sigma} \int_{x-\sigma}^{x+\sigma} n_{2c}(t, x) \, dx \, dt. \tag{37}
\]
Substituting the result given by equation (35) into the latter integral inequality we can conclude that
\[
\int_{t-\sigma}^{t+\sigma} \int_0^1 \varphi(x) n_{2c}(t, x) \, dx \, dt = 0
\]
for every smooth test function that satisfies conditions (32). This implies that the result for \( n_2 \) given by equation (29) holds.

\textbf{Proof that if} \( R_1(t, \cdot) < 0 \) \textbf{on} \([0, 1]\) \textbf{then} \( n_i(t, \cdot) = 0 \) \textbf{a.e. on} \([0, 1]\) \textbf{for all} \( i = 3, \ldots, M - 1 \). The fact that
\[
\lim_{\varepsilon \to 0} \int_{t-\sigma}^{t+\sigma} \int_{x-\sigma}^{x+\sigma} n_{ic}(t, x) \, dx \, dt = 0 \quad \text{for} \quad i = 3, \ldots, M - 1, \tag{38}
\]
and the results for \( n_i \) with \( i = 3, \ldots, M - 1 \) given by equation (29) can be proved through a bootstrap argument from \( i = 3 \) to \( i = M - 1 \) using the same method of proof that we have used for the case \( i = 2 \).

**Proof that if** \( R_1(t, \cdot) < 0 \) **on** \([0, 1] \) **then** \( n_M(t, \cdot) = 0 \) **a.e. on** \([0, 1] \). **Multiplying both sides of equation (7) for** \( n_{M\varepsilon}(t, x) \) **by a smooth test function** \( \varphi : [0, 1] \rightarrow \mathbb{R} \) **and integrating over the set** \([\hat{t} - \sigma, \hat{t} + \sigma] \times [0, 1] \) **we obtain**

\[
\begin{align*}
\varepsilon \left[ \int_0^1 \varphi(x) n_{M\varepsilon}(\hat{t} + \sigma, x) \, dx - \int_0^1 \varphi(x) n_{M\varepsilon}(\hat{t} - \sigma, x) \, dx \right] \\
= \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} \int_0^1 Q_{M-1}(\rho_{M\varepsilon}(t), x) \varphi(x) n_{M-1\varepsilon}(t, x) \, dx \, dt \\
- \frac{d}{dt} \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} \int_0^1 \varphi(x) n_{M\varepsilon}(t, x) \, dx \, dt.
\end{align*}
\]

Since the uniform upper bound given by equation (24) guarantees that

\[
\lim_{\varepsilon \to 0} \varepsilon \left[ \int_0^1 \varphi(x) n_{M\varepsilon}(\hat{t} + \sigma, x) \, dx - \int_0^1 \varphi(x) n_{M\varepsilon}(\hat{t} - \sigma, x) \, dx \right] = 0,
\]

we can conclude that

\[
\lim_{\varepsilon \to 0} \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} \int_0^1 Q_{M-1}(\rho_{M\varepsilon}(t), x) \varphi(x) n_{M-1\varepsilon}(t, x) \, dx \, dt \\
- \frac{d}{dt} \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} \int_0^1 \varphi(x) n_{M\varepsilon}(t, x) \, dx \, dt = 0,
\]

for every smooth test function \( \varphi \). Hence, choosing a test function that satisfies conditions (32) and using the result given by equation (38) for \( n_{M-1\varepsilon}(t, x) \) together with the fact that \( Q_{M-1}(\rho_{M\varepsilon}(t), x) > 0 \) on \([0, T] \times [0, 1] \) we achieve

\[
\lim_{\varepsilon \to 0} \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} n_{M\varepsilon}(t, x) \, dx \, dt = 0. \tag{39}
\]

The weak convergence result (14) for \( n_{M\varepsilon}(t, x) \) guarantees that

\[
\int_{\hat{t} - \sigma}^{\hat{t} + \sigma} \int_0^1 \varphi(x) n_M(t, x) \, dx \, dt = \lim_{\varepsilon \to 0} \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} \int_0^1 \varphi(x) n_{M\varepsilon}(t, x) \, dx \, dt \tag{40}
\]

for every smooth test function \( \varphi : [0, 1] \rightarrow \mathbb{R} \). Therefore, choosing a test function \( \varphi \) that satisfies conditions (32) and using the fact that the function \( n_{M\varepsilon}(t, x) \) is non-negative we find

\[
\int_{\hat{t} - \sigma}^{\hat{t} + \sigma} \int_0^1 \varphi(x) n_M(t, x) \, dx \, dt \leq \lim_{\varepsilon \to 0} \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} \int_{\hat{t} - \sigma}^{\hat{t} + \sigma} n_{M\varepsilon}(t, x) \, dx \, dt. \tag{41}
\]
Substituting the result given by equation (39) into the latter integral inequality we can conclude that
\[
\int_{\hat{t}-\sigma}^{\hat{t}+\sigma} \int_{0}^{1} \varphi(x)n_{M}(t,x) \, dx \, dt = 0
\]
for every smooth test function that satisfies conditions (32). This implies that the result for \( n_{M} \) given by equation (29) holds.

**Part 5 – Step 3.** We prove that
\[
\max_{x \in [0,1]} R_{1}(t,x) = 0 \quad \text{for any } t \in [0,T], \tag{42}
\]
\[
\rho_{1}(t) \equiv \|n_{1}(t,\cdot)\|_{L^{1}([0,1])} > 0 \quad \text{a.e. on } [0,T], \tag{43}
\]
and
\[
\arg \max_{x \in [0,1]} R_{1}(t,x) = \arg \max_{x \in [0,1]} a_{1}(x) \quad \text{for any } t \in [0,T]. \tag{44}
\]

We begin by noting that, since \( p_{1}(\cdot) > 0 \) [vid. assumption (5)], if
\[
\max_{x \in [0,1]} R_{1}(t,x) = 0
\]
for some \( t \in [0,T] \) then [vid. the definition of the function \( R_{1} \) given by equation (26)]
\[
\max_{x \in [0,1]} \int_{0}^{t} \left( \frac{2a_{1}(x)}{1 + K \rho_{M}(s)} - 1 \right) \, ds = 0
\]
and, therefore,
\[
\arg \max_{x \in [0,1]} R_{1}(t,x) = \arg \max_{x \in [0,1]} a_{1}(x). \tag{45}
\]
Hence, if the result given by equation (42) holds true then the result given by equation (44) is verified.

