A Personalized Framework for Dynamic Modeling of Disease Trajectories in Chronic Lymphocytic Leukemia

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Abstract—Chronic Lymphocytic Leukemia (CLL) is the most common peripheral blood and bone marrow cancer in the developed world. This manuscript proposes mathematical model equations representing the disease dynamics of B-cell CLL. We interconnect delay differential cell cycle models in each of the tumor-involved disease centers using physiologically-relevant cell migration. We further introduce 5 hypothetical case studies representing CLL heterogeneity commonly seen in clinical practice and demonstrate how the proposed CLL model framework may capture disease pathophysiology across patient types. We conclude by exploring the capacity of the proposed temporally- and spatially-distributed model to capture the heterogeneity of CLL disease progression. By using Global Sensitivity Analysis, the critical parameters influencing disease trajectory over space and time are: (i) the initial number of CLL cells in peripheral blood, the number of involved lymph nodes, the presence and degree of splenomegaly; (ii) the migratory fraction of non-proliferating as well as proliferating CLL cells from bone marrow into blood and of proliferating CLL cells from blood into lymph nodes; (iii) the parameters inducing non-proliferative cells to proliferate. The proposed model offers a practical platform which may be explored in future personalized patient protocols once validated.

1. INTRODUCTION

Chronic Lymphocytic Leukemia (CLL) is the most common peripheral blood and bone marrow cancer in the developed world, usually afflicting older individuals [1]. It is a heterogeneous disease consisting of abnormal proliferation and reduced apoptosis in mutated clones, most commonly of mature B-cells, resulting in accumulation in 4 compartments: the bone marrow, peripheral blood, lymph nodes and spleen. Initial chromosomal mutations differ between patients and malignant transformations

progress variably. Subclonal mutations may arise and overtake the dominant clone because of other stressors, such as chemotherapy used to treat the original disease [2]. The site of primary CLL pathogenesis is the lymph nodes and/or bone marrow with cells then circulating in peripheral blood and homing preferentially towards bone marrow, lymph nodes, or spleen depending on expression of chemokine receptors or intracellular proteins [3]–[5]; this heterogeneity results in variable presentations of lymphocytosis, bone marrow failure, lymphadenopathy and splenomegaly [6, 7] with the clinical stages of disease defined using the Binet [8] or Rai [9] systems and WHO [10] criteria for diagnosis (Table 1). The disease typically progresses slowly with most CLL cells arrested in the resting (G0) or growth (G1) phases of the cell cycle [11]. Since CLL has defined mutations predictive of prognosis and a chronic trajectory often spanning decades, we hypothesize that mathematical models may characterize CLL disease dynamics with the potential to act as a framework for personalized treatment strategies.

Diverging from previous mathematical models for B-cell CLL (hereafter referred to as CLL), this manuscript presents the first personalized, physiologically-relevant mathematical framework modeling CLL with features that (1) consider each of the most relevant disease centers including those of bone marrow, peripheral blood, lymph nodes and spleen, (2) interconnect the affected tissues via descriptors for cell migration, (3) calculate migration rates between affected tissues and (4) provide a framework to predict CLL progression over the disease course, in the absence of treatment. This work belongs to an ongoing effort developing building blocks for modeling and optimizing biomedical systems [12] such as that for Acute Myeloid Leukemia [13, 14], Type 1 Diabetes Mellitus [15] and for the delivery of anesthesia [16, 17]. We previously performed a more limited global sensi-

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TABLE 1: Clinical Staging and Corresponding PB Lymphocyte Doubling Time (LDT) [8]–[10, 19, 20]

Symptoms	CLL Clinical Stage		LDT [months ± SEM]
	Rai	Binet	
Lymphocytosis ($> 5 \cdot 10^9$ cells/L)	0		
Lymphocytosis and nodal involvement	I	A (< 3 areas of lymph nodes)	69.6 ± 20.5
Lymphocytosis and organomegaly	II	B (Lymphadenopathy and splenomegaly)	10.8 ± 2.1
Anemia (Hgb < 11 g/dL)	III	C	10.8 ± 4.4
Lymphocytosis and thrombocytopenia (PLT < 10^{11} /L)	IV		

WHO classification: Lymphocytosis $\geq 5 \times 10^9$ cells/L for ≥ 3 mon with:
Immunophenotype: SIG, CIG $^{-/+}$, CD5, CD23, CD43, CD19, CD20, CD79a;
Genetics: IgVH, trisomy 12, [del(13q14.1)], [del(11q)], [del(6q)], [del(17p)]
 SEM $\equiv$ Standard Error Mean

TABLE 2: Expected lymphocyte count[†] [21, 22]

Body Part	Number of lymphocytes	Distri- bution	% cell type		
			B	T	NK
Blood (PB)	10×10^9	2.2%	25	70	5
Lymph nodes (LN)	190×10^9	41.3%	20	70	10
Spleen (S)	70×10^9	15.2%	50	40	10
Gut Lymphoid Tissue	20×10^9	4.3%			
Thymus	50×10^9	10.9%	< 1	> 95	
Bone Marrow (BM)	50×10^9	10.9%	50	50	
Other tissue	70×10^9	15.2%			

[†] Assuming a 70 kg human with 460×10^9 total lymphocytes

Fig. 1: CLL modeling framework incorporates the major disease compartments

tivity analysis than that described in Online Supplement Appendix F [18]; otherwise the manuscript has all new material.

2. MATHEMATICAL MODEL

The model (Fig. 1) consists of 3 cell cycle models and the interconnectivity between them; we assume that CLL cells are distributed in bone marrow (BM), peripheral

Fig. 2: Normalized feedback function (Eq 2) in semi-logarithmic plot ($n = 3$) [23]

blood (PB), and lymph nodes (LN) and spleen (S) at the point of diagnosis and that S is aggregated with LN.

A. Chronic Lymphocytic Leukemia (CLL)

Our model (Fig. 1) is a general framework which captures the following dynamics: as CLL cells proliferate in BM (as captured at diagnosis – the pre-diagnosis disease-initiating mutation(s) may occur in either LN or S and then populate BM), the cells may either migrate to PB and then LN or stay in BM. It includes 3 cell cycles and migration rates for both proliferative (P) and non-proliferative (N) CLL cells from BM to PB (BM2PB) and then to LN (PB2LN); this model applies to naïve stage and Binet stages A – C (SN, SA, SB, SC). Eqs (1) – (12) represent the specific case where: (a) the Mackey [23] G0 model (Appendix A) is correct and (b) we have data regarding lymphocyte counts and proliferation in affected tissues (Tables 1 & 2). While we limit our analysis to a proliferating/non-proliferating model [23], an equivalent development could be constructed for a detailed $G1 \rightarrow S \rightarrow G2/M$ cell cycle model, e.g., [24].

Before defining the 3 interconnected G0 models in Eqs (7) – (12), we define a time delay (Eq 1), proliferation feedback function (Eq 2 & 3), and the relationship between Ki-67 expression, i.e., percent proliferation, and cell numbers for each disease center $i \in \{BM, PB, LN\}$ and disease stage $z \in \{SN, SA, SB, SC\}$.

$$N_{i,\tau_{i,z}} = N_i(t - \tau_{i,z}) \quad (1)$$

$$\beta_i(N_i) = \beta_{N_i,0} \cdot \theta_i^{n_i} / (\theta_i^{n_i} + N_i^{n_i}) \quad (2)$$

$$\theta_i = \theta_{i,1,z}(t - t_{in,z}) + \theta_{i,2,z} \quad (3)$$

$$\text{Ki-67}_i = P_i / (P_i + N_i) \quad (4)$$

In the Eq (2) feedback function, $\beta_{N_i,0}$ is the maximum recruitment in each tissue [25]. Fig. 2 illustrates Eq (2) for $n = 3$ and demonstrates that control parameter θ_i modulates recruitment from non-proliferative (N_i) and proliferative (P_i) phases. The θ_i value is larger in CLL proliferation centers, i.e., BM, LN and S, than in the accumulation compartment (PB) where the expression of proliferative marker Ki-67 is lowest [26].

The BM disease center has restricted volume ($BM_{\max} \approx 13.5 \times 10^{11}$ lymphocytes; Appendix B). Assuming that the normal lymphocyte count in BM remains constant, carrying capacity, cc , represents restricted volume [27], a_{BM} is the percentage of normal lymphocytes in BM (see Appendix B), and $C_{NC, BM}$ is the number of lymphocytes in normal BM (Table 2):

$$\%BM_{\text{lymph}} = \frac{C_{NC, BM} + N_{BM} + P_{BM}}{\frac{1}{a_{BM}} C_{NC, BM} + P_{BM} + N_{BM}} \quad (5)$$

$$cc(N_{BM}, P_{BM}) = 1 - \frac{C_{NC, BM} + N_{BM} + P_{BM}}{BM_{\max}} \quad (6)$$

BM Eqs (7 – 8) hold $\forall z \in \{SN, SA, SB, SC\}$:

$$\begin{aligned} \frac{dN_{BM}}{dt} = & -\delta_{BM, z} N_{BM} \\ & - \beta_{BM}(N_{BM}) N_{BM} cc(N_{BM}, P_{BM}) \\ & + 2 \beta_{BM}(N_{BM}, \tau_{BM, z}) N_{BM, \tau_{BM, z}} \\ & e^{-\gamma_{BM, z} \tau_{BM, z}} cc(N_{BM}, P_{BM}) \\ & - k_{N, BM2PB, z} N_{BM} \end{aligned} \quad (7)$$

$$\begin{aligned} \frac{dP_{BM}}{dt} = & -\gamma_{BM, z} P_{BM} \\ & + \beta_{BM}(N_{BM}) N_{BM} cc(N_{BM}, P_{BM}) \\ & - \beta_{BM}(N_{BM}, \tau_{BM, z}) N_{BM, \tau_{BM, z}} \\ & e^{-\gamma_{BM, z} \tau_{BM, z}} cc(N_{BM}, P_{BM}) \\ & - k_{P, BM2PB, z} P_{BM} \end{aligned} \quad (8)$$

