Successful Therapy Must Eradicate Cancer Stem Cells

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ABSTRACT

Despite significant improvements in cancer therapy, tumor recurrence is frequent and can be due to a variety of mechanisms, including the evolution of resistance and tumor progression. Cancer stem cells have been postulated to maintain tumor growth similar to normal stem cells maintaining tissue homeostasis. Recently, the existence of these malignant stem cells has been proven for hematological as well as some solid tumors. Tumor stem cells are not targeted by standard therapy and might be responsible for treatment failure and tumor recurrence in many patients. We designed a simple mathematical model to demonstrate the importance of eliminating tumor stem cells. We explored different therapeutic scenarios to illustrate the properties required from novel therapeutic agents for successful tumor treatment. We show that successful therapy must eradicate tumor stem cells. STEM CELLS *2006;24:2603–2610*

INTRODUCTION

Human bone marrow is a site of very active cell proliferation to maintain homeostasis of circulating blood cells. The healthy bone marrow has considerable reserves for an acute increase in demand for cellular output in response to bleeding or infection. Normal cell turnover translates in the production of approximately 10^{12} cells every day [1]. Cellular proliferation harbors the risk of acquiring mutations because the genome replication machinery is not perfect. Serial accumulation of mutations increases the probability of malignant transformation, especially if the mutations occur in long-lived cells [2]. The hierarchical structure of the hematopoietic system might have evolved to prevent the development of cancer: the long-lived stem cells divide rarely, and only a small number of stem cells might actively contribute to hematopoiesis at any time [3, 4]. Such a tissue organization minimizes the risk of malignant transformation and is seen not only in the bone marrow but also in epithelia with significant cell turnover [2, 5–7].

Stem cells display several phenotypic characteristics that are considered critical for the acquisition of the tumor phenotype. These include the potential for unlimited cell replication, self-sufficiency, and long-term survival [4, 8]. It is thus likely that many hematologic malignancies, such as myeloproliferative disorders and chronic myeloid leukemia (CML), originate from the hematopoietic stem cell compartment [9]. There is increasing evidence that solid tumors also have a hierarchical structure with a small tumor stem cell compartment that maintains the growth of the rest of the tumor [4, 10, 11]. As compared with mature blood cells, stem cells are intrinsically less sensitive to the effects of chemotherapy, due to a variety of mechanisms, including adhesion to the extracellular matrix and the production of membrane proteins that pump out many different chemotherapeutic agents [12–14]. Unfortunately, tumor stem cells similarly express these phenotypic characteristics. Tumor stem cell insensitivity to therapy may be a critical determinant of success or failure when a patient is treated.

Mathematical modeling of cancer was initiated in the 1950s [15–18] and has led to considerable insight into the disease [5, 11, 19–23]. In the meantime, specific theories for cancer treatment and resistance have been studied [24–31]. In this paper, we develop a quantitative understanding of the dynamics of cancer therapy. We designed a simple mathematical model to study the response to treatment, and we demonstrate the critical need for tumor stem cell eradication for successful therapy. Although the model is inspired by malignancies of the hematopoietic system [11], it applies to all tumors that are maintained by tumor stem cells and provides significant insights into the treatment effects of diverse types of drugs.