To prove the results given by equations (42) and (43) we proceed as follows. Assume by contradiction that there exist \( \hat{t} \in [0,T] \) and \( \sigma > 0 \) with \( \hat{t} + \sigma \leq T \) such that
\[
\max_{x \in [0,1]} R_{1}(t,x) = 0 \quad \forall t \in [0,\hat{t}] \quad \text{and} \quad \rho_{1}(t) > 0 \quad \text{a.e. on } [0,\hat{t}] \tag{46}
\]
whereas
\[
\max_{x \in [0,1]} R_{1}(t,x) < 0 \quad \forall t \in (\hat{t}, \hat{t} + \sigma). \tag{47}
\]
Under assumptions (46) and (47), the result on \( n_{M}(t,x) \) given by equation (29) allows one to conclude that
\[
\rho_{M}(t) = 0 \quad \text{for a.e. } t \in (\hat{t}, \hat{t} + \sigma). \tag{48}
\]
We take $\hat{x} \in \arg\max_{x \in [0,1]} R_1(\hat{t}, x)$. Using the result given by equation (48) we find that under assumptions (46) and (47)

$$R_1(\hat{t} + \sigma, \hat{x}) = \int_{t}^{\hat{t} + \sigma} P_1(\rho_M(t), \hat{x}) \, dt = R_1(\hat{t}, \hat{x}) + \int_{t}^{\hat{t} + \sigma} P_1(0, \hat{x}) \, dt,$$

that is,

$$R_1(\hat{t} + \sigma, \hat{x}) = \int_{t}^{\hat{t} + \sigma} p_1(\hat{x}) \left[ 2a_1(\hat{x}) - 1 \right] \, dt.$$

Due to assumption (4) this yields $R_1(\hat{t} + \sigma, \hat{x}) > 0$, which contradicts the result on $R_1$ given by equation (27). Hence,

$$\max_{x \in [0,1]} R_1(t, x) = 0 \text{ for any } t \in (\hat{t}, \hat{t} + \sigma) \text{ and } \rho_1(t) > 0 \text{ a.e. on } (\hat{t}, \hat{t} + \sigma).$$

Therefore, the results given by equations (42) and (43) are verified, i.e. we can conclude that both the result given by equation (16) and the result on $n_1$ given by equation (18) hold.

**Part 5 – Step 4.** Since $\text{supp}(n_1(t, \cdot)) \neq \emptyset$, the uniform lower bounds for $\rho_\varepsilon(t)$ with $i = 2, \ldots, M - 1$ and $\rho_{M\varepsilon}(t)$ given by equations (21) and (23) allow one to conclude that $\rho_\varepsilon(t)$ does not vanish in the limit $\varepsilon \to 0$ for all $i = 2, \ldots, M$, that is,

$$\text{supp}(n_i(t, \cdot)) \neq \emptyset \text{ for all } i = 2, \ldots, M.$$

Based on the calculations carried out in Part 5 – Step 2, one can conclude that

$$\text{supp}(n_i(t, \cdot)) \subseteq \arg\max_{x \in [0,1]} R_1(t, x) \text{ for a.e. } t \in [0, T] \text{ and all } i = 2, \ldots, M.$$

Moreover, since $\max_{x \in [0,1]} R_1(t, x) = 0$ for any $t \in [0, T]$ and, therefore,

$$\arg\max_{x \in [0,1]} R_1(t, x) = \arg\max_{x \in [0,1]} a_1(x),$$

we have

$$\text{supp}(n_i(t, \cdot)) \subseteq \arg\max_{x \in [0,1]} a_1(x) \text{ for a.e. } t \in [0, T] \text{ and all } i = 2, \ldots, M.$$

Hence, the limits $n_i$ for $i = 2, \ldots, M$ are such that the results given by equation (18) hold.

The general asymptotic result established by Theorem 1 put on a rigorous mathematical basis the idea that clonal selection is driven by the self-renewal fraction of leukemic stem cells, as exemplified by Corollaries 2-4 given in the next subsection.
3.2 Biological implications of Theorem 1

Building upon the ideas presented in previous studies [57, 62, 63], we focus here on the case where there are $M = 3$ possible maturation stages, that is, stem cells ($i = 1$), progenitor cells ($i = 2$) and mature cells/leukemic blasts ($i = 3$).

In this case, Corollary 2 of Theorem 1 given below shows that if

$$\arg \max_{x \in [0, 1]} a_1(x) = \{ \bar{x} \}$$

then in the limit $\varepsilon \to 0$ the population density functions of stem cells $n_{1\varepsilon}(t, x)$, progenitor cells $n_{2\varepsilon}(t, x)$ and mature cells/leukemic blasts $n_{3\varepsilon}(t, x)$ become concentrated as Dirac masses centred at the point $\bar{x}$. Analogously, Corollary 3 of Theorem 1 given below shows that if

$$\arg \max_{x \in [0, 1]} a_1(x) = \{ \bar{x}_1, \ldots, \bar{x}_N \},$$

then when $\varepsilon \to 0$ the cell population density functions $n_{1\varepsilon}(t, x)$, $n_{2\varepsilon}(t, x)$ and $n_{3\varepsilon}(t, x)$ become concentrated as weighted sums of Dirac masses centred at the points $\{ \bar{x}_1, \ldots, \bar{x}_N \}$. In both cases, the centres of the Dirac masses do not depend on the functions $p_1(\cdot)$, $a_2(\cdot)$ and $p_2(\cdot)$.

From a biological point of view, the centres of the Dirac masses can be understood as the leukemic clones that are selected for in the limit of many cell generations. Therefore, the asymptotic results of Corollary 2 and Corollary 3 formalise the idea that clonal selection is controlled by the self-renewal fraction of leukemic stem cells, as the leukemic clone(s) with the highest stem cell self-renewal fraction are ultimately selected.