PB Eqs (9) & (10) hold $\forall z \in \{SN, SA, SB, SC\}$:

$$\begin{aligned} \frac{dN_{PB}}{dt} = & -\delta_{PB, z} N_{PB} - \beta_{PB}(N_{PB}) N_{PB} \\ & + 2 \beta_{PB}(N_{PB}, \tau_{PB, z}) N_{PB, \tau_{PB, z}} \\ & e^{-\gamma_{PB, z} \tau_{PB, z}} \\ & + k_{N, BM2PB, z} N_{BM} - k_{N, PB2LN, z} N_{PB} \end{aligned} \quad (9)$$

$$\begin{aligned} \frac{dP_{PB}}{dt} = & -\gamma_{PB, z} P_{PB} + \beta_{PB}(N_{PB}) N_{PB} \\ & - \beta_{PB}(N_{PB}, \tau_{PB, z}) N_{PB, \tau_{PB, z}} \\ & e^{-\gamma_{PB, z} \tau_{PB, z}} \\ & + k_{P, BM2PB, z} P_{BM} - k_{P, PB2LN, z} P_{PB} \end{aligned} \quad (10)$$

LN Eqs (11) & (12) hold $\forall z \in \{SN, SA, SB, SC\}$:

$$\begin{aligned} \frac{dN_{LN}}{dt} = & -\delta_{LN, z} N_{LN} - \beta_{LN}(N_{LN}) N_{LN} \\ & + 2 \beta_{LN}(N_{LN}, \tau_{LN, z}) N_{LN, \tau_{LN, z}} \\ & e^{-\gamma_{LN, z} \tau_{LN, z}} + k_{N, PB2LN, z} N_{PB} \end{aligned} \quad (11)$$

$$\begin{aligned} \frac{dP_{LN}}{dt} = & -\gamma_{LN, z} P_{LN} + \beta_{LN}(N_{LN}) N_{LN} \\ & - \beta_{LN}(N_{LN}, \tau_{LN, z}) N_{LN, \tau_{LN, z}} \\ & e^{-\gamma_{LN, z} \tau_{LN, z}} + k_{P, PB2LN, z} P_{PB} \end{aligned} \quad (12)$$

B. Algorithm for Determining Cell Cycle Parameters

The model is an index-1 system with 6 differential and 23 algebraic equations, 76 parameters (22 assumed,

TABLE 3: Data sources for determining input parameters

Parameter	Experimental Method	Source
Experiments in Common Clinical Practice		
$d_{LN, CLL}$	Ultrasound; CT scan	[7]
d_S, CLL	CT scan	[7]
l_S, CLL	CT scan	[7]
$V_{CLL, LN}$	Ultrasound	Est.
$V_{CLL, S}$	CT scan or from enlarged dimensions	[7]
$N_{ULN, d_{LN, CLL}}$	CT scan	[7]
Ki-67	Flow cytometry	[26]
%BM	Bone marrow biopsy	[28]
Other Possible Experiments		
τ	Response to PHA	[29]
δ, γ	<i>In vivo</i> : Labelled 2H_2O ; <i>Ex vivo</i> : Trypan blue, PI staining and Noxa expression	[30, 31] [32, 33]
Calculating Initial Conditions		
N_{BM}, P_{BM}	%Ki-67 _{BM} and %BM in Eq 5	
N_{PB}, P_{PB}	%Ki-67 _{PB} and blood tests	
N_{LN}, P_{LN}	%Ki-67 _{LN} and LN / S measurements by CT scan	

38 calculated, 10 measured, 6 initial conditions), 6 state variables and 33 variables. Table 7 defines the symbols. Table 1 outlines PB lymphocyte doubling time (LDT) and clinical staging [19]. The 3 parameter types are:

- (a) *Assumed* parameters from average human physiology or mathematical analysis – these parameters would be typically clinically unavailable,
- (b) *Measured* parameters may be directly determined – Table 3 proposes experimental methods and Table 7 lists typical values. Note that some measurements, e.g., from PB, may be taken more frequently than from others, such as BM or LN,
- (c) *Calculated* parameters that are deduced indirectly – this section proposes a relevant algorithm.

Fig. 3 is an example of patient data trajectory within a disease stage. Transition time, or CLL stage duration $\Delta t_z = t_{fin, z} - t_{in, z}$, is estimated by treating physicians based on changes in PB LDT, increased lymphadenopathy or splenomegaly and low levels of hemoglobin or platelets. Appendices D – E describe an algorithm we developed in GAMS and MATLAB which finds values of $\theta_{i, 1, z}$, $\theta_{i, 2, z}$, $\delta_{i, z}$, $\gamma_{i, z}$ and $k_{N, BM2PB, z}$, $k_{P, BM2PB, z}$, $k_{N, PB2LN, z}$, $k_{P, PB2LN, z}$, $\forall i \in \{BM, PB, LN\}$ and $\forall z \in \{SN, SA, SB, SC\}$; this algorithm takes the measurable parameters and deduces reasonable values for the calculated parameters. We seek *reasonable parameters* rather than *guaranteed parameter estimates* of the best possible values; our purpose is to capture disease dynamics. We define *reasonable parameters* as satisfying the Table 1 disease characteristics and the Table 7 *Source* column; further model building could augment these restrictions.

C. Global Sensitivity Analysis

We apply random sampling - high dimensional model representation (RS-HDMR; Appendix F) global sensitivity analysis [35, 36] to the nominal parameter values. The minimum and maximum values of parameters included in Table 4 are used as inputs to the RS-HDMR model analysis (Table F.1 in Appendix F). The RS-HDMR

TABLE 4: Cell cycle calculated and assumed parameters for the 5 hypothetical patients (P1 – P5)

			P1		P2		P3		P4		P5	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Transitions			Naïve	A	A	B	A	B	B	C	A	C
Calculated	$\gamma_{BM}^{\dagger}$	1/day	0.774	0.774	0.823	0.091	0.780	0.720	0.252	0.810	0.655	0.094
Parameters	$\gamma_{LN}^{\dagger}$	1/day	0.861	0.861	0.861	0.860	0.841	0.866	0.586	0.629	0.530	0.847
	γ_{PB}	1/day	0.001	0.001	0.001	0.003	0.001	0.006	0.001	0.001	0.001	0.006
	δ_{BM}	1/day	0.001	0.001	0.001	0.034	0.001	0.001	0.001	0.001	0.001	0.037
	$\delta_{LN}^{\dagger}$	1/day	0.001	0.001	0.001	0.001	0.001	0.001	0.020	0.020	0.020	0.001
	δ_{PB}	1/day	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001
	τ_{BM}	day	0.792		0.792		0.792		0.625		0.792	
	τ_{LN}	day	0.792		0.792		0.792		0.792		0.792	
	τ_{PB}	day	0.792		0.625		0.625		0.625		0.625	
	$\theta_{BM,1}^{\dagger}$	$\cdot 10^8$ cells	0.008	0.029	0.057	0.481	0.278	0.693	0.558	3.145	0.004	6.209
	$\theta_{BM,2}$	$\cdot 10^8$ cells	91.987	100.082	173.649	258.275	172.191	273.513	280.398	485.678	80.412	117.593
	$\theta_{LN,1}^{\dagger}$	$\cdot 10^8$ cells	0.265	0.372	0.154	0.451	1.134	1.223	10.811	19.297	1.120	5.160
	$\theta_{LN,2}$	$\cdot 10^8$ cells	0.001	265.039	329.114	607.436	327.621	745.614	303.030	4788.246	281.928	714.329
	$\theta_{PB,1}$	$\cdot 10^8$ cells	0.001	0.008	0.007	0.104	0.063	0.132	0.677	1.259	0.018	0.224
	$\theta_{PB,2}$	$\cdot 10^8$ cells	15.000	16.450	20.000	33.050	20.000	43.100	210.000	457.000	45.000	51.450
	$k_{N,BM2PB}^{\dagger}$	1/day	0.003	0.003	0.001	0.004	0.002	0.005	0.035	0.011	0.005	0.001
	$k_{P,BM2PB}^{\dagger}$	1/day	0.003	0.001	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.001
	$k_{N,PB2LN}^{\dagger}$	1/day	0.003	0.002	0.001	0.001	0.001	0.003	0.005	0.001	0.001	0.001
	$k_{P,PB2LN}^{\dagger}$	1/day	0.033	0.007	0.013	0.007	0.008	0.006	0.001	0.001	0.001	0.014
Assumed	t_{in}	day	0	1000	0	1800	0	365	0	365	0	365
Parameters	t_{fin}	day	1000	3190	1800	2165	365	730	365	730	365	730

[†] Critical parameters along with the patients initial conditions in RS-HDMR analysis (Appendix F, Figure F.2)

TABLE 5: Initial characteristics of the 5 hypothetical patients (more details in Appendix Table E.1)

Patient	P1	P2	P3	P4	P5	CLL
Bone Marrow Infiltration	37.3%	46.7%	46.7%	52.1%	37.4%	
CLL in PB (cells/ μ L)	6018	8016	8016	84168	18040	≥ 5000 for at least 3 months [†]
LN & S (cells)	0	$1340 \cdot 10^8$	$1340 \cdot 10^8$	$1326 \cdot 10^8$	$1047 \cdot 10^8$	
Ki-67 % in PB	0.30%	0.20%	0.20%	0.20%	0.22%	0.36 ± 0.34 % [‡]
LN & S	0.00%	2.99%	2.99%	2.00%	4.50%	4.95 ± 0.55 % [‡]
						4.50 ± 2.50 % [‡]
BM	2.70%	1.95%	1.95%	3.00%	1.76%	2.48 ± 0.77 % [‡]
Expected LDT (months)	$\infty \rightarrow 60$	$60 \rightarrow 12$	$12 \rightarrow 12$	$12 \rightarrow < 12$	$60 \rightarrow < 12$	

[†]Values for CLL published by the World Health Organization, [‡]Average values of Ki-67 in tissues

output variables are the number of proliferative and non-proliferative CLL cells in BM, PB and LN including S.