The Mathematical Model

Our mathematical model considers two layers of the differentiation hierarchy of the hematopoietic system: stem cells have the potential for indefinite self-renewal and give rise to differentiated cells that perform the functions of mature blood. Here, we
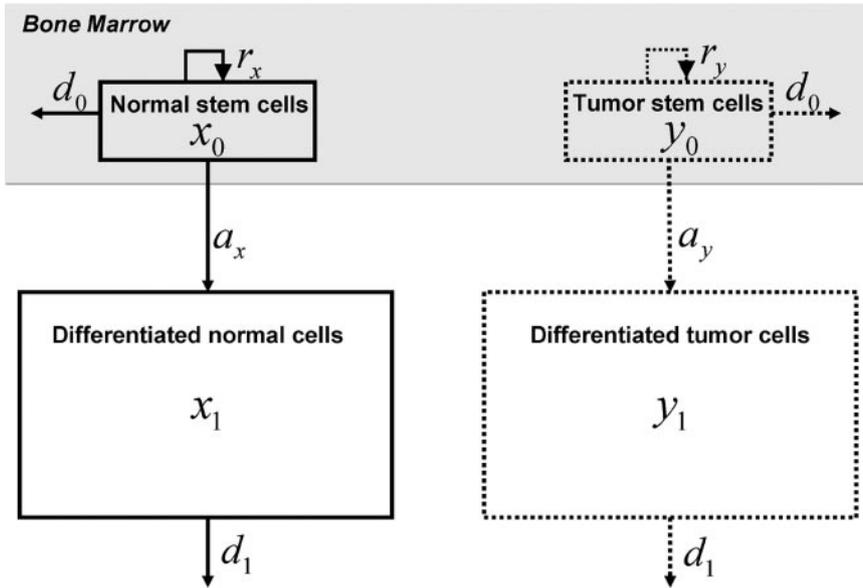
Figure 1. Schematic representation of a two-compartment model of hematopoiesis. Normal and tumor stem cells proliferate and give rise to progeny mature cells that form the bulk of the tumor. Both stem cells and mature cells die, and steady-state levels of normal stem cells in the absence of disease are due to feedback from the stem cell population itself. Details of the model are as given in the text.

define differentiated cells as all cells that do not have stem cell characteristics. This hierarchy applies both to normal and tumor cells (Fig. 1). Denote the abundances of normal stem cells and differentiated cells by x_0 and x_1, and the abundances of tumor stem cells and differentiated cells by y_0 and y_1, respectively. Normal stem cells divide at rate r_x per day and die at rate d_0 per day. Normal differentiated cells are produced from normal stem cells at rate a_x per day and die at rate d_1 per day. Tumor stem cells divide at rate r_y per day and die at rate d_0 per day. Differentiated tumor cells are produced from tumor stem cells at rate a_y per day and die at rate d_1 per day. Hence, the basic model is given by

$$\dot{x}_0 = [r_x \phi - d_0]x_0$$

$$\dot{x}_1 = a_x x_0 - d_1 x_1$$

$$\dot{y}_0 = [r_y \varphi - d_0]y_0$$

$$\dot{y}_1 = a_y y_0 - d_1 y_1 \qquad (1).$$

Here, $\dot{x}_0 = dx_0/dt$ denotes the time derivative. Homeostasis of normal stem cells is achieved by the function $\phi = 1/[1 + c_x (x_0 + y_0)]$. Similarly, homeostasis of tumor stem cells is achieved by the function $\psi = 1/[1 + c_y (x_0 + y_0)]$. The terms ϕ and ψ introduce competition within the stem cell compartment. In the model, c_x and c_y are dimensionless parameters that simulate the crowding effect that is seen in the bone marrow microenvironment. In the absence of tumor cells, normal cells remain at their equilibrium abundances, given by

$$x_0^* = \frac{1}{c_x}\left(\frac{r_x}{d_0} - 1\right) \text{ and } x_1^* = \frac{a_x x_0^*}{d_1}.$$

Tumor stem cells can grow only if

$$\frac{r_y}{1 + c_y x_0^*} - d_0 > 0$$

This condition can also be written as

$$\frac{1}{c_y}\left(\frac{r_y}{d_0} - 1\right) > \frac{1}{c_x}\left(\frac{r_x}{d_0} - 1\right)$$

If this inequality holds, the tumor initially grows exponentially as $y_0(t) = \exp[(r_y - d_0)t]$. In the basic model, we assume that normal and tumor stem cells as well as normal and tumor differentiated cells have the same respective death rates. This assumption can be changed by assigning individual death rates to the four cell types, d_{x0}, d_{x1}, d_{y0}, and d_{y1}.

Parameter estimates for the model are based on the observation that in a healthy adult, the numbers of hematopoietic stem cells and differentiated cells are approximately 2×10^4 and 1×10^{12}, respectively, although there is some controversy regarding the size of stem cell compartment [32, 33]. Normal stem cells are assumed to divide every 200 days, $r_x = 0.005$ per day, and to die every 500 days, $d_0 = 0.002$ per day. These estimates are based on previous reports in relation to CML [11]. Homeostasis is achieved by setting $c_x = 0.75 \times 10^{-4}$. Normal differentiated cells are produced by normal stem cells at rate $a_x = 1.065 \times 10^7$ per day and die at rate $d_1 = 1$ per day [11]. Tumor stem cells have a selective advantage as compared with normal stem cells. They might have a larger intrinsic growth rate, $r_y > r_x$, or might be less sensitive to environmental crowding, $c_y < c_x$.