The results of Corollary 2 and Corollary 3 are complemented by Corollary 4 of Theorem 1 given below, which shows that if the function $a_1(x)$ is constant, i.e. if

$$a_1(x) = A_1 \in \mathbb{R}_+ \text{ with } \frac{1}{2} < a_2(x) < A_1 < 1 \text{ for all } x \in [0, 1],$$

then in the asymptotic regime $\varepsilon \to 0$ the cell population density functions $n_{1\varepsilon}(t, x)$, $n_{2\varepsilon}(t, x)$ and $n_{3\varepsilon}(t, x)$ do not become concentrated as Dirac masses. Biologically, this indicates that if the stem cell self-renewal fraction is the same for all leukemic clones, then clonal selection will not occur and no specific clones will be selected.

**Corollary 2 (Selection of one single clone)** Let the assumptions of Theorem 1 and the additional assumption given by equation (49) hold. Moreover, let $M = 3$ and assume

$$n_{1,2,3}^0(x) \in C([0, 1]) \text{ with } n_{1,2,3}^0(x) > 0 \text{ for all } x \in [0, 1].$$

Then, the measures $n_1$, $n_2$ and $n_3$ given by Theorem 1 are such that

$$n_i(t, x) = \rho_i(t) \delta(x - \bar{x}) \text{ for a.e. } t \in [0, T] \text{ with } i = 1, 2, 3.$$
Proof For $M = 3$, under the additional assumptions (49) and (52), the result given by equation (53) is a straightforward consequence of the general asymptotic result given by equation (18).

Corollary 3 (Selection of multiple clones) Let the assumptions of Theorem 1 and the additional assumption given by equation (50) hold. Moreover, let $M = 3$ and assume

$$n^0_{1,2,3}(x) \in C([0,1]) \quad \text{with} \quad n^0_{1,2,3}(x) > 0 \quad \text{for all} \quad x \in [0,1].$$

Then, the measures $n_1$, $n_2$ and $n_3$ given by Theorem 1 are such that

$$n_i(t,x) = \sum_{j=1}^{N} \rho_j(t) \delta(x - \pi_j) \quad \text{for a.e.} \quad t \in [0,T] \quad \text{with} \quad i = 1,2,3.$$  (55)

Proof For $M = 3$, under the additional assumptions (50) and (54), the result given by equation (55) is a straightforward consequence of the general asymptotic result given by equation (18).

Corollary 4 (Absence of clonal selection) Let the assumptions of Theorem 1 and the additional assumption given by equation (51) hold. Moreover, let $M = 3$ and assume

$$n^0_{1,2,3}(x) \in C([0,1]) \quad \text{with} \quad n^0_{1,2,3}(x) > 0 \quad \text{for all} \quad x \in [0,1].$$

Then, the measures $n_1$, $n_2$ and $n_3$ given by Theorem 1 are such that

$${\text{supp}}(n_i(t,\cdot)) = [0,1] \quad \text{for a.e.} \quad t \in [0,T] \quad \text{with} \quad i = 1,2,3.$$  (57)

Proof For $M = 3$, under the additional assumptions (51) and (56), the result given by equation (57) is a straightforward consequence of the general asymptotic result given by equation (18).

4 Numerical solutions

In this section, we integrate the asymptotic results established by Corollaries 2 - 4 of Theorem 1 with numerical solutions of the Cauchy problem defined by the IDE system (2) for $M = 3$ complemented with biologically relevant initial conditions (see Subsection 4.2). We parametrise the model equations based on patient data from the existing literature (see Subsection 4.1).
4.1 Setup of numerical simulations and model calibration

We approximate the IDE system for the population density functions $n_1(t,x)$, $n_2(t,x)$ and $n_3(t,x)$ using the forward Euler method with step size $10^{-4}$. We choose a uniform discretisation of the interval $[0, 1]$ that consists of 1000 points. We assume $t \in [0, 10^4]$. Under the parameter settings considered here, such an interval corresponds to a time frame of approximately 30 years after the appearance of the first leukemic clones. This is biologically reasonable since acute leukemia has a pronounced peak of incidence in late adulthood.

Numerical computations are performed in MATLAB for two main parameter settings: one under which the total cell density functions $\rho_1(t)$, $\rho_2(t)$ and $\rho_3(t)$ converge to some stable values (Model calibration 1 described in Subsection 4.1.1), and the other such that the total cell density functions undergo periodic oscillations (Model calibration 2 described in Subsection 4.1.2).

4.1.1 Model calibration 1

To model an initial scenario in which, due to clonal heterogeneity, all possible leukemic clones are present at small numbers in every maturation compartment, and the total densities of healthy cells are close to cell counts at physiological equilibrium, we use the following initial data

$$n_i^0(x) = N_i \exp \left(-\frac{x^2}{0.2}\right) \quad \text{for } i = 1, 2, 3,$$

with $N_1 \approx 2.5 \times 10^7$, $N_2 \approx 3.8 \times 10^9$ and $N_3 \approx 10^8$. (58)

Such initial conditions satisfy the assumptions of Theorem 1, as well as the additional assumptions (52), (54) and (56).