3. CASE STUDIES

The initial characteristics of 5 hypothetical patients (P1 – P5) are posited in Table 5. The 5 hypothetical patients represent a range of clinically-relevant physiological conditions (disease heterogeneity) based on common disease presentation (Table 1 and Appendices C – G). The only exception is P3 which represents the model outcome described by Molica *et al.* [37] where a patient in Binet Stage A has a lymphocyte doubling time, $LDT \leq 12$ months (Appendix E). The 5 hypothetical patient trajectories (P1 – P5) we model are:

- P1: Naïve Stage (LDT: ∞) $\rightarrow$ Binet Stage A (LDT= 60 months)
- P2: Binet Stage A (LDT = 60 months) $\rightarrow$ Binet Stage B (LDT = 12 months)
- P3: Binet Stage A (LDT = 12 months) $\rightarrow$ Binet Stage B (LDT = 12 months)

- P4: Binet Stage B (LDT = 12 months) $\rightarrow$ Binet Stage C (LDT = 12 months)
- P5: Binet Stage A (LDT = 60 months) $\rightarrow$ Binet Stage C (LDT = 7 months)

We also make the following assumptions: (a) normal lymphocyte count in each tissue is stable, (b) zero net migration of normal lymphocytes, (c) normal bone marrow lymphocytes have percentage $a_{BM} = 25\%$ [38], (d) peripheral blood volume is 5 L [39], (e) the maximum recruitment rate $\beta_{N,0}$ equals 3.35 /day in each tissue [25]; (f) CLL disease stage duration, Δt_z , is based on LDT (Table 1) and has values [days]: 1000, 1800, 365, 365 and 365 for the 5 case studies, P1 – P5, respectively.

We apply the Section 2.B algorithm for determining parameters to the 5 case studies, simulate the trajectories of Eq (1) – (12) and predict future patient signs/sequelae via correlation with the simulated CLL cells number in each disease center. For more detail,

Fig. 3: Example patient trajectory within a disease stage; horizontal lines show PB LDT calculation and vertical lines show transition from initial diagnosis at time t_{in} to the point of progression to a new phase at t_{fin}

see Appendix D. We consider 4 types of physical signs: (a) high infiltration pattern of BM predicted from Eq (5), (b) only splenomegaly, (c) only lymphadenopathy, and (d) combined splenomegaly and lymphadenopathy (Appendices C and G). We implemented the model in MATLAB R2013a (8.1.0.604) and GAMS 24.1.3 (solver: CONOPT) on a 4.40 GHz Intel Core i7-4770 processor with 8GB RAM running 64-bit Windows 7; each simulation took 0.1 – 0.2 CPU s.

4. RESULTS AND DISCUSSION

Results are in Tables 4 & 6 and Fig. 4. The clinical predictions in Table 6 are based on analyses in Appendices C and G; Fig. 4 diagrams the MATLAB outputs. The sequel comments on each of the 5 case studies.

P1 starts from a naïve stage with high LDT and progresses to Binet Stage A (LDT 60 months); the proliferation increase of ≈ 33 months after initial diagnosis correlates with the increased recruitment rates into the bonemarrow and lymph node cell cycles. P2 and P3 have similar initial disease characteristics (% CLL cells in bone marrow and %Ki-67) but diverge with respect to initial LDT; these two test cases correspond to work showing that LDT significantly impacts the probability of progression from Binet Stage A to B within a year [37]. The model captures this progression tempo; P2 and P3 progress to Stage B in 60 and 12 months, respectively.

P4 shows a trajectory from Binet Stage B to C where the bone marrow pattern changes from mixed to diffuse and the rate of lymphocyte doubling is roughly constant at 12 months; the fraction of CLL cells migrating decreased or remained stable in the P4 trajectory. Note here that, although the definition of Binet Stage C requires a descriptor of anemia and low platelets (Table 1), we use instead an indirect metric, assuming that the signs of Binet Stage C will follow from an increase in bone

marrow tumor burden. Finally, P5 is an unusual CLL case; the disease initiates slowly in Binet Stage A and bone marrow failure occurs quickly within 2 years.

RS-HDMR analysis of the CLL model in Binet Stages from A to C, illustrated in Fig. F.2 of Appendix F for 5000 HDMR samples is done in Matlab with dde23 solver. The parameters ($S_i \geq 0.1$) of this model which most significantly affect disease trajectory over space and time are: (i) the initial number of CLL cells in peripheral blood, the number of involved (enlarged) lymph nodes and the presence and degree of splenomegaly, (ii) the migratory fractions of non-proliferating as well as proliferating CLL cells from bone marrow into peripheral blood and of proliferating CLL cells from peripheral blood to lymph nodes and, (iii) the parameter inducing non-proliferative cells to proliferate. Several critical parameters identified by our model (enlarged lymph nodes, splenomegaly, LDT) are already used in staging systems, but none of: (i) total CLL cell number, (ii) migration rate and, (iii) propensity to induce proliferation (unless directly incurred by a known mutation, e.g., p53, or mutated pathway) is currently used. We therefore propose these factors for inclusion in multi-parameter models of disease progression, as targets for prevention of progression or therapy. In particular, the use of migration rate, e.g., using chemokine or integrin surface markers or other relevant serum biomarkers, and the induction of a cell proliferation profile, e.g., via mutated cell cycle checkpoints or intracellular pathways such as PI3K, could be important focal points for inclusion in future research. These parameters could be made readily available for input into models on a patient-specific basis over the disease course from peripheral blood tests done at the bedside (for migration assessments) and from sampling of the primary disease center, e.g., lymph node or bone marrow for proliferation parameters via fine needle aspirate or core biopsy - both routine procedures.

Expression of Ki-67, a proliferation marker found in cycle phases G1, S, G2, and M but not G0 [40], is found in tissues affected by CLL in varying percentage: peripheral blood $0.36 \pm 0.34\%$, bone marrow $2.48 \pm 0.77\%$, and lymph nodes and spleen $4.95 \pm 0.55\%$ [26]. In addition to having higher proliferative features than those of bone marrow or peripheral blood, lymph nodes and spleen disease centers in CLL also have lower death rates [33, 34], a feature which has been partially attributed to the microenvironment [41]. One of the earliest mathematical frameworks of CLL models the interaction between CLL cells and T-cells, a component of the microenvironment, via a Volterra predator-prey equation [42]. A kinetic model [30] has also been used to develop the first model of CLL incorporating interactions between CLL cells, natural killer (NK) cells, cytotoxic (CD8+) T-cells and helper (CD4+) T-cells [43]. This model implicitly as-

Fig. 4: Characteristics of CLL in theoretical patients P1 – P5. Each row represents one patient; the vertical dashed lines represent transitions between disease stages

TABLE 6: Possible characteristics of 5 hypothetical patients derived from the mathematical model

	Symptoms	Initially	Finally
P1: Naïve $\rightarrow$ Binet Stage A LDT: $\infty \rightarrow 60$ months	Bone marrow pattern Enlarged lymph nodes Splenomegaly	Interstitial or nodular No No	Interstitial or nodular [Diameter (cm), #] [3, 5], [4, 2], [5, 1] No
P2: Binet Stage A $\rightarrow$ B LDT: 60 months $\rightarrow$ 12 months	Bone marrow pattern Enlarged lymph nodes Splenomegaly	Interstitial or nodular Yes No	Mixed [Diameter (cm), #] [3, 2], [4, 1] Mild to moderate (5×12 cm)
P3: Binet Stage A $\rightarrow$ B LDT: 12 months $\rightarrow$ 12 months	Bone marrow pattern Enlarged lymph nodes Splenomegaly	Interstitial or nodular Yes No	Mixed [Diameter (cm), #] [3, 4], [4, 2] Mild to moderate (5×12 cm)
P4: Binet Stage B $\rightarrow$ C LDT: 12 months $\rightarrow$ 12 months	Bone marrow pattern Enlarged lymph nodes Splenomegaly	Mixed Yes No	Diffuse [Diameter (cm), #] [4, 11], [5, 6], [6, 5] Marked (7×14 cm)
P5: Binet Stage A $\rightarrow$ C LDT: 60 months $\rightarrow$ 7 months	Bone marrow pattern Enlarged lymph nodes Splenomegaly	Interstitial or Nodular No No	Diffuse [Diameter (cm), #] [3, 5], [4, 2] Mild to moderate (5×15 cm)

sumes that the entire body is 1-compartment and thereby does not account for the proliferation center microenvironment, disease center carrying capacity, or the cellular migration between disease centers [43]. CLL lymphocytosis kinetics have been studied using a 2-compartment model [30] that characterizes CLL cell redistribution dynamics from proliferation centers into the peripheral blood induced by the novel BTK inhibitor, Ibrutinib albeit there is little known about steady-state dynamics or of that which occurs during the natural course of disease [44]. Variations may assume different cell cycle models for each disease compartment. For example, immunophenotyping to distinguish between B- and T-cells in the peripheral blood lymphocyte compartment [41, 45] which may facilitate the incorporation of immune interactions and current immuno-chemotherapeutic targets [46]. Our model therefore *complements* the 1-compartment model of Nanda *et al.* [43]. If we could assume data on T-cell populations, i.e., CD4+, CD8+, the Nanda *et al.* [43] model could replace each G0 model in Fig. 1. This manuscript does not use the optional T-cell module and reflects data availability from typical clinical protocols with first-order approximation that 90% of lymphocytes in affected tissues are CLL cells [41].

