

## Cancer-Associated Mutations in Healthy Individuals: Assessing the Risk of Carcinogenesis

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### Abstract

Mutations associated with hematopoietic malignancies have been repeatedly identified in healthy individuals. For certain cases, such as the t(14;18) translocation and monoclonal B-cell lymphocytosis, no clear link between the presence of aberrant cells and the later development of cancer has been established. Intriguingly, longitudinal studies suggest that these abnormalities persist for long periods of time in some individuals, but in others are transient in which they disappear completely. Here, we present a mathematical model, based on cellular replication limits, that provides a possible explanation for these seemingly contradictory findings. It proposes that the transient and persistent nature of the phenotypes depends on the stage in the differentiation pathway of a given lineage in which the mutation originates. Our work suggests that cellular replication limits may not only prevent cancer by aborting clonal expansion of cells, but also by influencing the fate of altered but nonneoplastic cells in healthy tissue. *Cancer Res*; 74(6); 1661–9. ©2014 AACR.

### Major Findings

If the mutation originates in stem cells, long-term persistence of the mutants is likely, and this poses a significant risk for progression to cancer. If the mutation arises downstream, such as in progenitor cells, the presence of the mutants is likely to be transient and the risk of disease development is significantly lower. We propose experiments based on telomere length measurements that may make it possible to estimate the risk posed in individual patients by cancer-associated mutations found in healthy individuals.

### Introduction

The biologic significance of precancerous mutations in healthy individuals is not well understood. Dozens of chromosomal translocations associated with hematopoietic malignancy have been identified in healthy individuals (1–3). For example, the t(14;18) translocation between the BCL2 gene on chromosome 18 and the immunoglobulin heavy chain on chromosome 14 occurs in approximately 90% of follicular

lymphomas and is considered a hallmark of the disease (4). Yet, it is also found in 30% to 60% of healthy individuals (1) and no clear link between its presence and the later development of follicular lymphoma has been established (5). Another example is monoclonal B-cell lymphocytosis (MBL), which resembles chronic lymphocytic leukemia (CLL). MBL is found in 4% of the population older than 40 years of age (6). All cases of CLL seem to arise from MBL, although the majority of MBL cases do not give rise to proliferative disorders (6). Intriguingly longitudinal studies of both t(14;18) and MBL suggest that these abnormalities persist for long periods of time in some individuals (7–10) and are transient, completely disappearing in others (9–11). In this article, we present a cell lineage model based on the concept of fitness in evolutionary theory and cellular replication limits, which provides an explanation for these phenomena.

Normal somatic cells can undergo a limited number of divisions. If a cell hits this limit, proliferation is halted and the cell either becomes senescent or undergoes apoptosis (12, 13). This limit on the proliferative potential of cells is typically attributed to telomere shortening and is thought to have evolved as a protective mechanism against cancer development (14).

Although the role of telomeres is typically considered in the context of aborting clonal expansion of cells (14), there is significant correlative evidence of telomere attrition in precancerous tissues (15–18). This suggests that replication limits might also play a role in determining the fate of altered but nonneoplastic cells that can potentially be cancer precursors. Here, we analyze the role of replication limits in determining the fate of cancer-associated mutations that arise in cells at different stages in the differentiation pathway of a given lineage. We find that the different proliferative potentials within cells at different stages of differentiation can explain the dynamics of t(14;18) and MBL described above.

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## Quick Guide to Equations and Assumptions

### Major assumptions of the model

The tissue is organized into three fundamentally different types of cells: stem cells, progenitors, and differentiated cells. Stem cells have a full capacity to self-renew and maintain their own numbers through self-replication, whereas the self-renewal potential of progenitors is more limited and by itself insufficient to sustain a constant population size. It is also assumed that cells are subject to the effects of the Hayflick limit.

### Key equations

Equation 2 gives the expected number of mutants in the progenitor compartment when partial fixation takes place. It is expressed in terms of three variables: (i)  $f$ , the ratio of reproduction to elimination events in the progenitor cell population, (ii)  $P$  the number of progenitor cells, and (iii)  $r$  the fitness of mutants. This equation predicts on average what is the fraction of altered (mutant) cells in the progenitor population.

Equation 3 gives necessary conditions for partial fixation in terms of  $f$  and  $r$  (described above). Even for advantageous mutants ( $r > 1$ ), the possibility of establishing a successful colony depends on the degree of self-renewal in progenitors (expressed here in terms of  $f$ ).

Equation 5 gives the probability that partial fixation takes place. It is expressed in terms of the same variables described above and the expected number of mutants  $m$ . Even if a mutation is sufficiently advantageous (as described by Eq. 3) there is a chance that stochastically it will go extinct. This equation computes the probability that a sufficiently advantageous mutation is successful in establishing a long-lived colony.

Equation 6 describes the time-evolution of the average replication capacity of somatic cells, defined as the number of times a cell population can divide until cell division stops (as described by the Hayflick limit). It is expressed in terms of the same fundamental variables and  $\rho$  (the maximum replication capacity of stem cells).

Equations 7 and 8 give the probability of acquiring a subsequent mutation after  $M$  steps (which is a unit of time). It is expressed in terms of the same variables previously described and  $\mu$  is the mutation rate during cell division. In comparing this probability in the absence (Eq. 7) and presence (Eq. 8) of replication limits, it offers a metric to quantify the protection replication limits offer against precancerous mutations.