Multicompartment models of hematopoiesis after bone marrow transplantation allow one to estimate the proliferation rates and the self-renewal fractions of stem and progenitor healthy cells, as well as the degradation rate of the feedback signal by mature cells and leukemic blasts [57,59]. In particular, in agreement with the estimations performed in [59], we assume that

$$a_1(0) = 0.85, \quad a_2(0) = 0.84, \quad p_1(0) = 0.1/\text{day}, \quad p_2(0) = 0.4/\text{day}$$

and

$$K = 1.75 \times 10^{-9} \text{kg/cell.}$$

(60)

(61)

Clearance rates of mature cells and leukemic blasts can be estimated based on patient data and they appear to be between $0.1/\text{day}$ and $2.3/\text{day}$ [9,40,53]. For this reason, we set

$$d = 2/\text{day.}$$

(62)

In agreement with the estimations performed in [57,59], which are based on clinical data of blast dynamics in relapsing patients, we impose the following conditions

$$0.85 \leq a_1(x) \leq 0.99 \quad \text{and} \quad 0.1/\text{day} \leq p_1(x) < 1/\text{day} \quad \text{for all } x \in (0, 1].$$

(63)
The values of the function \( a_2(x) \) are restricted by assumption (6). Moreover, since it is well accepted that stem cells divide at lower rates compared to progenitor cells \([1, 12, 54]\), we impose the following additional conditions

\[
0 < p_1(x) \leq p_2(x) < 1 \quad \text{for all } x \in [0, 1]. \tag{64}
\]

We let the continuous structuring variable \( x \) parametrise cell proliferation rate and we make use of the following definitions

\[
p_1(x) = \alpha_1 + \beta_1 x \quad \text{and} \quad p_2(x) = \alpha_2 + \beta_2 x. \tag{65}
\]

In order to fulfill conditions (60), we set

\[
\alpha_1 = 0.1/\text{day} \quad \text{and} \quad \alpha_2 = 0.4/\text{day}. \tag{66}
\]

Moreover, we choose

\[
\beta_1 = 0.2/\text{day} \quad \text{and} \quad \beta_2 = 0.5/\text{day} \tag{67}
\]

so that the conditions given by equation (63) and equation (64) are satisfied. To take into account the complex relationship between proliferation and self-renewal observed in leukemic cells \([18, 23, 30, 33, 65, 69]\), we assume the correspondence between the self-renewal fraction and the cell proliferation rate to be non-bijective and – amongst all possible definitions which satisfy the conditions given by equations (6), (60) and (63) – we choose

\[
a_2(x) = 0.5 \exp \left[ - \frac{(x - 0.4)^2}{8.82} \right] + 0.349 \tag{68}
\]

and either

\[
a_1(x) = \exp \left[ - \frac{(x - 0.6)^2}{9.68} \right] - 0.1135 \tag{69}
\]

or

\[
a_1(x) = a_1^0 + 0.1 \sum_{j=1}^{4} \exp \left[ - \frac{(x - \bar{x}_j)^2}{0.0025} \right] \tag{70}
\]

with

\[
\bar{x}_j \in \{0.35, 0.55, 0.7, 0.85\} \quad \text{and} \quad a_1^0 = 0.85 - 0.1 \sum_{j=1}^{4} \exp \left[ - \frac{\bar{x}_j^2}{0.0025} \right], \tag{71}
\]

or

\[
a_1(x) \equiv 0.88. \tag{72}
\]

The definition given by equation (68) model a biological scenario where the leukemic clone identified by \( x = 0.4 \) has the highest self-renewal fraction amongst progenitor cells. Moreover, the definitions given by equation (69), equations (70) and (71), or equation (72) model, respectively, three distinct situations whereby, due to differential gene expression at different maturation stages:
- the leukemic clone identified by \( x = 0.6 \) has the highest self-renewal fraction amongst stem cells;

- the leukemic clones corresponding to \( x = 0.35, x = 0.55, x = 0.7 \) and \( x = 0.85 \) have the highest self-renewal fraction amongst stem cells;

- all leukemic clones in the stem-cell compartment have the same self-renewal fraction.

4.1.2 Model calibration 2

On the basis of considerations analogous to those presented in Subsection 4.1.1, we use the initial data given by equation (58) with

\[
N_1 \approx 4.37 \times 10^6, \quad N_2 \approx 5 \times 10^8, \quad N_3 \approx 4.28 \times 10^8
\]

and we set

\[
K = 1.75 \times 10^{-9} \text{kg/cell}, \quad d = 0.15 \text{/day.} \tag{74}
\]

Moreover, we make use of the definitions for the functions \( p_1(x) \) and \( p_2(x) \) given by equation (65) with

\[
\alpha_1 = 0.975 \text{/day}, \quad \alpha_2 = 0.04 \text{/day} \tag{75}
\]

and

\[
\beta_1 = 0.025 \text{/day}, \quad \beta_2 = 0.0333 \text{/day}. \tag{76}
\]

Also, we assume

\[
a_1(x) = \frac{0.7}{0.8865} \left\{ \exp \left[ -\frac{(x - 0.6)^2}{9.68} \right] - 0.1135 \right\} \tag{77}
\]

and

\[
a_2(x) = \frac{0.6}{0.8467} \left\{ 0.5 \exp \left[ -\frac{(x - 0.4)^2}{8.82} \right] + 0.349 \right\}, \tag{78}
\]

\( i.e. \) we consider a biological scenario whereby the leukemic clones identified by \( x = 0.6 \) and \( x = 0.4 \) have the highest self-renewal fraction amongst stem cells and progenitor cells, respectively. The parameters and functions given by equations (75)-(78) are such that the values of the functions \( p_1(x), p_2(x), a_1(x) \) and \( a_2(x) \) at the point \( x = 0.6 \) \( [i.e. \] the maximum point of the function \( a_1(x) \)] coincide with the parameter values for which the solutions of the ODE system (79) with \( M = 3 \) are known to undergo periodic oscillations [31].
Fig. 2: Selection of one single clone. Dynamics of the normalised cell population density functions $n_1(t, x)/\rho_1(t)$ (left panel), $n_2(t, x)/\rho_2(t)$ (central panel) and $n_3(t, x)/\rho_3(t)$ (right panel) under the parameter setting given in Subsection 4.1.1 with $a_1(x)$ defined by equation (69). The black lines indicate the clone with the maximum self-renewal fraction in the stem-cell compartment [i.e. the maximum point of the function $a_1(x)$], while the white lines indicate the clone with the maximum self-renewal fraction in the progenitor-cell compartment [i.e. the maximum point of the function $a_2(x)$]. The transient behaviour of the solutions from the initial conditions is highlighted by the plots in the insets, which show the dynamics of the normalised population density functions for $t \in [0, 500]$. The colour scale ranges from blue (low density) to yellow (high density).