Our model meaningfully adapts to each of the disparate case studies and the parameters and variables adhere to physiologically-relevant values. The proposed framework has been developed using a variety of hypothetical test cases. However, used in tandem with real patient data and other clinical diagnostic tools [47, 48], the developed algorithm may enable patient-specific, leukemia-specific disease dynamic modeling for actual patients with CLL. It also provides insight into the trajectory of disease progression and presents CLL cell migration and propensity towards proliferation as avenues of future research.

5. CONCLUSIONS

This paper develops the first physiologically-relevant mathematical model of CLL connecting the most af-

fected tissues. The mathematical novelty stems from the integration of both temporal and spatial CLL aspects. The delay differential equations incorporate the temporal aspect of cell division. The model is practical in that (1) measurable input parameters have been taken from common clinical protocols, (2) from data which is readily accessible in current standard clinical practice, (3) it captures physiologically-relevant transitions from Binet Stage A to C and, (4) it predicts outcomes which may be clinically useful for expectant patient care if validated using primary patient data. Using the model, we found that CLL cell migration between disease centers and the induction of CLL cells into proliferative phase are critical parameters determining disease evolution and should be evaluated in future research.

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TABLE 7: Symbol Definition

	Symbol	Value	Units	Description	Source
Indices	CLL			B-Cell Chronic Lymphocytic Leukemia Cells	
	$t \in \{\text{in}, \text{fin}\}$			Time points t ; in $\equiv$ initial; fin $\equiv$ final	
	$d \in \{\text{LB}, \text{AV}, \text{UB}\}$			Data d : LB $\equiv$ Lower Bound; AV $\equiv$ Average; UB $\equiv$ Upper Bound	
	$h \in \{\text{BM2PB}, \text{PB2LN}\}$			Net flow h of CLL cells: BM2PB $\equiv$ bone marrow $\rightarrow$ peripheral blood; PB2LN $\equiv$ peripheral blood $\rightarrow$ lymph nodes and spleen	
	$i \in \{\text{NC}, \text{BM}, \text{PB}, \text{LN}, \text{S}\}$			Cell types i ; NC $\equiv$ Normal lymphocyte in any tissue; BM $\equiv$ CLL in bone marrow (proliferating region); PB $\equiv$ CLL in peripheral blood (non-proliferating region); LN $\equiv$ CLL in lymph node (proliferating region); S $\equiv$ CLL in spleen (proliferating region, grouped with LN in our analysis); LC $\equiv$ lymphocytes	
State Variables	$z \in \{\text{SN}, \text{SA}, \text{SB}, \text{SC}\}$			Stage of the disease: SN $\equiv$ Naïve Stage; SA $\equiv$ Binet Stage A; SB $\equiv$ Binet Stage B; SC $\equiv$ Binet Stage C	
	P_i		cells	Proliferating cells in compartments $i \in \{\text{NC}, \text{BM}, \text{PB}, \text{LN}\}$	
Assumed Parameters	N_i		cells	Non-proliferating cells in compartments $i \in \{\text{NC}, \text{BM}, \text{PB}, \text{LN}\}$	
	a_{BM}	24.7 ± 9.7	%	Percentage of lymphocytes in healthy bone marrow	[38]
Measured Parameters at $t = 0$ day	$\beta_{N_i, 0}$	3.35	1/day	Maximum recruitment rate	[23, 25]
	$d_{\text{LN}, \text{NC}}$	[1, 2]	cm	Normal lymph node diameter	[49]
	$d_{\text{S}, \text{NC}}$	5	cm	Normal spleen diameter	Table G.1 [†]
	$l_{\text{S}, \text{NC}}$	11	cm	Normal spleen length	Table G.1 [†]
	n_i	3	–	Control shape of the Eq (D.7) feedback function	[23, 25]
	$V_{\text{LN}, \text{NC}}$	0.52	cm ³	Normal lymph node volume	Eq (G.1) [†]
	V_{S}	215.86	cm ³	Normal spleen volume (147.50 $\pm$ 81.46)	[50]
	V_{CLL}	166×10^{-12}	cm ³	Volume of a CLL cell	[51]
	$t_{\text{in}, z}$		day	Time when disease stage z starts	
	$t_{\text{fin}, z}$		day	Time when disease stage z finishes	
	$C_{\text{NC}, \text{BM}}$	50×10^9	cells	Normal number of lymphocytes in bone marrow	Table 2
	BM_{max}	13.5×10^{11}	cells	Maximum number of cells in bone marrow	Appendix B [†]
	$\% \text{Ki-67}_{i, \text{AV}}$		%	Average Ki-67 of CLL in tissues. Ki-67 _{BM, AV} = 2.48 $\pm$ 0.77%; Ki-67 _{PB, AV} = 0.36 $\pm$ 0.34%; Ki-67 _{LN, AV} = 4.95 $\pm$ 0.55%;	[26]
	$d_{\text{S}, \text{CLL}}$	[5, 10]	cm	Enlarged spleen diameter	Table G.1 [†]
	$l_{\text{S}, \text{CLL}}$	[11, 28]	cm	Enlarged spleen length	Table G.1 [†]
	$d_{\text{LN}, \text{CLL}}$	$\geq 1, 6$	cm	Enlarged lymph node diameter	[7]
Initial Conditions	$\% \text{Ki-67}_i$		%	Percentage of Ki-67 in compartments $\forall i \in \{\text{NC}, \text{BM}, \text{PB}, \text{LN}\}$	Eq (4)
	$\% \text{BM}_{\text{lymph}}$		%	Percentage of lymphocytes in bone marrow	Eq (5)
	$V_{\text{CLL}, \text{LN}}$		cm ³	Volume of an enlarged lymph node	Eq (G.2) [†]
	$N_{\text{uLN}, d_{\text{LN}, \text{CLL}}}$			Number of enlarged lymph nodes of diameter $d_{\text{LN}, \text{CLL}}$	Eq (G.5) [†]
	$V_{\text{CLL}, \text{S}}$		cm ³	Volume of CLL cells in spleen	Eq (G.7) [†]
	$N_{\text{BM}}, P_{\text{BM}}$		cells	$\% \text{Ki-67}_{\text{BM}}$ and $\% \text{BM}$ in Eq 5	
	$N_{\text{PB}}, P_{\text{PB}}$		cells	$\% \text{Ki-67}_{\text{PB}}$ and blood tests	
	$N_{\text{LN}}, P_{\text{LN}}$		cells	$\% \text{Ki-67}_{\text{LN}}$ and CT scans	
	$\delta_{i, z}$		1/day	Cell death rate in quiescent phase $\forall i \in \{\text{NC}, \text{BM}, \text{PB}, \text{LN}\}$ $\delta_{\text{BM}} \in [0.001, 0.080]$; $\delta_{\text{PB}} \in [0.001, 0.020]$; $\delta_{\text{LN}} \in [0.001, 0.020]$	[23, 25] [30, 32]
	$\gamma_{i, z}$		1/day	Rate of cell loss from proliferating phase $\forall i \in \{\text{NC}, \text{BM}, \text{PB}, \text{LN}\}$ $\gamma_{\text{BM}} \in [0.001, 1.000]$; $\gamma_{\text{PB}} \in [0.001, 1.000]$; $\gamma_{\text{LN}} \in [0.001, 1.000]$	[23, 25] [30, 32]
Calculated Parameters	$\tau_{i, z}$	[0.625, 0.792]	day	Time spent in proliferative phase in compartments $i \in \{\text{NC}, \text{BM}, \text{PB}, \text{LN}\}$	[29]
	$k_{\text{N}, \text{h}, z}; k_{\text{P}, \text{h}, z}$		1/day	Migration rate of (non-)proliferative (N/P) cells for net flows h	Appendix D [†]
	$\theta_{i, 1, z}; \theta_{i, 2, z}$		cells	Parameters of θ_i function	Appendix D [†]
	θ_i		cells	Control shape of the feedback function $\forall i \in \{\text{NC}, \text{BM}, \text{PB}, \text{LN}\}$	Eq (3)
	β_i		1/day	Hill feedback function	Eq (D.7) [†]
	$V_{\text{CLL}, \text{LN}}^*$		cm ³	Volume of an enlarged lymph node based on lymphocytes	Eq (G.3) [†]
	$(N+P)_{\text{CLL}, \text{LN}}$		cells	Number of CLL cells per lymph node	Eq (G.4) [†]
	$N_{\text{uLN}, d_{\text{LN}, \text{CLL}}}$			Number of enlarged lymph nodes of diameter $d_{\text{LN}, \text{CLL}}$	Eq (G.5) [†]
	$V_{\text{CLL}, \text{S}}^*$		cm ³	Volume of CLL cells in spleen	Eq (G.7) [†]
	$(N+P)_{\text{CLL}, \text{S}}$		cells	Total number of CLL cells in spleen	Eq (G.8) [†]
Variables	cc			Bone marrow carrying capacity	Eq (6)
	$d_{\text{S}, \text{CLL}}$	[5, 10]	cm	Enlarged spleen diameter	Table G.1 [†]
	$l_{\text{S}, \text{CLL}}$	[11, 28]	cm	Enlarged spleen length	Table G.1 [†]
	$d_{\text{LN}, \text{CLL}}$	$\geq 1, 6$	cm	Enlarged lymph node diameter	[7]
	$\% \text{Ki-67}_i$		%	Percentage of Ki-67 in compartments $\forall i \in \{\text{NC}, \text{BM}, \text{PB}, \text{LN}\}$	Eq (4)
	$\% \text{BM}_{\text{lymph}}$		%	Percentage of lymphocytes in bone marrow	Eq (5)
	$V_{\text{CLL}, \text{LN}}$		cm ³	Volume of an enlarged lymph node	Eq (G.2) [†]
	$V_{\text{CLL}, \text{S}}$		cm ³	Volume of CLL cells in spleen	Eq (G.7) [†]
	$N_{i, \tau_{i, z}}$		cells	Number of CLL cells in tissues at time $t - \tau_i$ at stage z	Eq (1)
	$N_i(t - \tau_{i, z})$		cells	Number of CLL cells in tissues at time $t - \tau_i$ at stage z	Eq (1)