Human tissues are hierarchically organized into lineages consisting of different cell compartments (19). At the starting points one finds stem cells, which give rise to more differentiated progenitor or transit-amplifying cells (20). The end products are the fully differentiated cells that are mostly postmitotic. If we define a precancerous mutation as one that does not lead to immediate growth, we find that replication limits are unlikely to protect against precancerous mutations that originate in stem cells. A successful stem cell mutant can establish a long-lived colony that spreads into the more differentiated cell compartments. This would explain the persistent mutations found in both t(14;18) and MBL among healthy individuals. Replication limits, however, offer significant protection against mutations occurring downstream in the cell lineage. On the basis of its fitness, a precancerous mutant arising in a progenitor has a certain probability of partially colonizing its compartment. We call this phenomenon partial fixation. When it occurs, the number of mutants remains as a near constant fraction of the entire population (in which the value of this fraction is determined by the biologic parameters of the system). However, there is a very high chance that partial fixation will only be transient, and that mutants will eventually be driven to extinction by the exhaustion of the cellular replicative capacity. These dynamics would seem as a transient mutation in healthy individuals.

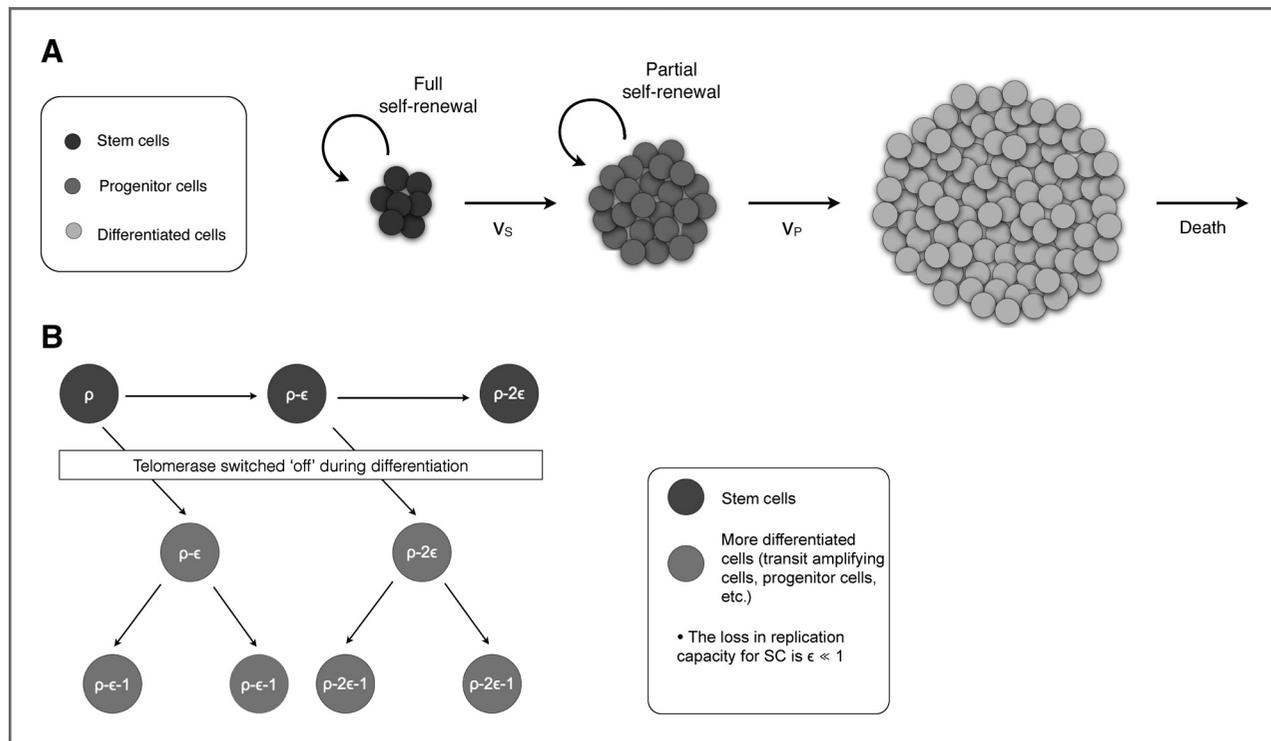
We found necessary conditions for the partial fixation of mutant progenitors and computed the probability and dura-

tion of this type of fixation based on the biologic parameters of the system. Moreover, we quantified the protection that replication limits offer against precancerous mutations by comparing the probabilities of acquiring a second subsequent mutation in the absence and presence of replication limits. Our results strongly suggest that replication limits play a role in reducing the accumulation of sequential mutations at early precancerous stages of tumor development. Furthermore, our findings provide a possible explanation for the poorly understood dynamics of precancerous mutations among healthy individuals. We end by discussing these results in light of existing studies and examining possible clinical applications for determining the risk posed by precancerous mutations in individual patients.