Our model describes the whole differentiation hierarchy of the hematopoietic system by only two layers, stem cells and differentiated cells. This approach, although simple, is sufficient to demonstrate the basic principles that we want to show in this paper. Our results carry over to more elaborate models with many layers of the differentiation hierarchy. The model can also be interpreted as describing stem cells and progenitor cells rather than fully differentiated cells, but the implications will be similar.

We perform numerical simulations of Equation 1 to investigate the impact of a therapeutic agent on the success of therapy. For the sake of clarity, therapeutic scenarios are assumed to change one parameter at a time. In the model, therapy decreases a given parameter linearly; for example, imatinib decreases the rate of production of progenitor and mature cells
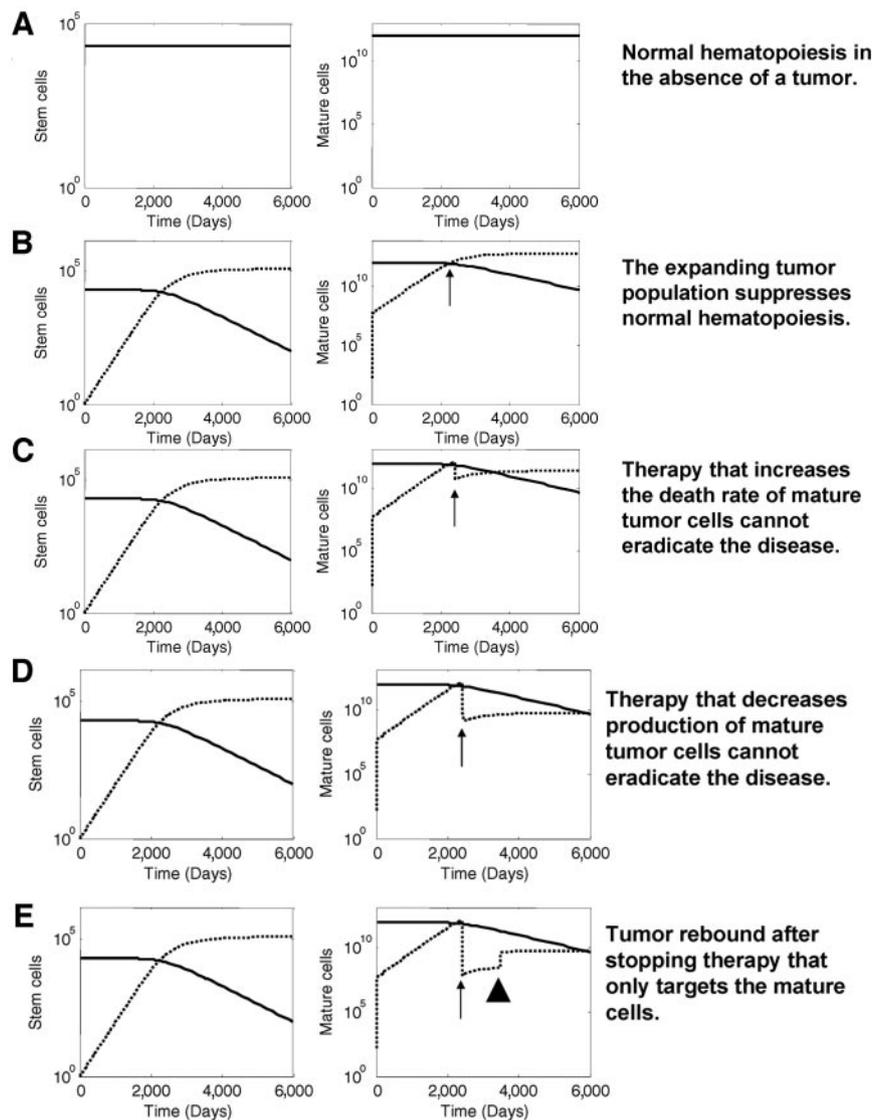
STEM CELLS