[†] Material in the Online Supplementary Material

Supplementary Material

A Personalized Framework for Dynamic Modeling of Disease Trajectories in Chronic Lymphocytic Leukemia

Savvopoulos • Misener • Panoskaltsis • Pistikopoulos
• Mantalaris

APPENDIX A BASIC G0 CELL CYCLE MODEL

Fig. A.1: The G0 cell cycle model [23, 25]

We represent lymphocyte proliferation using the 2-compartment delay differential equation (DDE) model illustrated in Fig. A.1 [23, 25]. The non-proliferative compartment N [cells] represents the resting (G0) phase and the proliferative compartment P [cells] aggregates the remaining cell cycle phases (G1, S, G2, M). Non-proliferative cells migrate to the proliferative compartment where they divide in fixed time τ (day); the time delay is represented $N_\tau = N(t - \tau)$. In the case of normal lymphocytes (NC):

$$\frac{dN_{NC}}{dt} = -\delta_{NC} N_{NC} - \beta_{NC}(N_{NC}) N_{NC} + 2 \beta_{NC}(N_{\tau_{NC}, NC}) N_{\tau_{NC}, NC} e^{-\gamma_{NC} \tau_{NC}} \quad (A.1)$$

$$\frac{dP_{NC}}{dt} = -\gamma_{NC} P_{NC} + \beta_{NC}(N_{NC}) N_{NC} - \beta_{NC}(N_{\tau_{NC}, NC}) N_{\tau_{NC}, NC} e^{-\gamma_{NC} \tau_{NC}} \quad (A.2)$$

The feedback from non-proliferative to proliferative compartment is given by β (1/day) with maximum entry rate $\beta_{N,0}$ (1/day) and control shape n, θ (cells) of Eq (D.7). The apoptotic rate in the non-proliferative and proliferative compartments is δ (1/day) and γ (1/day), respectively [23, 25].

APPENDIX B

PERCENTAGE OF LYMPHOCYTES IN BONE MARROW AND BONE MARROW CARRYING CAPACITY

The mean lymphocyte percentage in healthy human bone marrow biopsies is $a_{BM} = 25\%$ (Range: 9 – 37%) [38]. From Table 2, the total number of lymphocytes in bone marrow is $C_{NC, BM} = 50 \times 10^9$. If healthy bone marrow could be evacuated, the total number of analysed cells would be roughly $C_{NC, BM}/a_{BM}$ and we can use

Fig. C.1: CLL BM infiltration patterns [6, 28]; BM infiltration pattern, $\%BM_{CLL}$, is typically nodular or interstitial in Naïve stage or Binet Stage A & B, mixed in Binet Stage B & C and diffuse in Binet Stage C

Eq 5 to calculate $\%BM_{lymph}$, the increased bone marrow lymphocyte percentage due to CLL. Observe in Figure F.2 that a_{BM} is relatively unimportant in the model

The maximum fraction of CLL cells in BM is 90%. Assuming a constant number (50×10^9) of normal BM lymphocytes [21, 22], we estimate a maximum of 13.5×10^{11} lymphocytes in heavily-infiltrated BM:

$$\begin{aligned} \%BM_{lymph} &= \frac{C_{NC, BM} + N_{BM} + P_{BM}}{\frac{1}{a_{BM}} C_{NC, BM} + P_{BM} + N_{BM}} \Leftrightarrow \\ 90\% &= \frac{50 \times 10^9 + N_{BM} + P_{BM}}{\frac{1}{25\%} 50 \times 10^9 + N_{BM} + P_{BM}} \Leftrightarrow \\ N_{BM} + P_{BM} &= 13.0 \times 10^{11} \end{aligned}$$

APPENDIX C

CALCULATING THE BONE MARROW INFILTRATION PATTERN

Accumulating CLL cells may increase the proportion and change the infiltration pattern of BM lymphocytes; lymphocytes typically represent $24.7 \pm 9.6\%$ of white blood cells (WBCs) on healthy human BM aspirates but may be $> 80\%$ in later CLL stages [38]. Fig. C.1 diagrams the four CLL BM infiltration patterns: interstitial; nodular; mixed; diffuse [28]. In the interstitial pattern, the lymphocyte percentage ranges 30 – 50% (light BM infiltration) [6, 28]; mature lymphocytes replace hematopoietic tissue to some degree but BM structure and fat cells are preserved. In the nodular pattern, the lymphocyte percentage also ranges 30 – 50%, fat cells are preserved and there is no interstitial infiltration [6, 28], but nodules of mature lymphocytes

appear with diameter longer than the lymphoid follicles. In mixed pattern, the percentage of lymphocytes ranges 50 – 80% (moderate BM infiltration) [6, 28] and there is both interstitial and nodular infiltration. In diffuse pattern, the percentage of lymphocytes is > 80% (heavy BM infiltration) [6, 28] and there is massive replacement of hematopoietic tissue by mature lymphocytes.

APPENDIX D PARAMETER ESTIMATION

In a CLL disease stage $z \in \{SN, SA, SB, SC\}$, both $t = t_{in}$ and $t = t_{fin}$ should require:

$$\begin{aligned} & -\delta_{BM,z} N_{BM,t,z} - \beta_{BM}(N_{BM,t,z}) N_{BM,t,z} c c t, z \\ & + 2 \beta_{BM}(N_{BM,t,z}) N_{BM,t,z} e^{-\gamma_{BM,z} \tau_{BM,z} c c t, z} \\ & - k_{N, BM2PB,z} N_{BM,t,z} = \frac{N_{BM,fin,z} - N_{BM,in,z}}{t_{fin,z} - t_{in,z}} \end{aligned} \quad (D.1)$$

$$\begin{aligned} & -\delta_{PB,z} N_{PB,t,z} - \beta_{PB}(N_{PB,t,z}) N_{PB,t,z} \\ & + 2 \beta_{PB}(N_{PB,t,z}) N_{PB,t,z} e^{-\gamma_{PB,z} \tau_{PB,z}} \\ & + k_{N, BM2PB,z} N_{BM,t,z} - k_{N, PB2LN,z} N_{PB,t,z} \\ & = \frac{N_{PB,fin,z} - N_{PB,in,z}}{t_{fin,z} - t_{in,z}} \end{aligned} \quad (D.2)$$

$$\begin{aligned} & -\delta_{LN,z} N_{LN,t,z} - \beta_{LN}(N_{LN,t,z}) N_{LN,t,z} \\ & + 2 \beta_{LN}(N_{LN,t,z}) N_{LN,t,z} e^{-\gamma_{LN,z} \tau_{LN,z}} \\ & + k_{N, PB2LN,z} N_{PB,t,z} = \frac{N_{LN,fin,z} - N_{LN,in,z}}{t_{fin,z} - t_{in,z}} \end{aligned} \quad (D.3)$$

$$\begin{aligned} & -\gamma_{BM,z} P_{BM,t,z} + \beta_{BM}(N_{BM,t,z}) N_{BM,t,z} c c t, z \\ & - \beta_{BM}(N_{BM,t,z}) N_{BM,t,z} e^{-\gamma_{BM,z} \tau_{BM,z} c c t, z} \\ & - k_{P, BM2PB,z} P_{BM,t,z} = \frac{P_{BM,fin,z} - P_{BM,in,z}}{t_{fin,z} - t_{in,z}} \end{aligned} \quad (D.4)$$

$$\begin{aligned} & -\gamma_{PB,z} P_{PB,t,z} + \beta_{PB}(N_{PB,t,z}) N_{PB,t,z} \\ & - \beta_{PB}(N_{PB,t,z}) N_{PB,t,z} e^{-\gamma_{PB,z} \tau_{PB,z}} \\ & + k_{P, BM2PB,z} P_{BM,t,z} - k_{P, PB2LN,z} P_{PB,t,z} \\ & = \frac{P_{PB,fin,z} - P_{PB,in,z}}{t_{fin,z} - t_{in,z}} \end{aligned} \quad (D.5)$$

$$\begin{aligned} & -\gamma_{LN,z} P_{LN,t,z} + \beta_{LN}(N_{LN,t,z}) N_{LN,t,z} \\ & - \beta_{LN}(N_{LN,t,z}) N_{LN,t,z} e^{-\gamma_{LN,z} \tau_{LN,z}} \\ & + k_{P, PB2LN,z} P_{PB,t,z} = \frac{P_{LN,fin,z} - P_{LN,in,z}}{t_{fin,z} - t_{in,z}} \end{aligned} \quad (D.6)$$

where $i \in \{BM, PB, LN\}$ and $z \in \{SN, SA, SB, SC\}$

$$\beta_i(N_{i,t,z}) = \beta_{N_i,0} / (1 + (u_{i,t,z})^{n_i}) \quad (D.7)$$

$$u_{i,t,z} = N_{i,t,z} / \theta_{i,t,z} \quad (D.8)$$

$$\theta_{i,2,z} = \theta_{i,t_{in},z} \quad (D.9)$$

$$\theta_{i,1,z} = \frac{\theta_{i,t_{fin},z} - \theta_{i,t_{in},z}}{t_{fin,z} - t_{in,z}} \quad (D.10)$$

$$\theta_i = \theta_{i,1,z} (t - t_{in,z}) + \theta_{i,2,z}. \quad (D.11)$$

Furthermore, we assume:

(a) The constraints of $u_{i,t,z}$ are:

$$u_{BM}^{LB} = 2 \leq u_{BM,t,z} \leq u_{BM}^{UB} = 20 \quad (D.12)$$

$$u_{PB}^{LB} = u_{PB}^{UB} = 20 \quad (D.13)$$

$$u_{LN}^{LB} = 2 \leq u_{LN,t,z} \leq u_{PB,t,z} \quad (D.14)$$

If $N_{LN,t_{in},z} + P_{LN,t_{in},z} \simeq 0$ then $u_{LN,t_{in},z} = u_{PB,t_{in},z}$

(b) Apoptotic rates bounds from Table 7 [1/day]:

$$\delta_{BM}^{LB} = 0.001 \leq \delta_{BM,z} \leq \delta_{BM}^{UB} = 0.080 \quad (D.15)$$

$$\delta_{PB}^{LB} = 0.001 \leq \delta_{PB,z} \leq \delta_{PB}^{UB} = 0.020 \quad (D.16)$$

$$\delta_{LN}^{LB} = 0.001 \leq \delta_{LN,z} \leq \delta_{LN}^{UB} = 0.020 \quad (D.17)$$

$$\gamma_i^{LB} = 0.001 \leq \gamma_{i,z} \leq \gamma_i^{UB} = 1.000 \quad (D.18)$$

(c) Apoptotic rates for proliferating cells may be high when they lose contact with T cells [52].

$$\gamma_{i,z} \geq \delta_{i,z} \quad (D.19)$$

Fig. D.1: Iterative procedure for disease stage $z1$; subsequent stages are equivalent. Intermediate MATLAB outputs (marked *) should match Table E.1 (no *)

(d) Migration rate lower and upper bounds are 0.001 and 1.000, respectively. We assume lower migration rates for proliferating cells than non-proliferating cells in bone marrow because of the niche:

$$k_{P, BM2PB,z} \leq k_{N, BM2PB,z} \quad (D.20)$$

(e) If CLL cells are accumulating in PB, use Eq (D.21), otherwise use Eq (D.22):

$$N_{BM,fin,z} \geq \frac{k_{N, PB2LN,z}}{k_{N, BM2PB,z}} N_{PB,fin,z} \quad (D.21)$$

$$N_{BM,fin,z} \leq \frac{k_{N, PB2LN,z}}{k_{N, BM2PB,z}} N_{PB,fin,z} \quad (D.22)$$

- (f) Migration rates from PB to LN are related to the Ki-67 fraction listed in Table 5 for each tissue:

$$\frac{k_{P, PB2LN, z}}{k_{N, PB2LN, z}} \cdot \frac{P_{PB}}{N_{PB}} \leq \text{Ki-67}_{LN, AV} \Rightarrow$$

$$k_{P, PB2LN, z} \leq k_{N, PB2LN, z} \frac{\text{Ki-67}_{LN, AV}}{\text{Ki-67}_{PB, AV}}; \quad (\text{D.23})$$

- (g) Time [day] spent in proliferative phase [29]:

$$\tau_i^{LB} = 0.625 \leq \tau_{i, z} \leq \tau_i^{UB} = 0.792 \quad (\text{D.24})$$

- (h) Only in the parameter estimation:

$$cc_{t, z} = 1 \quad (\text{D.25})$$

Eqs (D.1) – (D.25), seeded with the initial and final number of cells measured at different time points (Tables 5 & E.1), are solved as a feasibility problem in GAMS. Final conditions at $t = t_{\text{fin}}$ are set by expected values depending on the disease stage (see Tables 1 & C.1 and Appendix C). The GAMS output is a set of parameter values $\tau_{i, z1}$, $\delta_{i, z1}$, $\gamma_{i, z1}$, $\theta_{i, 1, z1}$, $\theta_{i, 2, z1}$, $k_{N, BM2PB, z1}$, $k_{P, BM2PB, z1}$, $k_{N, PB2LN, z1}$ and $k_{P, PB2BM, z1} \quad \forall i \in \{\text{BM}, \text{PB}, \text{LN}\}$, $z1 \in \{\text{SN}, \text{SA}, \text{SB}\}$ satisfying the constraints.

We enter the output parameters from the GAMS feasibility problem into the set of DDEs in MATLAB and check that final conditions agree with expected values. If the MATLAB output is not as expected, we vary the parameters first in BM and then in LN:

- (a) If the relative error of Ki-67, $\varepsilon_{\text{Ki-67}_{k, t_{\text{fin}}, z1}} > 15\%$ for $k \in \{\text{BM}, \text{LN}\}$, we minimize $\varepsilon_{\text{Ki-67}_{k, t_{\text{fin}}, z1}}$ by sampling over the lower and upper bound of $\gamma_{k, z1}$:

$$\varepsilon_{\text{Ki-67}_{k, t_{\text{fin}}, z1}} = \frac{|\Delta \text{Ki-67}_{k, z1}(t_{\text{fin}})|}{\text{Ki-67}_{k, z1}(t_{\text{fin}})} \quad (\text{D.26})$$

- (b) If the relative error of N_k , $\varepsilon_{N_k, t_{\text{fin}}, z1} > 20\%$, we increase or decrease control parameter $\theta_{k, 1, z1}$ for insufficiency or hypersufficiency, respectively. We increment $\theta_{k, 1, z1}$ by increasing the highest non-zero digit of the initial $\theta_{k, 1, z1}$ by 1 until the flowsheet in Fig. D.1 is satisfied:

$$\varepsilon_{N_k, t_{\text{fin}}, z1} = \frac{|\Delta N_{k, z1}(t_{\text{fin}})|}{N_{k, z1}(t_{\text{fin}})} \quad (\text{D.27})$$

For trajectories spanning two Binet stages, the final values of the first stage are initial conditions for the second disease stage (Table E.1) and, for the second disease stage, only parameters varying within a disease stage, e.g., the control function and migration rates) are calculated by GAMS. Solving for the second disease stage is similar except that we tighten the tolerance in step (a) above to 10%. Recall here that our goal is finding reasonable rather than optimal parameters; Appendix H demonstrates the results using a multistart strategy since there are multiple possible outputs of the GAMS feasibility problem.

APPENDIX E

INITIAL AND FINAL EXPECTED CONDITIONS

Representing patient heterogeneity, Table 5 posits symptoms of 5 hypothetical patients. For each tissue in each disease stage, we estimate the fraction of proliferative cells using expected Ki-67 expression (Table 5). PB lymphocyte counts are based on expected LDT values (Table 1) and BM infiltration patterns are chosen with respect to CLL disease progression (Table C.1).

This study focusses on cells migrating from BM to PB and then from PB to LN and S; initial numbers of enlarged LN in the 5 patients correspond to expected disease stage. Final CLL cell count is based on expected lymphadenopathy and splenomegaly in CLL. As in PB and BM, the expected proliferative cell fraction in LN and S is based on expected values of Ki-67 (Table 5).

APPENDIX F

GLOBAL SENSITIVITY ANALYSIS

RS-HDMR, like the Sobol global sensitivity analysis method, decomposes model output into summands of increasing dimensionality [36].

$$f(\mathbf{x}) = f_0 + \sum_{i=1}^n f_i(x_i) + \sum_{i,j}^n f_{i,j}(x_i, x_j) + \dots + f_{1,2,\dots,n}(\mathbf{x})$$

where:

$$f_0 = \int_{K^n} f(\mathbf{x}) \, d\mathbf{x}$$

$$f_i(x_i) = \int_{K^{n-1}} f(\mathbf{x}) \, d\mathbf{x}^i - f_0$$

$$f_{ij}(x_i, x_j) = \int_{K^{n-2}} f(\mathbf{x}) \, d\mathbf{x}^{ij} - f_i(x_i) - f_j(x_j) - f_0$$

$$\dots$$

and $\mathbf{x} = [x_1, x_2, \dots, x_n]$ is the input of parameters whose values change; $d\mathbf{x}^i$ is the product $dx_1 dx_2 \dots dx_n$; $d\mathbf{x}^{ij}$ is the same product without dx_i and dx_j . This decomposition, also called analysis of variance (ANOVA), creates unique, orthogonal terms and reveals both first- and higher-order effects to model output via variance-based methods. The total variance D is [53]:

$$D = \int_{K^n} f^2(\mathbf{x}) \, d\mathbf{x} - f_0^2$$

and the partial variances $D_{i_1, \dots, i_s}$ are calculated by:

$$D_i = \int_0^1 f_i^2(x_i) \, dx_i$$

$$D_{ij} = \int_0^1 \int_0^1 f_{i,j}^2(x_i, x_j) \, dx_i \, dx_j \quad \dots$$

TABLE E.1: Initial and final conditions in hypothetical patients P1 – P5 and initial steps in GAMS