## Materials and Methods

### Model

We considered a system with three types of cells: stem cells, progenitor cells, and differentiated cells. When a stem cell divides the offspring may be two stem cells, two progenitors or one of each. Similarly, division of a progenitor results in two progenitors, two differentiated cells or one of each. On average stem cells produce one stem cell per cell division allowing them to maintain their own numbers through self-replication. The self-renewal potential of progenitors is more limited and by itself insufficient to sustain a constant population size. The flux



**Figure 1.** A, the cell lineage model. The cell population is divided into three compartments: stem cells, progenitor, and differentiated cells. Stem cells have a full capacity to self-renew and maintain their own number through self-replication. Progenitors have only a limited capacity to self-renew. The final products are the fully differentiated cells that die at a certain rate. B, somatic cells have a limited replication capacity and are able to undergo only a few rounds of division. Adult stem cells express telomerase, which significantly increases their replication capacity. However, if the level of telomerase expressed is insufficient to keep a stable telomere length, the replication capacity of stem cells will decrease upon cell division. In either case, the loss in replication capacity of stem cells  $\epsilon$  would be smaller than the loss in progenitor cells (here assumed to be equal to one).

of cells from the stem cell compartment into the progenitor one offsets this loss, keeping the number of progenitors at a constant level. Differentiated cells cannot divide any further and die at a certain rate (See Fig. 1A). This scheme describes a healthy tissue at homeostasis and is, thus, appropriate to model the dynamics of early precancerous mutations that precede abnormal growth. It is important to note, however, that the same scheme (Fig. 1A) might not apply to cancerous tissue in which several factors (e.g., overexpression of specific oncogenes or cellular hypoxia; refs. 21–23) might cause the dedifferentiation of transit-amplifying cells.

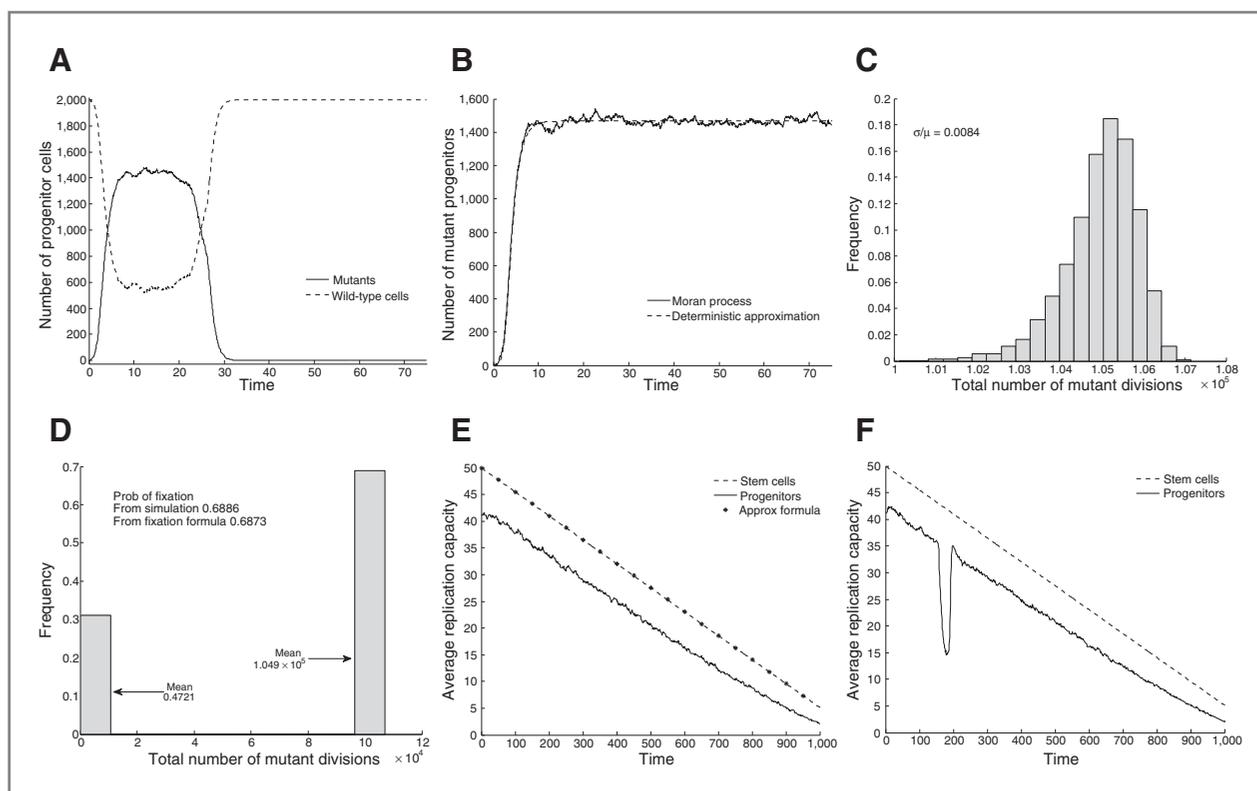
Somatic cells are able to undergo only a few rounds of divisions (typically between 40–60 for embryonic cells; ref. 24). Higher levels of telomerase expression allow adult stem cells to divide significantly more times (25); however, the number of divisions of which they are capable might still be limited (25, 26). We modeled these features by associating to every cell a number that we call its replication capacity (Fig. 1B). When a cell divides, the replication capacity of the offspring will decrease by one unit if the dividing cell is a progenitor or by  $\epsilon$  if it is a stem cell (where  $\epsilon \ll 1$ ).

We were interested in the population dynamics of mutants for a tissue at homeostasis. In the process of carcinogenesis, this corresponds to mutational events that precede abnormal growth. To model this scenario, we kept the number of cells in each compartment constant. This is the same approach used in

the Moran process, which has been extensively used to study mutations in the stem cell compartment (see e.g., refs. 27, 28). In each Moran step, two individuals are chosen: one for reproduction and one for elimination (the same individual can be chosen for both). Mutants have a given fitness  $r$  compared with a fitness of one for wild-type individuals. If the size of the entire population is  $N$  and there are  $m$  mutants at a given time, then the probability of being selected for reproduction is  $r/(rm + N - m)$  for a mutant and  $1/(rm + N - m)$  for a wild-type cell. All cells have the same probability to be selected for elimination.