Figure 2. Numerical simulation of normal (solid line) and tumor (broken line) hematopoiesis. **(A):** In the absence of leukemia, normal stem cells and mature cells are in a steady state ($r_x = 0.005$, $d_{xO} = 0.002$, $a_x = 1.065 \times 10^7$, $c_x = 0.75 \times 10^{-4}$, and $d_{x1} = 0.213$). The tumor is initiated with one tumor stem cell at time $t = 0$. Initially, tumor stem cells expand exponentially and eventually suppress the population of normal stem cells. **(B):** As a result, the number of differentiated tumor cells increases while differentiated normal cells decrease in abundance ($r_y = 0.0115$, $d_{yo} = 0.002$, $a_y = 1.065 \times 10^7$, $c_y = 0.38 \times 10^{-4}$, and $d_{y1} = 0.213$). **(C):** A therapeutic agent that increases the death rate of differentiated tumor cells, $d_{y1} = 5.0$, administered from day 2,200 (arrow) onwards, leads to an initial decrease in tumor burden, but the continued expansion of the tumor stem cells will ultimately lead to tumor regrowth. **(D):** Therapy that suppresses the rate of generation of mature tumor cells, $a_y = 1.065 \times 10^4$, administered from day 2,200 onwards, cannot control the tumor either. **(E):** If therapy is stopped after 3 years (arrowhead), the tumor burden returns quickly due to the persistent expansion of the stem cell pool. Here, therapy increases the death rate of mature tumor cells, $d_{y1} = 4.26$.

at least 5,000-fold [11]. We define cure as $y_O < 1$ cell, although in practice, the tumor stem cell population perhaps need not die out for a cure to be operationally defined.

To test the sensitivity of the results to the input model parameters, we have studied the outcome of simulations in which each parameter was varied by ±10%. All the results were robust across this range of values.

RESULTS

Basic Model Characteristics

In the absence of tumor cells, $y_0(t) = y_1(t) = 0$ for all times t, Equation 1 gives rise to a steady state with normal numbers of stem cells, $x_0 = 2 \times 10^4$, and mature cells, $x_1 = 10^{12}$ (Fig. 2A). At time $t = 0$, the tumor is initiated with one tumor stem cell, $y_0(0) = 1$ (Fig. 2B). Initially, the tumor stem cell population increases exponentially following $y_0(t) = \exp[(r_y - d_0)t]$. For the purpose of the analysis, we assume that normal and tumor stem cells differentiate at the same rate, $a_x = a_y$. The growth rate and density parameter of tumor stem cells, r_y and c_y, are chosen

such that the tumor stem cells surpass the normal stem cells 6 years after the appearance of the first tumor stem cell. It is thought that this is the time required for CML to be clinically manifest [34]. As soon as the number of tumor stem cells approaches that of normal stem cells, homeostatic mechanisms prevent a large increase of the total stem cell abundance and normal stem cells decline. Both tumor and normal stem cells differentiate to produce mature blood cells. Differentiated normal cells track the decline of normal stem cells, whereas differentiated tumor cells track the increase of the tumor stem cell population (Fig. 2B). In the absence of therapy, the tumor takes over and would ultimately drive the normal blood system to extinction.