Patient 1 (P1)				
	Naïve initial (0 day)	Naïve final (1000 day)	Binet A initial (1000 day)	Binet A final (3190 day)
N_{BM} ($\cdot 10^8$ cells)	380.000	380.010	380.010	667.000
N_{LN} ($\cdot 10^8$ cells)	0.010	912.000	912.000	3662.000
N_{PB} ($\cdot 10^8$ cells)	300.000	329.000	329.000	660.000
P_{BM} ($\cdot 10^8$ cells)	10.500	13.500	13.500	19.000
P_{LN} ($\cdot 10^8$ cells)	0.000	42.000	42.000	172.500
P_{PB} ($\cdot 10^8$ cells)	0.900	1.500	1.500	2.380
What changed to 2^{nd} stage	only θ s and migration rates change in GAMS			
Patient 2 (P2)				
	Binet A initial (0 day)	Binet A final (1800 day)	Binet B initial (1800 day)	Binet B final (2165 day)
N_{BM} ($\cdot 10^8$ cells)	800.000	1108.000	1108.000	1815.000
N_{LN} ($\cdot 10^8$ cells)	1300.000	2075.000	2075.000	2556.000
N_{PB} ($\cdot 10^8$ cells)	400.000	661.000	661.000	1423.000
P_{BM} ($\cdot 10^8$ cells)	15.900	33.110	33.110	60.000
P_{LN} ($\cdot 10^8$ cells)	40.000	97.800	97.800	132.000
P_{PB} ($\cdot 10^8$ cells)	0.810	2.380	2.380	5.140
What changed to 2^{nd} stage	all parameters change in GAMS except τ s			
Patient 3 (P3)				
	Binet A initial (0 day)	Binet A final (365 day)	Binet B initial (365 day)	Binet B final (730 day)
N_{BM} ($\cdot 10^8$ cells)	800.000	1108.000	1108.000	1750.000
N_{LN} ($\cdot 10^8$ cells)	1300.000	2550.000	2550.000	3877.000
N_{PB} ($\cdot 10^8$ cells)	400.000	862.000	862.000	1826.000
P_{BM} ($\cdot 10^8$ cells)	15.900	33.110	33.110	58.000
P_{LN} ($\cdot 10^8$ cells)	40.000	120.158	120.158	212.000
P_{PB} ($\cdot 10^8$ cells)	0.810	3.113	3.113	6.591
What changed to 2^{nd} stage	only θ s and migration rates change in GAMS			
Patient 4 (P4)				
	Binet B initial (0 day)	Binet B final (365 day)	Binet C initial (365 day)	Binet C final (730 day)
N_{BM} ($\cdot 10^8$ cells)	1100.000	1933.000	1933.000	4934.000
N_{LN} ($\cdot 10^8$ cells)	1300.000	15806.000	15806.000	38334.000
N_{PB} ($\cdot 10^8$ cells)	4200.000	9140.000	9140.000	18332.000
P_{BM} ($\cdot 10^8$ cells)	34.129	57.110	57.110	158.000
P_{LN} ($\cdot 10^8$ cells)	26.000	744.158	744.158	2317.000
P_{PB} ($\cdot 10^8$ cells)	8.410	33.114	33.114	59.591
What changed to 2^{nd} stage	only θ s and migration rates change in GAMS			
Patient 5 (P5)				
	Binet A initial (0 day)	Binet A final (365 day)	Binet C initial (365 day)	Binet C final (730 day)
N_{BM} ($\cdot 10^8$ cells)	390.000	390.410	390.410	7030.000
N_{LN} ($\cdot 10^8$ cells)	1000.000	2455.150	2455.150	7832.000
N_{PB} ($\cdot 10^8$ cells)	900.000	1029.000	1029.000	2666.000
P_{BM} ($\cdot 10^8$ cells)	7.000	7.390	7.390	210.000
P_{LN} ($\cdot 10^8$ cells)	47.110	115.500	115.500	545.000
P_{PB} ($\cdot 10^8$ cells)	2.000	3.713	3.713	10.000
What changed to 2^{nd} stage	all parameters change in GAMS except τ s			

The sensitivity indices are given by:

$$S_{i_1, \dots, i_s} = \frac{D_{i_1, \dots, i_s}}{D}, 1 \leq i_1 < \dots < i_s \leq n$$

so that all terms add up to 1:

$$\sum_{i=1}^n S_i + \sum_{1 \leq i < j \leq n} S_{i,j} + \dots + S_{1,2,\dots,n} = 1$$

The main effect of the input variable x_i on the output is calculated by the first order sensitivity index S_i while the effect of x_i and x_j on the output is given by the second order sensitivity indices and so on. Fig. parameter ranking is done for each of the model outputs.

Global sensitivity analysis of two compartments, like that in Fig. A.1 with initial conditions $N_0 = 1809 \cdot 10^8$ cells, $P_0 = 54 \cdot 10^8$ cells and $N/\theta \ll 20$ (proliferation), shows that the most important parameters in the non-proliferative population are $\theta_1 > \theta_2 > \gamma > \beta_0 > \tau > \delta$ (Fig. F.1) and the most important parameters in the proliferative population are $\gamma > \theta_1 > \tau > \beta_0 > \theta_2 > \delta$ (Fig. F.1). θ_1 increases both the non-proliferative and proliferative populations but γ decreases the non-proliferative population and increases the proliferative population because it decreases the rate of cells which pass through the proliferative phase.

Table F.1 contains parameters and their relevant ranges for sensitivity analysis; we have previously analyzed a

TABLE F.1: Ranges of parameters used in RS-HDMR analysis of CLL framework from Binet Stages A to C

Parameter (Units)	Nominal Value	Minimum Value	Maximum Value	Ref.
γ_{BM} (1/day)	0.565	0.091	0.823	Table 4
γ_{LN} (1/day)	0.706	0.530	0.866	Table 4
γ_{PB} (1/day)	0.001	0.001	0.003	Table 4
δ_{BM} (1/day)	0.001	0.001	0.037	Table 4
δ_{LN} (1/day)	0.001	0.001	0.020	Table 4
δ_{PB} (1/day)	0.001	0.001	0.003	Table 4
τ_{BM} (day)	0.792	0.625	0.792	[29]
τ_{LN} (day)	0.792	0.625	0.792	[29]
τ_{PB} (day)	0.792	0.625	0.792	[29]
$\theta_{BM,1}$ ($\cdot 10^8$ cells)	0.010	0.004	6.209	Table 4
$\theta_{BM,2}$ ($\cdot 10^8$ cells)		$3 \leq N_{BM,0}/\theta_{BM,2} \leq 5$		Table 4
$\theta_{LN,1}$ ($\cdot 10^8$ cells)	0.279	0.154	19.297	Table 4
$\theta_{LN,2}$ ($\cdot 10^8$ cells)		$3 \leq N_{LN,0}/\theta_{LN,2} \leq 4.5$		Table 4
$\theta_{PB,1}$ ($\cdot 10^8$ cells)	0.001	0.001	1.259	Table 4
$\theta_{PB,2}$ ($\cdot 10^8$ cells)		$N_{PB,0}/\theta_{PB,2} \geq 20$		Table 4
$k_{N,BM2PB}$ (1/day)	0.003	0.001	0.035	Table 4
$k_{P,BM2PB}$ (1/day)	0.001	0.001	0.003	Table 4
$k_{N,PB2LN}$ (1/day)	0.003	0.001	0.005	Table 4
$k_{P,PB2LN}$ (1/day)	0.013	0.001	0.033	Table 4
n_{BM}	3	2	4	[23, 43]
n_{LN}	3	2	4	[23, 43]
n_{PB}	3	2	4	[23, 43]
$\beta_{N_{BM},0}$ (1/day)	3.350	1.690 (-50%)	5.070 (+50%)	[25]
$\beta_{N_{LN},0}$ (1/day)	3.350	1.690 (-50%)	5.070 (+50%)	[25]
$\beta_{N_{PB},0}$ (1/day)	3.350	1.690 (-50%)	5.070 (+50%)	[25]
a_{BM}	0.25	0.09	0.37	[38]
BM_{max} ($\cdot 10^8$ cells)	13500	6750(-50%)	20250(+50%)	Appendix B
$C_{NC,BM}$ ($\cdot 10^8$ cells)	500	250(-50%)	750(+50%)	Table 2
$N_{BM,0}$ ($\cdot 10^8$ cells)	380.000	380.000	2000.000	Table 4
$N_{LN,0}$ ($\cdot 10^8$ cells)	1000.000	1000.000	15000.000	Table 4
$N_{PB,0}$ ($\cdot 10^8$ cells)	300.000	300.000	9000.000	Table 4

 Fig. F.1: Global sensitivity analysis of a two compartmental model in which all the parameters change $\pm 50\%$ except $\tau \in [0.625, 0.792]$ day for 2000 steps

subset of these parameters on a different CLL model [18]. Table F.1 lists ranges for non-proliferative initial conditions but not for proliferative initial conditions because non-proliferative and proliferative tissue are related with respect to Ki-67 measurements (Table 5). In the analysis, the maximum order for approximation of the first and second order component functions is 10 and 5, respectively. Ten iterations are used for both the

first and second order component functions.

APPENDIX G

CALCULATING THE NUMBER OF ENLARGED LYMPH NODES AND PREDICTING THE SPLEEN PATTERN

TABLE G.1: Cases of splenomegaly and spleen size [54]

Severity	Size (width $\times$ length)
Mild to Moderate	$\geq 5 \times 11$ cm
Marked	$\geq 6 \times 16$ cm
Extreme	$\geq 8 \times 20$ cm

The body has $\approx Nu_{LN} = 800$ LN which are the primary defense mechanism against infection [55]; each LN typically has diameter $d = 1 - 2$ cm and is connected to other LN via lymphatic vessels [49]. We approximate LN as spheres and S as a cylinder. What causes the change of the size of LN and S is the increased number of migrated CLL cells whose shape can be assumed as spheres with volume $V_{CLL} = 166 \cdot 10^{-12} \text{ cm}^3$ [51]. The sequel explains how we estimate the number of enlarged LN when either the S is normal or enlarged.