In the Moran process, we found that replication limits have little effect on stem cell dynamics. Even if cell division slightly diminishes the replication capacity of stem cells, the loss is probably too small to have a significant effect. In the colon crypt, for example, stem cells divide on average once every 10 days (29, 30), implying roughly 2,500 divisions in 70 years (and as many as 5,000 according to some estimates; ref. 31). On the other hand, the size of the stem cell compartment is small, with as little as four to six cells per crypt (30, 31). For a mutant in a compartment of  $N$  cells, the mean time to fixation is in the order of  $N$  Moran steps (32). Hence, in a small stem cell compartment, the large replication capacity would present no impediment to the ability of a mutant to colonize the entire population.

To study mutations in progenitors, we describe a variation of the Moran process. First, assume that stem cell division is



**Figure 2.** At time  $t = 0$ , a mutation originates in a progenitor. A, the number of mutants first steadily increases and then remains very close to a constant level (we call this partial fixation). Once the replication capacity of the mutant lineage is exhausted, their number drops and the lineage becomes extinct. B, the level of partial fixation depends on mutant fitness and the ratio of reproduction over elimination events (Eq. 1). C, distribution of the total number of mutant divisions when partial fixation occurs (by an arbitrary time  $t = 75$ ). Note the small SD compared with the mean (6,886 simulations). D, total number of divisions up to time  $t = 75$ . Either the mutant progeny is extinguished early on or partial fixation occurs. The estimated probability of partial fixation (Eq. 5) is in excellent agreement with the simulation results ( $10^4$  simulations). E, average replication capacity of stem cells and progenitors as a function of time. F, a mutant is introduced at time  $t = 150$  and partial fixation occurs. As mutants reproduce, the average replication capacity decreases. Eventually, mutants become extinct and are replaced by wild-type cells, causing the average replication capacity to bounce back and resume the expected course. Parameters:  $P = 2,000$ ;  $r = 4$ ;  $f = 0.8$ . Cell-cycle duration equals one unit of time. In A, E, and F, the maximum replication capacity  $\rho_{\max} = 50$ . No replication limits in B to D. See the text for discussion.

asymmetrical, resulting in one stem and one progenitor cell (we considered later symmetrical division). A reproduction event in the Moran process is modeled as a self-renewal event in a progenitor (self-replication). Similarly, a progenitor cell dividing into two differentiated cells results in the elimination of the parent cell. At every time step in the progenitor compartment, we chose a cell for differentiation. We could not, however, pick a cell for self-replication at every step, doing so would imply that progenitors have a full capacity to self-renew and the influx of cells from the stem cell compartment would cause uncontrolled growth. Instead at regular time intervals, we skipped the progenitor reproduction step and randomly selected a stem cell for division to replenish the population. More precisely, let  $f$  be the ratio of reproduction over elimination events in progenitors and  $M$  an arbitrary number of steps. Then stem cell division occurs  $(1 - f)M$  times during the course of the  $M$  steps. As before, cell division caused the replication capacity of the offspring to decrease by one unit in progenitors and by  $\epsilon$  in stem cells. If a cell exhausts its replication capacity it is no longer eligible for reproduction, only for elimination. Let  $P_a$  be the total number of progenitors

who have not exhausted their replication capacity and  $m_a$  the corresponding number of mutant cells. Then, the probability of selecting a mutant during a reproduction event is  $rm_a / (rm_a + P_a - m_a)$ . For a related deterministic model that studies the dynamics of neutral mutants in hierarchically organized tissues (including the extinction of mutations originating in non-stem cells), see refs. 33, 34.

## Results

As we previously discussed, replication limits are unlikely to interfere with the ability of a mutant stem cell to colonize its compartment. Hence, from now on, we focus on mutations that originate in progenitor cells. Figure 2A exemplifies the basic features of the system. At time  $t = 0$ , one advantageous mutant ( $r > 1$ ) emerges among progenitors. The number of mutants first steadily increases and then remains very close to a constant level (we call this partial fixation), eventually the mutant replicative capacity is exhausted and the phenotype goes extinct. Note that fixation is only partial because there is a constant reseeding of wild-type cells from the stem cell

compartment. Another possible outcome (not shown) is that the mutant lineage dies very early on before few (if any) reproduction events take place.

In the next sections, we study the behavior of the system. First, we assume that there are no replication limits and calculate the partial fixation level and the probability that it occurs. Then, we study the effects of replication limits and quantify the protection they offer against precancerous mutations. Finally, we present an agent-based model with more freedom than the Moran process and compare the results.