Therapeutic Scenarios

Cancer therapy can affect the mature cell compartment, the stem cell compartment, or both. The therapeutic effect may decrease the proliferation rate of tumor stem cells, increase their death rate, slow the rate of production of differentiated tumor cells, increase their death rate, and restore sensitivity to environmental
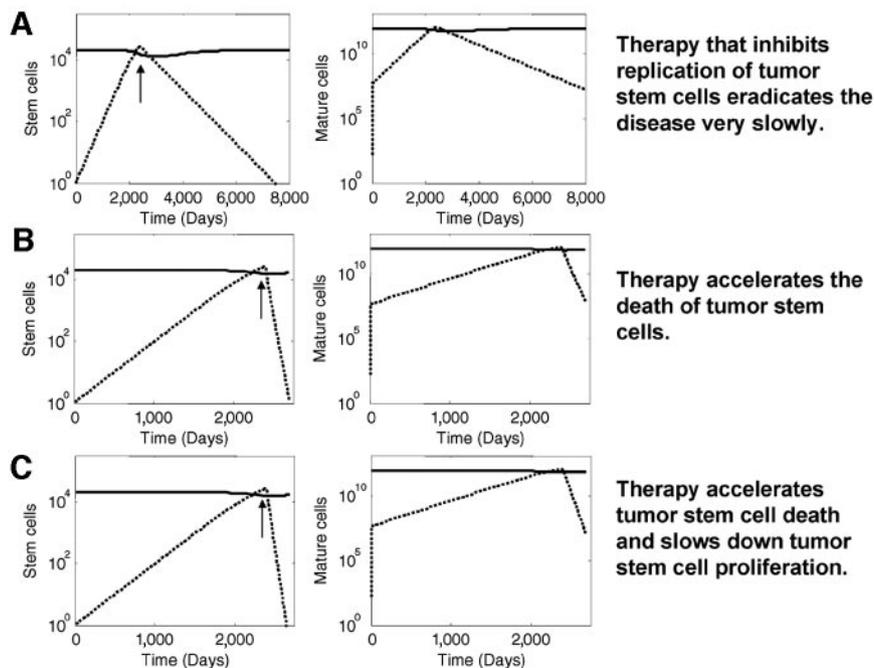
Figure 3. Therapy directed at the tumor stem cell compartment. **(A):** Therapy completely inhibits tumor stem cell proliferation, $r_y = 0$, but the time to cure the disease is very long (for all other parameters, see Figure 2). **(B):** Therapy increases the death rate of the tumor stem cells, $d_{yO} = 0.04$. **(C):** If the drug both decreases the proliferation rate and increases the death rate, $r_y = 0.0006$ and $d_{yO} = 0.04$, of the tumor stem cells, the clone is eliminated more quickly. All other parameters are as in Figure 2B. Arrows indicate the start of therapy.

cues of tumor stem cells. In the following, we explore the impact of therapy on these parameters in isolation or in combination in various therapeutic scenarios.

Therapy Directed at Mature Tumor Cells. Therapy that only increases apoptosis in the mature tumor cells (e.g., hydroxyurea and imatinib in CML) leads to an initial decrease in the tumor burden, but because the tumor stem cell pool continues to expand, therapy will ultimately fail due to the continuous amplification that occurs in the bone marrow (Fig. 2C). Similarly, a therapeutic agent that decreases the rate at which mature tumor cells are generated (e.g., imatinib) cannot cure the disorder (Fig. 2D). Therapy that both increases the death rate and suppresses generation of mature tumor cells will also fail for the same reason. If therapy is stopped, the mature tumor cells rapidly reappear and reach higher levels than those before initiation of therapy, due to continuous expansion of the tumor stem cells (Fig. 2E). This effect has been observed in some patients with CML who stopped imatinib [11, 35]. Therefore, any treatment that is designed to eradicate the tumor must target the tumor stem cells.

Therapy Directed at Tumor Stem Cells. A therapeutic agent designed to selectively inhibit the replication of tumor stem cells (e.g., interferon [IFN]) can in principle lead to tumor eradication. In the best-case scenario, tumor stem cell proliferation is completely inhibited, $r_y = 0$, and the tumor stem cell pool decreases exponentially at a rate $\exp[(-d_0)t]$. Such an agent, however, has to be continuously administered for a prolonged period to eradicate the tumor cell clone (Fig. 3A). Because the presence of the tumor stem cell clone increases the risk of additional mutations and therefore more aggressive disease, the aim of therapy should be to eradicate the stem cell clone as rapidly as possible and minimize the risk of treatment failure [36].

One of the characteristics of tumor cells is that they lose responsiveness to their surroundings. This effect is captured by the parameter c_y in our model. We assume that the sensitivity to crowding of tumor stem cells is half that of the normal stem cells. Therapy that only returns stem cell responsiveness to environmental cues to normal levels, $c_y = c_x$, has very little impact on the time to tumor eradication (data not shown). If this sensitivity is coupled with additional treatment effects, it can hasten treatment response.