4.2 Main results

In agreement with the asymptotic results established by Corollary 2 and Corollary 3 of Theorem 1, the numerical solutions presented in Fig. 2 and Fig. 3 show that, under the parameter setting given in Subsection 4.1.1, the population density functions of stem cells $n_1(t, x)$, progenitor cells $n_2(t, x)$ and mature cells/leukemic blasts $n_3(t, x)$ become progressively concentrated at the maximum point(s) of the function $a_1(x)$. Also, the plots in the insets of Fig. 2 and Fig. 3 highlight the existence of leukemic clones which grow transiently before becoming ultimately extinct. Moreover, the numerical results displayed in Fig. 4 illustrate how, in agreement with the asymptotic results of Corollary 4 of Theorem 1, if the function $a_1(x)$ is constant, the long-term limits of the population density functions $n_1(t, x)$, $n_2(t, x)$ and $n_3(t, x)$ are not concentrated at any particular point.

As mentioned earlier, these results communicate the biological notion that the self-renewal fraction of leukemic stem cells determines the faith of clonal selection. In fact, the leukemic clones characterised by the highest stem cell self-renewal fraction are selected, regardless of their properties in terms of stem cell proliferation rate, progenitor cell proliferation rate and progenitor cell self-renewal fraction. This supports the idea that interclonal variability in
the self-renewal fraction of leukemic stem cells provides the necessary substrate for clonal selection to act on.

Under the parameter setting given in Subsection 4.1.1, the total density of stem cells $\rho_1(t)$, progenitor cells $\rho_2(t)$ and mature cells/leukemic blasts $\rho_3(t)$ converge to some stable values (vid. Fig. 5). However, it is known that for given parameter choices the solutions of the ODE system (79) with $M = 3$ undergo periodic oscillations, which result from the occurrence of Hopf bifurcation [31].

The numerical results presented in Fig. 7 show that, under the parameter setting given in Subsection 4.1.2, periodic oscillations emerge in the integrals of the solutions to the IDE system (2) with $M = 3$ (i.e. in the total cell densities $\rho_1(t)$, $\rho_2(t)$ and $\rho_3(t)$). In analogy with the previous cases, the numerical solutions presented in Fig. 6 indicate that the cell population density functions $n_1(t, x)$, $n_2(t, x)$ and $n_3(t, x)$ become progressively concentrated at the maximum point of the function $a_1(x)$ – i.e. the leukemic clone characterised by the highest stem cell self-renewal fraction is ultimately selected.
Fig. 4: **Absence of clonal selection.** Dynamics of the normalised cell population density functions $n_1(t, x)/\rho_1(t)$ (left panel), $n_2(t, x)/\rho_2(t)$ (central panel) and $n_3(t, x)/\rho_3(t)$ (right panel) under the parameter setting given in Subsection 4.1.1 with $a_1(x)$ defined by equation (72). The white lines indicate the clone with the maximum self-renewal fraction in the progenitor-cell compartment [i.e. the maximum point of the function $a_2(x)$]. The colour scale ranges from blue (low density) to yellow (high density).

Fig. 5: **Dynamics of the total cell densities.** Left panel. Dynamics of the total density of stem cells $\rho_1(t)$ (blue line), progenitor cells $\rho_2(t)$ (red line) and mature cells/leukemic blasts $\rho_3(t)$ (red line) under the parameter setting given in Subsection 4.1.1 with $a_1(x)$ defined by equation (69) (left panel), equations (70) and (71) (central panel) and equation (72) (right panel). Values are in units of $10^7$.

5 Discussion and conclusions

Recent progress in genetic techniques has shed light on the complex coevolution of malignant cell clones in leukemias [2, 5, 17, 35]. However, several aspects of clonal selection still remain unclear. In this work, we have adopted a mathematical modelling approach to study clonal selection in acute leukemias, with the aim of supporting a better understanding of the biological mechanisms which underpin observable clonal dynamics.
Fig. 6: Clonal selection when the total cell densities undergo periodic oscillations. Dynamics of the normalised cell population density functions $n_1(t,x)/\rho_1(t)$ (left panel), $n_2(t,x)/\rho_2(t)$ (central panel) and $n_3(t,x)/\rho_3(t)$ (right panel) under the parameter setting given in Subsection 4.1.2. The black lines indicate the clone with the maximum self-renewal fraction in the stem-cell compartment [i.e. the maximum point of the function $a_1(x)$], while the white lines indicate the clone with the maximum self-renewal fraction in the progenitor-cell compartment [i.e. the maximum point of the function $a_2(x)$]. The colour scale ranges from blue (low density) to yellow (high density).

Fig. 7: Emergence of periodic oscillations in the total cell densities. Dynamics of the total density of stem cells $\rho_1(t)$ (blue line), progenitor cells $\rho_2(t)$ (red line) and mature cells/leukemic blasts $\rho_3(t)$ (red line) under the parameter setting given in Subsection 4.1.2. Values are in units of $10^7$.

Our model consists of a system of coupled IDEs that describe the dynamics of cells at different maturation stages – from stem cells to increasingly mature progenitor cells to mature-cells/blasts – seen as distinct compartments. Each maturation compartment is structured by a continuous variable that identifies the clone of the cells. In order to incorporate into our model the high degree of interclonal heterogeneity which is observed in leukemia patients, we let the cellular proliferation rate and self-renewal fraction (i.e. the fraction of progeny cells adopting the same fate as their parent cell) in each maturation compartment be functions of the structuring variable.
In the framework of this model, we have established a number of analytical results which give answers to the open questions Q1–Q3 posed in the introduction of this paper. In summary:

A1 Clonal selection is driven by the self-renewal fraction of leukemic stem cells. Theorem 1 rigorously shows that all leukemic clones with non-maximal stem cell self-renewal fraction ultimately become extinct, independently of their proliferation rates.

A2 Non-stem cell properties do not have a substantial impact on clonal selection. The result established by Corollary 4 formalises the idea that, in a scenario where the stem cells of all clones have the same self-renewal fraction, one should expect the stable coexistence of all clones to occur.

A3 Only the clones whose stem cells are endowed with the highest self-renewal fraction can stably coexist in the presence of interclonal heterogeneity. Corollaries 2 and 3 put on a rigorous basis the notion that the leukemic clones whose stem cells have the highest self-renewal fraction will ultimately be selected.