### Partial fixation level

In this and the next section, we assume that there are no replication limits. To calculate the partial fixation level, we considered a deterministic approximation of the model. Let  $m_k$  be the number of mutants at time step  $k$  and  $P$  the total number of progenitors. By making  $m_{k+1} - m_k$  equal to the expected change in the stochastic model, we have

$$m_{k+1} = m_k + \frac{f m_k}{r m_k + P - m_k} - \frac{m_k}{P} \quad (1)$$

The partial fixation level equals the nonzero steady-state number of mutants  $\tilde{m}$ , which is

$$\tilde{m} = \frac{r f - 1}{r - 1} P \quad (2)$$

To analyze the stability of the fixed point  $\tilde{m}$ , consider the function  $F$  that satisfies  $m_{k+1} = F(m_k)$ . The fixed point is stable if and only if  $|F'(\tilde{m})| < 1$ . We have

$$|F'(\tilde{m})| = \left| 1 + \frac{1}{r P f} - \frac{1}{P} \right| < 1 \Leftrightarrow 1 < r f \quad (3)$$

Hence, we found that there is a unique nonzero stable number of mutant cells if and only if the fitness of mutants  $r$  and the ratio of reproduction events to differentiation events satisfy  $1 < r f$ . [Note that because of  $f < 1$ , this implies that only advantageous mutants ( $r > 1$ ) are capable of partial fixation.] In Fig. 2B, we compared results from the deterministic method with a stochastic simulation. The value of  $\tilde{m}$  predicts with remarkable accuracy the partial fixation level observed in the simulation.

It is important to understand why in the stochastic setting the number of mutants remains close to  $\tilde{m}$ . Note that the expected change in the mutant population size equals

$$\frac{(f r m - f m)(P - m)}{(r m + P - m) P} - \frac{(1 - f) m}{P} \quad (4)$$

Hence, if  $m > \tilde{m}$ , then on average the next event will decrease the value of  $m$ , and if  $m < \tilde{m}$  on average the next event will increase the value of  $m$ . Therefore, in the vicinity of  $\tilde{m}$  a control mechanism is essentially in place. The control on the number of mutant progenitors is apparent when we look at the very small variance relative to the expected total number of mutant divisions (Fig. 2C).

### Probability of partial fixation

There is an inherent control mechanism (Eq. 4) that drives the system toward the steady state. However, its effectiveness

depends on having a relatively large number of mutants so that the law of large numbers applies. Given that a mutation originates in just one cell, the fate of the mutant lineage is initially governed by the effects of random fluctuations. To estimate the probability of partial fixation, we calculated the probability that the mutant population reaches the steady state  $\tilde{m}$  before it goes extinct.

After each full Moran step, there are three possible outcomes: the value of  $m$  increases by one, decreases by one, or remains the same. If there are  $m$  mutants at time step  $t$ , the probability that at time  $t + 1$  the value of  $m$  increases by 1 is  $a(m) = \frac{f r m}{r m + P - m} \times \frac{P - m}{P}$ . The probability that  $m$  decreases by 1 is  $b(m) = \frac{f(P - m)}{r m + P - m} \times \frac{m}{P} + \frac{(1 - f) m}{P}$ . Now using a result from birth-death processes (35), the probability that the mutant population reaches the steady state  $\tilde{m}$  before it reaches zero is  $\left[ 1 + \sum_{k=1}^{\tilde{m}-1} \prod_{j=1}^k \frac{b(j)}{a(j)} \right]^{-1}$ . The estimated probability of reaching partial fixations  $R$  is then equal to

$$R = \left( 1 + \sum_{k=1}^{\tilde{m}-1} \prod_{m=1}^k \frac{(P - m) + (1 - f) r m}{f(P - m) r} \right)^{-1} \quad (5)$$

Simulation results suggest that Eq. 5 gives a very good approximation. In Fig. 2D, we plotted the frequency of times mutants reached partial fixation. The results differed in less than 0.2% from the estimated probability.

### Replication limits

We then focused on the replication capacity of cells. First, assume that stem cells express enough telomerase to keep telomere length constant (i.e.,  $\epsilon = 0$ ). This translates into stem cells having a constant replication capacity  $\rho$ . Without mutants the expected replication capacity "a" of progenitors satisfies,  $a P = a P - f a + 2 f(a - 1) - a + (1 - f)(\rho - 1)$ , which means that  $a = \rho - 1 - 2 f / (1 - f)$ .

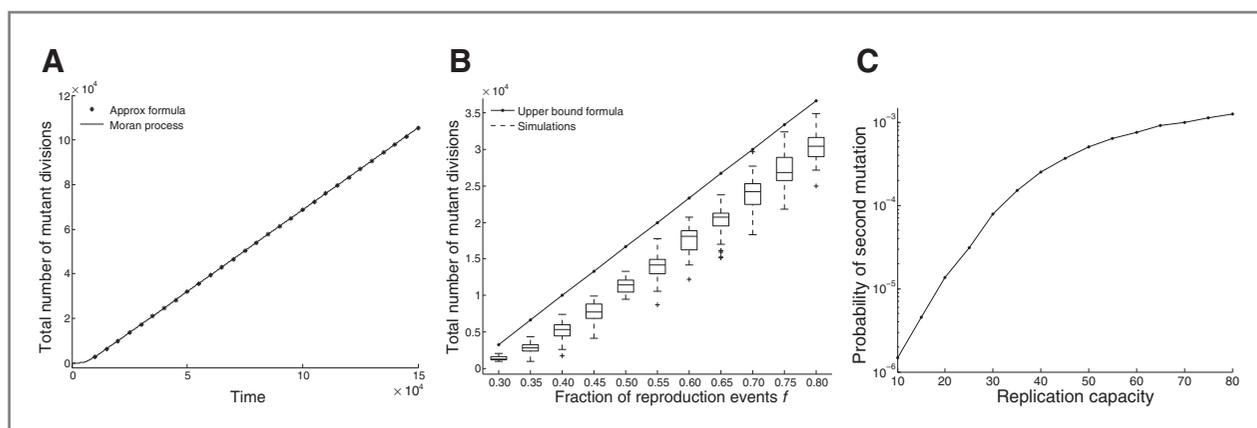
Now assume that when a stem cell divides its replication capacity, it decreases by  $\epsilon > 0$ . In the deterministic approximation (Eq. 1) call  $a_k$  the expected replication capacity of progenitors at time step  $k$ . We arrived at the following recursive relation,  $a_{k+1} = f a_k - \epsilon (1 - f) k + (1 - f)(\rho - 1) - 2 f$ . Calling  $\alpha = f$ ,  $\beta = -\epsilon (1 - f)$  and  $\gamma = (1 - f)(\rho - 1) - 2 f$  we had  $a_{k+1} = \alpha a_k + \beta k + \gamma$ . From where we found