Therapeutic agents may increase the death rate of the tumor stem cells. This effect can lead to relatively fast tumor eradication (Fig. 3B). An agent that decreases the proliferation rate of the stem cell pool concomitantly can improve the outcome even further (Fig. 3C). If the tumor stem cells die rapidly, the effect of sensitivity to the environment is not very important. Mutations leading to the evolution of resistance to therapy might also arise in progenitor cells. Therefore, an agent that can eliminate both tumor stem and mature cells quickly has the additional advantage that it minimizes the potential emergence of such resistance mutations.

Side Effects. Although targeted therapy is designed to eliminate toxicity to normal cells, complete absence of toxicity is rarely the case. Some side effects are observed with agents such as imatinib mesylate, which can lead to neutropenia [37]. We therefore studied the therapeutic outcome if a particular drug is only relatively selective to the tumor stem cells. If the selectivity of the drug is at least 1,000-fold higher for tumor stem cells, then for practical purposes, its effect is almost identical to a drug with no side effects on normal stem cells. As the selectivity of the drug decreases, the normal stem cell pool is suppressed and therefore toxicity increases (Fig. 4A). The model can accommodate toxicity as understood in a broad sense and does not refer strictly to either stem cell proliferation or survival. Genotoxic agents, for example, can change the fitness of the stem
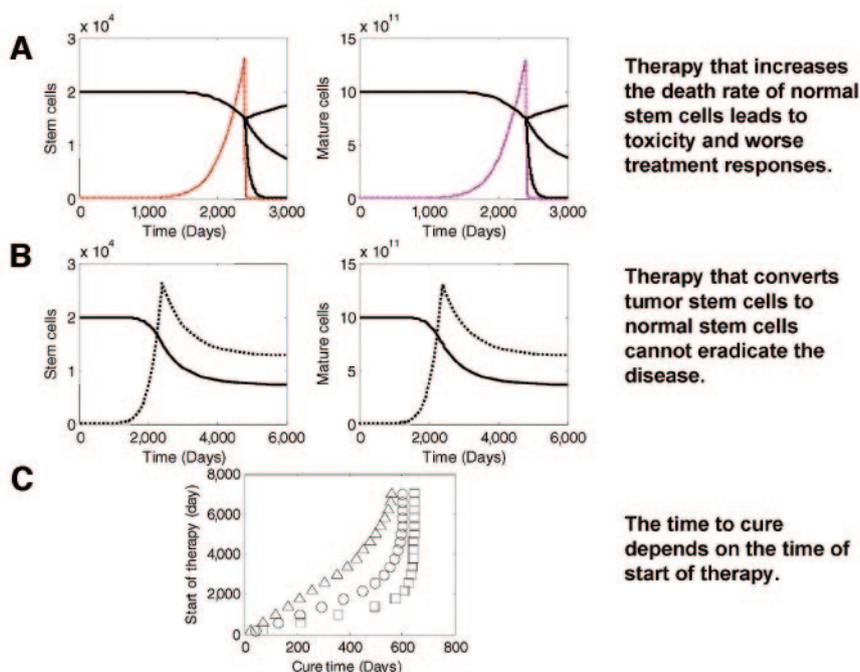
Figure 4. Additional therapeutic scenarios. **(A):** The therapeutic agent increases the death rate of normal stem cells, d_{x0}, but to a lesser extent than the death rate of the tumor stem cells, d_{y0}. A differential sensitivity of 0.1, 0.05, and 0.001 is shown ($d_{y0} = 0.04$, $d_{x0} = 0.004$, $d_{x0} = 0.002$, and $d_{x0} = 0.000004$ for $r_x = 0.005$, $a_x = 1.065 \times 10^7$, $c_x = 0.75 \times 10^{-4}$, $d_{x1} = d_{y1}0.213$, $r_y = 0.0115$, $a_y = 1.065 \times 10^7$, and $c_y = 0.38 \times 10^{-4}$). Any therapy that has a difference in selectivity between tumor and normal stem cells greater than 1,000-fold has very little toxicity. **(B):** If a therapeutic agent induces "normal" behavior on tumor stem cells, both normal and tumor stem cells contribute to hematopoiesis with the ratio dependent on their abundances at the time of the initiation of therapy ($r_y = r_x = 0.005$, $d_{x0} = d_{y0} = 0.002$, $a_y = a_x = 1.065 \times 10^7$, $c_y = c_x = 0.75 \times 10^{-4}$, and $d_{x1} = d_{y1} = 0.213$). Such therapy cannot eradicate the tumor population. **(C):** We show the dependence of the time to cure on the start of therapy. With a large tumor burden, the duration of therapy is almost independent of the time of start of therapy and is determined by the efficiency with which therapy destroys the tumor cells. Sensitivity to environmental cues limits the size of the tumor and has an impact on the duration of therapy (triangles: $c_y = 0.75 \times 10^{-4}$; circles: $c_y = 0.5 \times 10^{-4}$; squares: $c_y = 0.25 \times 10^{-4}$). Here, $d_{y0} = 0.04$ and all other parameter values are as in **(A)**.