We have integrated our asymptotic results with numerical solutions of a calibrated version of the model based on real patient data. In agreement with the theoretical results of Stiehl et al. [56], our numerical results reveal the existence of leukemic clones which display transient growth before becoming extinct. Such an emergent behaviour was not captured by the IDE model considered by Busse et al. [7], who studied the case where cells of different clones at the same maturation stage share the same proliferation rate. This suggests that interclonal heterogeneity in the cell proliferation rate may have an impact on transient clonal dynamics.

It has been shown by means of genetic techniques that, in most leukemia patients, the majority of leukemic cells is derived from a relatively small number of clones [2,17]. Our results indicate that this may be due to the fact that only a few leukemic clones are characterised by a high stem cell self-renewal fraction.

There is accumulating experimental evidence that many of the different gene mutations involved in the development of leukemia increase the self-renewal of cells. Possible examples include the TIM-3/Gal-9 autocrine stimulation [30] or alterations of Wnt/β-Catenin signalling [65]. Moreover, it has been shown that genetic mutations can affect self-renewal and proliferation of cells simultaneously due to crosstalk between different signalling pathways. For instance, the NUP98-Ddx10 oncogene increases both cell proliferation and self-renewal [69]; hyperactivation of the mTOR pathway leads to S6K1-mediated increase in self-renewal and reduction in proliferation [23]; up-regulation of PLZF brings about increased self-renewal and reduced proliferation [18]. The outcome of our analysis, which ascribes a pivotal role to increased self-renewal in orchestrating clonal selection, is in line with the observation that all of the aforementioned genetic alterations lead to increased self-renewal, whereas they can have divergent effects on cell proliferation.
The result that clonal selection is not influenced by progenitor cell properties is biologically meaningful, since it implies that mutations which affect only progenitor cell properties without altering stem cell properties cannot lead to the selection of leukemic clones. This result is new and not self-evident, as progenitor cells can expand independently of the influx from the stem cell compartment – although they possess smaller self-renewal fractions than stem cells [52,59,68]. This insight is also clinically relevant. In fact, it is known that progenitor cells are more sensitive to treatment interventions than stem cells which are located in protective niches. However, our results suggest that manipulations of progenitor cells have no impact on clonal selection phenomena.

A rigorous mathematical understanding of clonal selection is needed since the potentially nonlinear interplay of different genetic and epigenetic hits [3, 21, 25] leads to complex fitness landscapes and non-trivial interdependencies between self-renewal fraction and proliferation rate of leukemic cells. In particular, there is evidence that combinations of leukemic mutations occur frequently in patients [46,49]. An \textit{in silico} approach can help to understand how the different detected mutations modify the stem cell properties. In this regard, our modelling approach can be further developed in several directions.

For instance, in line with what it was done for the ODE counterpart of the model presented here [56,61], we plan to extend our model to take into account the effect of multiple feedback mechanisms and incorporate the occurrence of \textit{de novo} mutations. Moreover, along the lines of the modelling method proposed by Doumic \textit{et al.} [19], it may be interesting to replace the discrete maturation structure considered in this work by a continuous age structure, which would lead to the definition of a fully-continuously structured population model of clonal selection in acute leukemias.

From a mathematical point of view, we plan to carry out a systematic investigation of the conditions for the occurrence of Hopf bifurcation leading to the emergence of periodic oscillations in the total cell densities, as shown by the numerical solutions presented in this paper.

**A Multicompartmental ODE model**

In a number of previous papers [56,57,60,61,63], it was shown that mathematical models defined in the framework of the following ODE system can effectively recapitulate clinical data from leukemia patients:

\[
\begin{align*}
\frac{d}{dt} N_1^j(t) &= 2 \left( 1 - \frac{a^j_1}{1 + K Z_M(t)} \right) p^j_1 N_1^j(t) - d N_1^j(t), \\
\frac{d}{dt} N_i^j(t) &= 2 \left( 1 - \frac{a^j_{i-1}}{1 + K Z_M(t)} \right) p^j_{i-1} N_{i-1}^j(t) \\
&\quad + \frac{2 a^j_i}{1 + K Z_M(t)} p^j_i N_i^j(t), \\
\frac{d}{dt} N_M^j(t) &= 2 \left( 1 - \frac{a^j_{M-1}}{1 + K Z_M(t)} \right) p^j_{M-1} N_{M-1}^j(t) - d N_M^j(t).
\end{align*}
\]
with \( i = 2, \ldots, M - 1 \), \( j = 0, \ldots, J \) and
\[
Z_M(t) = \sum_{j=0}^{J} N_M^j(t).
\]

The index \( i \) denotes the cell maturation stage while the index \( j \) indicates to which leukemic clone the cells belong. In particular, the stem-cell compartment is labelled by the index \( i = 1 \), the indexes \( i = 2, \ldots, M - 1 \) correspond to increasingly mature progenitor-cell compartments and the mature-cell/blast compartment is labelled by the index \( i = M \). Moreover, the index \( j = 0 \) refers to healthy cells whereas the different leukemic clones are labelled by the indexes \( j = 1, \ldots, J \).

In the system of equations (79), the function \( N_M^j(t) \) stands the density of cells of clone \( j \) at the maturation stage \( i \) which are present at the time instant \( t \geq 0 \). Cells in the compartment \( i = M \) do not divide and are cleared from the system at rate \( d > 0 \), which is assumed to be the same for healthy and leukemic cells [7, 40, 53]. On the other hand, the parameters \( p_i^j > 0 \) and \( a_i^j > 0 \), respectively, the proliferation rate and the self-renewal fraction of cells of clone \( j \) at the maturation stage \( i \). In agreement with biological findings presented in [32, 34, 55], the terms \( a_i^j \) are multiplied by the factor \( 1 + K Z_M(t) \) to model the fact that the signal which promotes the self-renewal of dividing cells is absorbed by mature cells and leukemic blasts at a rate that depends on their total density \( Z_M(t) \). The parameter \( K > 0 \) depends on the degradation rate of the feedback signal by mature cells and leukemic blasts. This has been shown to be a biologically consistent way of incorporating into the model the effects of feedback signals which control cell self-renewal [42, 59, 62, 63]. In principle, the effects of feedback signals which control cell proliferation could also be included. However, it has been demonstrated that such signals have only a small impact on the dynamics of the blood system [42, 58, 59].