1) Size & number of enlarged LN with normal S:

Fig. F.2: Average values of $S_i \pm SD$ indices in each non-proliferative and proliferative output for hypothetical patients P1 – P5

- (a) **Calculating LN volume:** Each LN has diameter $d_{LN,NC} \approx 1$ cm, so its volume is:

$$V_{LN} \approx \frac{4}{3} \pi \left(\frac{1 \text{ cm}}{2} \right)^3 = 0.52 \text{ cm}^3 \quad (\text{G.1})$$

- (b) **Counting CLL cells in enlarged LN:** The volume of LN with enlarged diameter $d_{LN,CLL}$ is:

$$V_{CLL,LN} = \frac{4}{3} \pi \left(\frac{d_{LN,CLL}}{2} \right)^3 \quad (\text{G.2})$$

We assume that normal lymphocyte count is constant, i.e., the tissue is not replaced, so the volume of CLL cells in an enlarged LN is $V_{CLL,LN}^*$:

$$V_{CLL,LN}^* = V_{CLL,LN} - V_{LN} \quad (\text{G.3})$$

The number of CLL cells per LN $((N + P)_{CLL,LN})$ are calculated by dividing the volume of CLL cells (V_{CLL}) in LN:

$$(N + P)_{CLL,LN} = V_{CLL,LN}^* / V_{CLL} \quad (\text{G.4})$$

Calculating the number of enlarged LN with diameter $d_{LN,CLL}$:

$$Nu_{LN, d_{LN,CLL}} = \frac{N_{LN} + P_{LN}}{(N + P)_{CLL,LN}} \quad (\text{G.5})$$

- 2) **Size & number of enlarged LN with enlarged S:**

- (a) **Calculating the total volume of S:** Normally, S has diameter $d_{S,NC} \approx 5$ cm and length $l_{S,NC} \approx 11$ cm; splenomegaly severity is given in Table G.1. Assuming S is a cylinder, the S volume (V_S) is:

$$V_S = \pi (5 \text{ cm}/2)^2 11 \text{ cm} = 215.86 \text{ cm}^3 \quad (\text{G.6})$$

Eq (G.6) agrees with experiment, $V_S = 147.50 \pm 81.46 \text{ cm}^3$ [50]. We assume that the normal lymphocyte count and the membrane volume are constant.

- (b) **Calculating the volume of enlarged S and the number of CLL cells.** The dimensions of the enlarged S are given by the physician. If the measured thickness of the enlarged S is $d_{S,CLL}$ and its measured length is $l_{S,CLL}$, the volume of CLL cells in S ($V_{CLL,S}$) is given by:

$$V_{CLL,S} = \pi (d_{S,CLL}/2)^2 l_{S,CLL} - V_S \quad (\text{G.7})$$

The number of CLL cells are calculated $(N + P)_{CLL,S}$, by dividing the volume of CLL cells in S with the volume of one CLL cell:

$$(N + P)_{CLL,S} = V_{CLL,S} / V_{CLL} \quad (\text{G.8})$$

For cases with enlarged S, the number of enlarged LN is calculated by subtracting the number of CLL

cells in S from the total number of CLL cells and repeating the steps for calculating $Nu_{LN, d_{LN, CLL}}$.

APPENDIX H INITIALIZATION

Because the Appendix D procedure begins with solving a feasibility problem, there are multiple starting points for the algorithm. We cannot prove that the algorithm will always converge to the same point, but this section demonstrates our computational experience that results of the Appendix D algorithm give answers within 10% of one another. Tables H.1 & H.2 provide our computational experience with respect to patients P1 and P2. After initialising the GAMS feasibility problem 50 times, we had 4 distinct P1 feasible points (Table H.1) and 3 distinct P2 feasible points (Table H.2). Running the distinct points through the Appendix D algorithm changed some of the parameters (as illustrated with $\rightarrow$ in the tables). See in Fig H.1 that, although the lines are not identical, the trajectories are within 10%.

Fig. H.1: Disease trajectories of P1 and P2 after multi-starting the Appendix D algorithm; observe that the trajectories are similar

TABLE H.1: Results of an initial multistart strategy: Patient 1

Transitions		Case 1		Case 2		Case 3		Case 4	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
		Naïve	A	Naïve	A	Naïve	A	Naïve	A
γ_{BM}	1/day	0.796	1.000	0.796	0.001 $\rightarrow$ 0.792	0.004 $\rightarrow$ 0.724	0.002 $\rightarrow$ 0.767	0.774	0.774
γ_{LN}	1/day	1.000	0.844 $\rightarrow$ 0.858	0.001 $\rightarrow$ 0.867	0.858	0.863	0.001 $\rightarrow$ 0.857	0.861	0.861
γ_{PB}	1/day	0.001	0.008	0.001	0.001	0.001	0.001	0.001	0.001
δ_{BM}	1/day	0.001	0.001	0.001	0.001	0.004	0.002	0.001	0.001
δ_{LN}	1/day	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
δ_{PB}	1/day	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
τ_{BM}	day	0.792	0.792	0.792	0.792	0.792	0.792	0.792	0.792
τ_{LN}	day	0.683	0.792	0.792	0.792	0.790	0.792	0.792	0.792
τ_{PB}	day	0.792	0.792	0.792	0.625	0.792	0.792	0.792	0.792
$\theta_{BM,1}$	$\cdot 10^8$ cells	0.008	0.031	0.008	0.012 $\rightarrow$ 0.052	0.052	0.058	0.008	0.029
$\theta_{BM,2}$	$\cdot 10^8$ cells	92.233	107.835	92.233	37.11	47.986	41.691	91.987	100.082
$\theta_{LN,1}$	$\cdot 10^8$ cells	0.279	0.368	0.059 $\rightarrow$ 0.219	0.368	0.265	0.438	0.265	0.372
$\theta_{LN,2}$	$\cdot 10^8$ cells	0.001	264.655	0.001	265.039	0.001	77.053	0.001	265.039
$\theta_{PB,1}$	$\cdot 10^8$ cells	0.001	0.008	0.001	0.008	0.001	0.008	0.001	0.008
$\theta_{PB,2}$	$\cdot 10^8$ cells	15.000	16.450	15.000	16.450	15.000	16.450	15.000	16.450
$k_{N,BM2PB}$	1/day	0.003	0.002	0.003	0.002	0.003	0.002	0.003	0.003
$k_{P,BM2PB}$	1/day	0.003	0.001	0.003	0.001	0.001	0.001	0.003	0.001
$k_{N,PB2LN}$	1/day	0.003	0.001	0.003	0.001	0.003	0.002	0.003	0.002
$k_{P,PB2LN}$	1/day	0.025	0.001	0.033	0.007	0.008	0.007	0.033	0.007

TABLE H.2: Results of an initial multistart strategy: Patient 2

Transitions		Case 1		Case 2		Case 3	
		Initial	Final	Initial	Final	Initial	Final
		A	B	A	B	A	B
γ_{BM}	1/day	0.770	0.740	0.796 $\rightarrow$ 0.823	0.740	0.002 $\rightarrow$ 0.794	0.091
γ_{LN}	1/day	1.000 $\rightarrow$ 0.995	0.002 $\rightarrow$ 0.619	0.001 $\rightarrow$ 0.859	0.855 $\rightarrow$ 0.860	0.001 $\rightarrow$ 0.858	0.860
γ_{PB}	1/day	0.001	0.001	0.005	0.001	0.001	0.003
δ_{BM}	1/day	0.001	0.001	0.001	0.001	0.002	0.034
δ_{LN}	1/day	0.001	0.020	0.001	0.001	0.001	0.001
δ_{PB}	1/day	0.001	0.001	0.001	0.003	0.001	0.003
τ_{BM}	day	0.792	0.792	0.792	0.792	0.792	0.792
τ_{LN}	day	0.699	0.792	0.792	0.792	0.792	0.792
τ_{PB}	day	0.625	0.625	0.625	0.625	0.792	0.625
$\theta_{BM,1}$	$\cdot 10^8$ cells	0.057	0.526	0.057	0.522	0.118	0.481
$\theta_{BM,2}$	$\cdot 10^8$ cells	173.123	274.733	173.649	274.325	80.337	258.275
$\theta_{LN,1}$	$\cdot 10^8$ cells	0.162	-0.193 $\rightarrow$ 0.293	0.025 $\rightarrow$ 0.225	0.450	0.256	0.451
$\theta_{LN,2}$	$\cdot 10^8$ cells	344.553	524.785	87.137	607.614	87.120	607.436
$\theta_{PB,1}$	$\cdot 10^8$ cells	0.007	0.104	0.007	0.104	0.007	0.104
$\theta_{PB,2}$	$\cdot 10^8$ cells	20.000	33.05	20.000	33.05	20.000	33.05
$k_{N,BM2PB}$	1/day	0.003	0.004	0.001	0.004	0.001	0.004
$k_{P,BM2PB}$	1/day	0.001	0.004	0.001	0.001	0.001	0.001
$k_{N,PB2LN}$	1/day	0.005	0.003	0.001	0.001	0.001	0.001
$k_{P,PB2LN}$	1/day	0.013	0.046	0.014	0.009	0.013	0.007