cells and alter their long-term behavior, including survival as well as their propensity to differentiate.

Normalization Therapy. There has been a lot of interest in the use of differentiating agents for cancer therapy with the aim of changing the phenotype of the tumor cells (e.g., all-trans retinoic acid [ATRA], valproic acid) [38–41]. This form of therapy is depicted in Figure 4B. In an ideal scenario, the therapeutic agent "converts" the tumor stem cells to normal stem cells in all their characteristics. In such a case, therapy will not be able to eradicate the clone even if it is started early, but the patient will have normal and malignant hematopoiesis as long as the tumor stem cells remain sensitive to therapy. The acquisition of a resistance mutation will lead to rapid progression of the disease.

Finally, we study the effect of early and late initiation of therapy and the duration of treatment necessary for tumor cell eradication. Here, we assume that the disease is diagnosed even before symptom development, and in principle, this is possible with molecular diagnostics (e.g., quantitative reverse transcription-polymerase chain reaction). Figure 4C shows the dependence of the time to cure on the start of therapy. Their relationship is nonlinear with a lower tumor burden requiring a shorter period of therapy. However, once the tumor burden is large, the time required to cure the disease is independent of the burden and is determined by the death rate of the cells with therapy.

DISCUSSION

In the last few years, our understanding of the molecular basis of hematopoietic neoplasms has expanded greatly. Molecular targets for CML [42], acute promyelocytic leukemia (APL) [43], hypereosinophilic syndrome/systemic mast cell disease [44], and a subtype of chronic myelomonocytic leukemia [45] can all be treated with imatinib mesylate or newer compounds designed to circumvent resistant mutants [46, 47]. It is only a matter of time before small molecule inhibitors for the JAKV617F mutation, which has now been shown to be the cause of polycythemia vera, will be identified [48]. These novel agents are associated with reduced toxicity, but they not only have to eliminate the hierarchy of tumor cells, but more importantly must destroy the malignant stem cell compartment [49]. This is illustrated by observations in CML in which withdrawal of imatinib therapy in patients who have been treated uninterruptedly for 3 years experience rapid relapse of the disease in the absence of mutations in the *abl* kinase [11, 35]. Similarly, differentiating agents that convert tumor stem cells into normal stem cells cannot eradicate the tumor. Although hematopoiesis may return to normal, cessation of therapy can lead to rapid relapse of the disease as has been observed in the initial studies of APL when ATRA was used alone [50].