$$a_k = \alpha^k \left( a_0 + \sum_{i=0}^{k-1} \frac{\beta i + \gamma}{\alpha^i + 1} \right)$$

Making  $x = 1/\alpha$ , the last expression may be written as

$$a_k = \frac{a_0}{x^k} + \frac{[\beta k + \gamma] x^{k+2} + [\beta(k+1) + \gamma] x^{k+1} + [\beta - \gamma] x^2 + \gamma x}{x^{k+2} - 2 x^{k+1} + x^k} \quad (6)$$

Because  $x > 1$ , for  $k$  large the expected replication capacity  $a_k \approx \beta k + \gamma = -\epsilon (1 - f) k + (1 - f)(\rho - 1) - 2 f$ . For small values of  $k$ , on the other hand, the value of  $a_k$  is governed by  $a_0/x^k$ . Hence,  $a_k \approx a_0 f^k$  for  $k$  small. We find then that the expected replication capacity of the progenitor cell population is characterized by a brief initial exponential phase followed by a quasi-linear phase independent of its initial value.



**Figure 3.** A, cumulative number of mutant divisions (no replication limits) from simulation and formula. B, cumulative number of mutant divisions (with replication limits). For each value of  $f$ , 50 simulations are considered, and the total number of mutant divisions up to time  $t = 75$  is used to produce the box plots. The upper bound formula is also plotted. C, probability of acquiring a second mutation as a function of the replication capacity of the original mutant (2,000 simulations/point). Parameters: In all,  $P = 2,000$ ; A,  $f = 0.8$  and  $r = 4$ ; B,  $r = 4$ ,  $\rho_{max} = 50$ , and  $\epsilon = 0.05$ ; C,  $f = 0.8$  and  $r = 2$ . Cell-cycle duration equals one unit of time.

In stochastic simulations, we observed the same patterns in the average replication capacity (Fig. 2E). In Fig. 2F, a mutant is introduced at time  $t = 150$  and partial fixation occurs. Note that the average replication capacity of progenitors sharply decreases during the period of partial fixation. As the number of mutant reproduction events increases, their reproduction capacities decrease. Eventually, it is completely depleted and the lineage becomes extinct. As mutants are replaced by wild-type individuals, the average replication capacity bounces back and resumes the expected course.

#### Protection against precancerous mutations

Next, we quantified the protection replication limits offer against precancerous mutations. Suppose that there were no replication limits, then any mutant lineage that reaches partial fixation would persist throughout the hosts life-span. Given that control mechanisms keep the number of mutants very close to the steady-state value, the variance in the number of mutant divisions is very small (Fig. 2C). Hence, we can predict the expected number of mutant divisions during a given time interval with great accuracy (Fig. 3A). After  $M$  time steps, this quantity equals  $Mfr\bar{m}/(r\bar{m} + P - \bar{m})$ .

The multistep theory of carcinogenesis requires that at least one additional mutation occurs in one of the altered (mutant) cells. If the mutation rate during cell division equals  $\mu$ , then the probability of acquiring a second mutation after  $M$  steps without replication limits is approximately

$$R[(1 - \mu)^{Mfr\bar{m}/(r\bar{m} + P - \bar{m})}] \quad (7)$$

If replication limits are in place, not only might partial fixation be impossible (depending on the replication capacity of the original mutant), but even if it occurs the mutant population would only be able to undergo a limited number of divisions. We looked for an upper bound to the expected number of mutant divisions with replication limits. Now, in a population of  $N$  individuals the expected loss in the average replication capacity after one Moran step is less than or equal

to  $2/N$ . This occurs because the expected replication capacity of the individual selected for reproduction is greater or equal than that of the individual selected for elimination. Hence, if we call  $\rho_{max}$  the maximum replication capacity of progenitors, an upper bound for the expected number of divisions is  $\rho_{max}\bar{m}/2$  (Fig. 3B). The probability of acquiring a second mutation is then less than or equal to

$$R[1 - (1 - \mu)^{\rho_{max}\bar{m}/2}] \quad (8)$$

Let us look at some numerical examples using Eqs. 7 and 8. Consider a mutation that confers a 50% growth advantage ( $r = 1.5$ ) in a population of  $P = 2,000$  cells, a ratio of reproduction to elimination events  $f = 0.8$  and maximum replication capacity  $\rho_{max} = 50$ . If cells divide on average once a day and the mutation rate  $\mu = 10^{-7}$ , having replication limits reduces the chances that a mutant acquires a second mutation by at least 93% within the first year. After 10 years, without replication limits the probability of a second mutation appearing in the mutant lineage is approximately 0.25. In contrast with replication limits the probability would be less than 0.0022, more than a 100-fold decrease. Figure 3C demonstrates the relationship between the probability of acquiring a second mutation and the replication capacity of the original mutant.