A version of the ODE model (79) with only one leukemic clone and three maturation stages (i.e. for \( M = 3 \) and \( J = 1 \)) has been fully analysed in [63], while a two compartmental version of the model for healthy hematopoiesis (i.e. for \( M = 2 \) and \( J = 0 \)) has been studied in [22, 45, 62]. Possible applications of this model to clinical data can be found in [56, 57], whereas applications to healthy hematopoiesis are provided in [58, 59]. Finally, a version of this model with a continuous differentiation structure has been proposed and studied in [19].

**B Proofs of the estimates given by equations (20)–(24)**

In this appendix, we give full details of the proof of the estimates for \( \rho_M(t) \) given by equations (20)–(24). In particular, in sections B.1–B.7 we prove some preliminary results that are used to prove such bounds in sections B.4–B.8.

**B.1 Upper and lower bounds for \( \rho_M(t) \) in terms of \( \rho_{M-1}(t) \)**

Integrating equation (7) for \( n_M(t, x) \) over \([0, 1]\) and estimating the RHS from above using assumptions (4) and (5) we obtain
\[
\varepsilon \frac{d}{dt} \rho_M(t) \leq 2 \|p_{M-1}\|_{L^\infty([0, 1])} \rho_{M-1}(t) - d \rho_M(t).
\]

In a similar way, estimating the RHS from below we find
\[
\varepsilon \frac{d}{dt} \rho_M(t) \geq 2 \inf_{p_{M-1}} \{ 1 - \|a_{M-1}\|_{L^\infty([0, 1])} \} \rho_{M-1}(t) - d \rho_M(t).
\]

Under assumptions (12) and (13), these differential inequalities allow us to conclude that for all \( t \in [0, T] \) and for any \( \varepsilon > 0 \)
\[
\rho_M(t) \geq \frac{2 \inf_{p_{M-1}} \{ 1 - \|a_{M-1}\|_{L^\infty([0, 1])} \}}{d} \rho_{M-1}(t) \geq 0
\]
(80)
and
\[ \rho_{M\varepsilon}(t) \leq \frac{2\|p_{M-1}\|_{L^\infty([0,1])}}{d} \rho_{M-1\varepsilon}(t). \quad (81) \]

B.2 Lower bounds for \( \rho_{i\varepsilon}(t) \) in terms of \( \rho_{i-1\varepsilon}(t) \) with \( i = 2, \ldots, M-1 \)

Integrating equation (7) for \( n_{i\varepsilon}(t,x) \) with \( i = 2, \ldots, M-1 \) over \([0,1]\) and estimating the RHS from below using assumptions (4) and (5) yields
\[
\varepsilon \frac{d}{dt} \rho_{i\varepsilon}(t) \geq 2 \left( 1 - \|a_{i-1}\|_{L^\infty([0,1])} \right) \inf_{p_{i-1}} \rho_{i-1\varepsilon}(t) - \|p_i\|_{L^\infty([0,1])} \rho_{i\varepsilon}(t).
\]

Under assumptions (9), the latter differential inequality guarantees that for all \( t \in [0,T] \) and for any \( \varepsilon > 0 \) we have
\[
\rho_{i\varepsilon}(t) \geq \frac{2 \left( 1 - \|a_{i-1}\|_{L^\infty([0,1])} \right) \inf_{p_{i-1}} \rho_{i-1\varepsilon}(t)}{\|p_i\|_{L^\infty([0,1])}} \geq 0, \quad i = 2, \ldots, M-1. \quad (82)
\]

B.3 Lower bounds for \( \rho_{M\varepsilon}(t) \) in terms of \( \rho_{i\varepsilon}(t) \) with \( i = 2, \ldots, M-2 \)

Under assumptions (9) and (12), we can substitute the lower bounds for \( \rho_{i\varepsilon}(t) \) given by equation (82) with \( i = 2, \ldots, M-2 \) into the lower bound for \( \rho_{M\varepsilon}(t) \) given by equation (80). In so doing, using a bootstrap argument, we find that for any \( t \in [0,T] \) and for any \( \varepsilon > 0 \)
\[
\rho_{M\varepsilon}(t) \geq \rho_{i\varepsilon}(t) \prod_{k=2}^{i} \frac{2 \left( 1 - \|a_{k-1}\|_{L^\infty([0,1])} \right) \inf_{p_{k-1}} \rho_{k-1\varepsilon}(t)}{\|p_k\|_{L^\infty([0,1])}} \geq 0, \quad i = 2, \ldots, M-2. \quad (83)
\]

B.4 Lower bounds for \( \rho_{i\varepsilon}(t) \) with \( i = 2, \ldots, M-1 \) in terms of \( \rho_{i\varepsilon}(t) \)

Under assumption (9), a bootstrap argument based on the lower bounds for \( \rho_{i\varepsilon}(t) \) given by equation (82) allows one to conclude that for all \( t \in [0,T] \) and for any \( \varepsilon > 0 \)
\[
\rho_{i\varepsilon}(t) \geq \rho_{i\varepsilon}(t) \prod_{k=2}^{i} \frac{2 \left( 1 - \|a_{k-1}\|_{L^\infty([0,1])} \right) \inf_{p_{k-1}} \rho_{k-1\varepsilon}(t)}{\|p_k\|_{L^\infty([0,1])}}, \quad i = 2, \ldots, M-1. \quad (84)
\]

B.5 Lower bound for \( \rho_{M\varepsilon}(t) \) in terms of \( \rho_{i\varepsilon}(t) \)