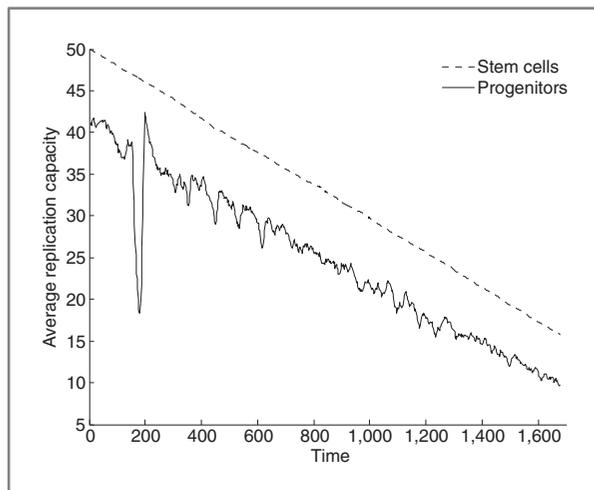
#### Agent-based model

Finally, we looked at an agent-based model in which the nature and time of each event is stochastically determined. This approach requires the introduction of feedback mechanisms to ensure homeostasis (36–38). One such mechanism operates through the secretion of negative feedback factors by differentiated cells, which inhibit division and self-renewal in stem cells and progenitors (39). We modeled feedback using Hill equations. Let  $S$ ,  $P$ , and  $D$  be the time-dependent number of stem cells, progenitors, and differentiated cells,  $v_S$  and  $v_P$  the division rates and  $d$  the death rate of differentiated cells. Stem cell division is symmetrical, resulting in two stem cells with

probability  $p_S$  or two progenitors with probability  $1 - p_S$ . Progenitor division results in two progenitors with probability  $p_P$  or two differentiated cells with probability  $1 - p_P$ . The division rates and self-renewal probabilities are functions of  $D$ ,  $p_S = p_0/(1 + h_0D)$ ,  $p_P = p_1/(1 + h_1D)$ ,  $v_S = v_0/(1 + g_0D)$  and  $p_S = v_1/(1 + g_1D)$ . Replication limits are dealt with as before. A full description of the algorithm is found in the Supplementary Information.

We discuss the link between the Moran process and the agent-based model. The probability of self-renewal in progenitors is related to the fraction  $f$  of reproduction over elimination events through the equation  $f = p_P/(1 - p_P)$ . At equilibrium the number of stem cell divisions per unit of time  $v_S S = v_P(1 - f)P$ , which corresponds to the interspacing of stem cell divisions used in the Moran process. Finally at equilibrium the self-renewal probability of stem cells is 0.5, which on average results in the same flux of cells from stem cells into the progenitor compartment than if we assume a fixed population size  $S$  and use asymmetric stem cell divisions.

The cell dynamics in the agent-based model exhibit the same fundamental features. The mutant lineage either fades away quickly or reaches partial fixation. If partial fixation occurs, replication limits ensure that eventually mutants go extinct and the average replication capacity resumes its projected course (Fig. 4). The probability and level of partial fixation are also accurately predicted by Eqs. 2 and 5 (Supplementary Fig. S1). The number of mutants might show greater oscillations around the steady state (Supplementary Fig. S1A), indicative of the fact that the number of progenitors is not constant. The cumulative number of mutant division, however, shows excellent agreement with the derived formulas (Supplementary Fig. S1B and S1C) supporting the accuracy of Eq. 7 and the overall validity of our results.



**Figure 4.** Agent-based model. Average replication capacity as a function of time. At time  $t = 150$ , a mutant is introduced. The overall cell dynamics follow the same pattern as the constant population model (compare with Fig. 2E). Partial fixation occurs. Parameters:  $P = 2,000$ ;  $f = 0.8$ ;  $r = 4$ ;  $\rho_{\max} = 50$ , and  $\epsilon = 0.02$ . Cell-cycle duration equals one unit of time.

## Discussion

In this article, we investigated how cellular replication limits can protect against the persistence of precancerous cell clones that could progress toward malignancy. Cellular replication limits offer very little protection against mutations that arise in stem cells due to their large proliferative potential and small size of the stem cell compartment. If a mutant stem cell colonizes its compartment, the mutation would persist for a long time, spread through the progenitor and differentiated cell populations and increase the chances of acquiring additional mutations that may lead to uncontrolled growth. Alternatively, if the mutation occurs in the progenitor population, then replication limits together with the tissue's architecture protect against the further accumulation of mutations. First, the mutant must be sufficiently advantageous to avoid being driven toward extinction (Eq. 3). Then there is a certain probability (Eq. 5) that it reaches partial fixation. This means that the number of mutants will remain as a near constant fraction of the entire population (Eq. 2), with a certain amount of wild-type cells remaining through reseeded from the stem cell compartment. This partial fixation, however, is transient, i.e., the population crashes to extinction after a time threshold due to exhaustion of the replicative capacity. This severely limits the ability of the cells to evolve and progress further, as clearly shown in our calculations (Eqs. 7 and 8). To bypass replication limits most cells would eventually require the activation of telomerase (14).