Under assumptions (9) and (12), the lower bound for \( \rho_{M\varepsilon}(t) \) given by equation (83) with \( i = 1 \) implies that for all \( t \in [0,T] \) and for any \( \varepsilon > 0 \) we have
\[
\rho_{M\varepsilon}(t) \geq \rho_{1\varepsilon}(t) \frac{2 \inf_{p_{M-1}} \left( 1 - \|a_{M-1}\|_{L^\infty([0,1])} \right) \prod_{k=2}^{M-2} \frac{2 \left( 1 - \|a_{k-1}\|_{L^\infty([0,1])} \right) \inf_{p_{k-1}} \rho_{k-1\varepsilon}(t)}{\|p_k\|_{L^\infty([0,1])}}}{d}. \quad (85)
\]
B.6 Upper bound for $\rho_{1e}(t)$

Under assumptions (9) and (12), integrating equation (7) for $n_{1e}(t,x)$ over $[0,1]$ and estimating the RHS from above using assumptions (4) and (5) together with the lower bounds for $\rho_{eM}(t)$ given by equation (23) we achieve

$$\varepsilon \frac{d}{dt} \rho_{1e}(t) \leq \left[ \frac{2 |a_1| L^\infty(0,1) \|p_1\| L^\infty(0,1)}{1 + G \rho_{1e}(t)} - \inf p_1 \right] \rho_{1e}(t),$$

with

$$G = 2 K \inf p_{M-1} \frac{1 - \|a_{M-1}\| L^\infty(0,1)}{d} \prod_{h=1}^{M-2} \frac{1 - \|a_{h}\| L^\infty(0,1)}{\|p_{h+1}\| L^\infty(0,1)} \inf p_h. \quad (86)$$

The latter differential inequality implies that for all $t \in [0,T]$ and for any $\varepsilon > 0$ we have

$$\rho_{1e}(t) \leq \max \left\{ \|n_1\| L^1(0,1), \frac{2 |a_1| L^\infty(0,1) \|p_1\| L^\infty(0,1)}{G \inf p_1} - \inf p_1 \right\}, \quad (87)$$

with $G$ being given by equation (86).

B.7 Upper bounds for $\rho_{ie}(t)$ in terms of $\rho_{i-1e}(t)$ with $i = 2, \ldots, M - 1$

Integrating equation (7) for $n_{ie}(t,x)$ over $[0,1]$ and estimating the RHS from above using assumptions (4) and (5) together with the lower bounds for $\rho_{eM}(t)$ given by equation (83) if $i = 2, \ldots, M - 2$ or equation (80) if $i = M - 1$ we find

$$\varepsilon \frac{d}{dt} \rho_{ie}(t) \leq 2 \|p_{i-1}\| L^\infty(0,1) \rho_{i-1e}(t) + 2 |a_i| L^\infty(0,1) \|p_i\| L^\infty(0,1) \frac{\rho_{ie}(t)}{1 + 2 B_i \rho_{ie}(t)} - \inf p_i \rho_{ie}(t),$$

with $B_i$ defined by equation (11). From the latter differential inequality, noting that

$$\frac{\rho_{ie}(t)}{1 + 2 B_i \rho_{ie}(t)} \leq \frac{1}{2 B_i},$$

we obtain

$$\varepsilon \frac{d}{dt} \rho_{ie}(t) \leq 2 \|p_{i-1}\| L^\infty(0,1) \rho_{i-1e}(t) + \frac{|a_i| L^\infty(0,1) \|p_i\| L^\infty(0,1)}{B_i \inf p_i} - \inf p_i \rho_{ie}(t).$$

Under assumptions (10), such a differential inequality allows one to conclude that for all $t \in [0,T]$ and for any $\varepsilon > 0$

$$\rho_{ie}(t) \leq \frac{2 \|p_{i-1}\| L^\infty(0,1) \rho_{i-1e}(t)}{\inf p_i} + \frac{|a_i| L^\infty(0,1) \|p_i\| L^\infty(0,1)}{B_i \inf p_i}, \quad i = 2, \ldots, M - 1, \quad (88)$$

where the constant $B_i$ is defined by equation (11).

B.8 Upper bounds for $\rho_{ie}(t)$ with $i = 2, \ldots, M - 1$ in terms of $\rho_{1e}(t)$

Under assumptions (10), a bootstrap argument based on the upper bound for $\rho_{ie}(t)$ given by equation (88) allows one to conclude that for all $t \in [0,T]$ and for any $\varepsilon > 0$

$$\rho_{ie}(t) \leq \frac{|a_i| L^\infty(0,1) \|p_i\| L^\infty(0,1)}{B_i \inf p_i} + \frac{2 \|p_{i-1}\| L^\infty(0,1)}{\inf p_i} F_i(\rho_{1e}(t)), \quad i = 2, \ldots, M - 1. \quad (89)$$

In equation (22), the constant $B_i$ is defined by equation (11) and $F_i(\cdot)$ is a positive linear functional of $\rho_{ie}(t)$. 
B.9 Upper bound for $\rho_{M^\varepsilon}(t)$ in terms of $\rho_{1^\varepsilon}(t)$

Under assumptions (10) and (13), we can substitute the upper bound for $\rho_{M^\varepsilon}(t)$ given by equation (88) into the upper bound for $\rho_{M^\varepsilon}(t)$ given by equation (81) and find that $\rho_{M^\varepsilon}(t)$ satisfies the following estimate for all $t \in [0, T]$ and for any $\varepsilon > 0$

$$\rho_{M^\varepsilon}(t) \leq \frac{2 \|p_{M-1}\|_{L^\infty([0,1])}}{d} \left( \frac{\|A_{M-1}\|_{L^\infty([0,1])} \|p_{M-1}\|_{L^\infty([0,1])}}{B_{M-1} \inf_{p_{M-1}}} + \frac{2 \|p_{M-2}\|_{L^\infty([0,1])}}{\inf_{p_{M-1}}} F_{M-1}(\rho_{1^\varepsilon}(t)) \right),$$

(90)

where the constant $B_{M-1}$ is defined by equation (11) and $F_{M-1}(\cdot)$ is a positive linear functional of $\rho_{1^\varepsilon}(t)$.

Acknowledgements TS and AM-C were supported by research funding from the German Research Foundation DFG (SFB 873; subproject B08). TL gratefully acknowledges support from Heidelberg Graduate School (HGS).

References