### Interpreting data that document the presence of cancer-associated mutations in healthy individuals

The dynamics described in this article, including the phenomenon of partial transient fixation, can be used to interpret clinical studies that document the occurrence of aberrant cells, typically associated with specific cancers, in a relatively large fraction of healthy individuals. This phenotype has been primarily observed in the hematopoietic system presumably due to the ease of sampling blood cells. Two interesting cases in which longitudinal data are available are the translocation  $t(14;18)$  and MBL. In a study of  $t(14;18)$ , 16 healthy individuals carrying the translocation were identified and their status tracked for between 6 and 50 months. In half of these individuals the mutation was recurrently observed at their last examination; in the other half it disappeared. Another study (9) followed a cohort of 76 MBL cases for approximately 3 years. Different types of MBL were distinguished. A subtype called CLL-like MBL persisted in 90% of the individuals throughout the investigation. In contrast, about 50% of the individuals harboring atypical CLL MBL and MBL  $CD5^{(-)}$  at the time of diagnosis lost this condition within 3 years, indicating that the presence of these cells is transient in nature. These results differed from a previous study (7) in which 12 atypical CLL MBL and MBL  $CD5^{(-)}$  cases were shown to persist 1 year after the initial diagnosis. According to our model, these seemingly different findings are not contradictory. The model predicts the possibility that aberrant cells can persist at stable levels for defined periods of time after which they go extinct due to the exhaustion of replicative potential. These observations underscore the need to conduct more longitudinal studies with longer time horizons and larger sample sizes.

Although chromosomal translocations are a defining feature of blood cancers and MBL is a precondition for CLL, they are evidently not sufficient on their own to drive uncontrolled proliferation. Understanding the nature of nonneoplastic cells with abnormalities is crucial to shed light onto their role as cancer precursors. The most important question is whether these cells could in some cases become the founders of uncontrolled growth, and how likely such a transformation could be. Our model indicates that clones originating in progenitors do not pose a significant risk and need not be considered cancer precursors. On the other hand, if the mutation arises in the tissue stem cells, then the cell clones are likely to persist longer and penetrate the progenitor cell population. In this case, the risk of these cells acquiring further mutations is significantly elevated.

Our model is also consistent with the puzzling weak correlation between the presence of t(14;18) in healthy individuals and age. Although some studies suggest an age-dependent increase in the frequency of t(14;18)-positive cells among healthy individuals (40, 41), most studies show no association with age (42–44), including one with a large sample of 204 individuals (5). If all mutations resulting in t(14;18) translocations were always persistent, one should expect to see a clear correlation between its incidence and age.

#### Translatable and testable insights

Our analysis suggests that telomere length can be used to distinguish between mutations originating in progenitors versus stem cells. According to our model, if the transient cases of these mutations indeed do originate in progenitors, then one should expect to see an increased rate of telomere shortening in these cells. In contrast, the persistent mutants, most likely originating in stem cells, should show less signs of telomere attrition.

This hypothesis can be validated with experiments, and if correct, can be translated into a clinical test to determine whether cancer-associated mutations in healthy individuals pose an increased risk for the development of cancer or not. To test this notion, cohorts of healthy people that harbor aberrant cells should be followed longitudinally to determine the fate of these cells, i.e., whether their presence is transient or not. This analysis can be performed using the same experimental techniques described in the studies discussed above (1–11). When testing for the presence of aberrant cells in the blood, the average telomere length should be determined both in the aberrant cells and in the normal cells of these individuals (in blood average, telomere length can be efficiently measured using flow FISH cytometry; ref. 45). If the mutation originated in progenitor cells, we expect to see that the telomere length of

the aberrant cells is significantly shorter than that of the normal cells, and that this difference becomes amplified over time. In these individuals, the presence of the aberrant cells should be transient, leading to their extinction over time. On the other hand, if the mutation originated in stem cells, then there should not be a significant difference in telomere length between aberrant and normal cells, and in these individuals, the aberrant cells are expected to remain present in the long-term without going extinct.

If such longitudinal studies confirm the model predictions presented here, then a single blood test in healthy people that carry cells with cancer-associated mutations could determine whether these cells pose an increased risk for cancer development or not. This blood test should compare the average telomere length in normal and aberrant cells in the patient. If the telomere length of aberrant cells is significantly shorter than that of normal cells, no increased cancer risk is indicated and no long-term monitoring is required. In contrast, if normal and aberrant cells show similar average telomere lengths, then an increased risk of carcinogenesis is indicated and the patient should be monitored regularly over time. Hence, the insights generated in this article can not only improve our knowledge about the meaning of cancer-associated mutations in healthy individuals, but can also give rise to relatively simple clinical tests to determine risk and long-term management strategies. At the moment, these tests and strategies are feasible mostly in the context of hematopoietic malignancies, in which transient and persistent cancer-associated mutations have been identified and cell sampling is easy.

The same principles, however, could also be relevant to cancer-associated mutations in solid tissues.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Authors' Contributions

**Conception and design:** I.A. Rodriguez-Brenes, D. Wodarz  
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**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** I.A. Rodriguez-Brenes, N.L. Komarova  
**Writing, review, and/or revision of the manuscript:** I.A. Rodriguez-Brenes, N.L. Komarova, D. Wodarz  
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** N.L. Komarova

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## Cancer-Associated Mutations in Healthy Individuals: Assessing the Risk of Carcinogenesis

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