Mathematical modeling of hematopoiesis and the suppressive effects of chemotherapy and acquired tumor resis-
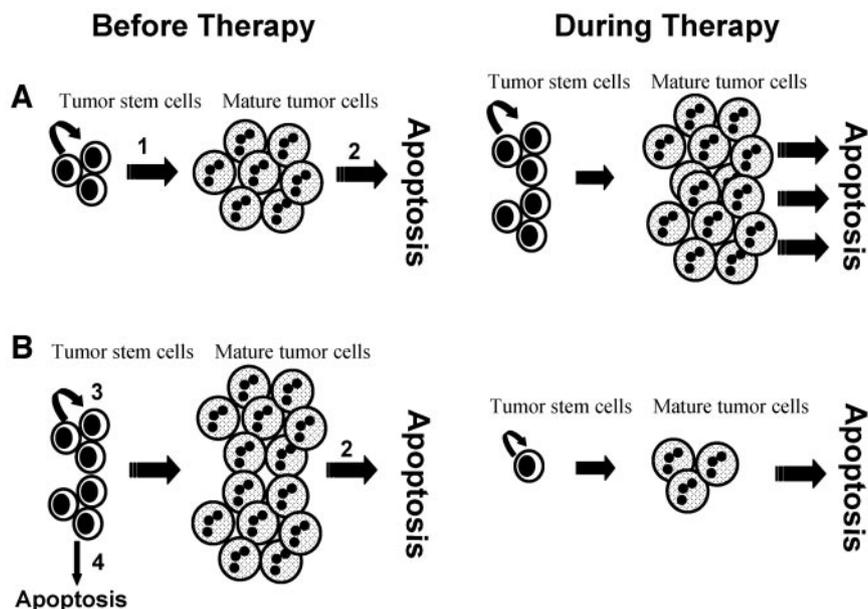
Figure 5. Therapeutic strategies for cancer. **(A):** If the therapeutic agent inhibits production of mature tumor cells (1) or increases their apoptosis rate (2), it cannot eliminate the tumor and the disease burden will continue to increase. **(B):** However, if therapy inhibits tumor stem cell replication (3) and/or induces apoptosis of tumor stem cells (4), the disease burden decreases and therapy can lead to a cure. The fastest eradication is obtained when both the stem cell and the mature cell compartments are targeted.

tance have a long history [27, 28, 51–56]. Often, multiple compartment models are invoked to explain the effects of chemotherapeutic agents on hematopoiesis [55]. To illustrate the importance of the malignant stem cell pool, we have developed a very simple compartment model of hematopoiesis in which a small stem cell pool drives expansion of the rest of the tumor. To keep the mathematics simple, we chose to ignore the impact of feedback loops whereby the mature cell compartment exerts negative feedback on the stem cell pool. However, a model that includes such a feedback function does not give results that lead to significant differences from the conclusions derived from this simple model.

Therapy directed at the stem cell pool can stop tumor stem cell proliferation, induce apoptosis, or return the cells' characteristics to those of normal stem cells. In the absence of mutations, a therapeutic agent that arrests tumor stem cell proliferation can lead to cure, but this requires prolonged therapy. Although antimitotic therapy can in principle transform the tumor into a chronic disorder, this approach will not solve all problems. One of the hallmarks of tumor cells is genetic instability, which can lead to the acquisition of additional mutations and select for drug-resistant clones or biologically more aggressive disease [57]. Thus, rapid elimination of the tumor stem cell pool is an important goal and to reach it, agents that increase stem cell death have to be developed. Furthermore, mutations causing resistance to therapy can also arise in mature tumor cells. To minimize the risk of resistance of mature cells, effective therapeutic agents must quickly kill mature tumor cells as well as decrease the tumor stem cell load. Only such agents can eliminate the tumor rapidly and cure the disease before it progresses (Fig. 5). It is also clear from these considerations that normalizing agents alone cannot provide satisfactory therapy of clonal hematological disorders.

Targeting the tumor stem cell is problematic, given the various mechanisms of resistance that these cells exhibit toward a wide variety of therapeutic agents. Some patients

with CML, for example, harbor tumor stem cells that do not express *bcr-abl* and presumably are not dependent on this oncogene for their survival [58]. On the other hand, CML stem cells have been identified that overexpress *bcr-abl* and are functionally resistant to the effects of the drug. Moreover, stem cells overexpress membrane pumps that actively efflux drugs from the cytoplasm and so neutralize their effects on this tumor compartment [59]. Some of these mechanisms of resistance can be addressed, and several investigators have attempted to block members of the ATP-binding cassette transporter family of proteins to inhibit efflux of drugs from the stem cell pool. However, so far this approach has not led to clinically significant results [60, 61]. It has not yet been determined whether the new *abl* kinase inhibitors have an effect on the CML stem cell pool. However, in an attempt to eradicate the CML stem cell, studies that combine imatinib with IFN-α are in progress because IFN is the only other drug that can lead to the disappearance of the Philadelphia chromosome in CML. Recent work suggests that tumor stem cells have a distinct immunophenotype that may aid in their isolation [62]. The novel cell surface marker CLL-1 (C-type lectin-like molecule-1) is expressed by the vast majority of tumor stem cells in acute myelogenous leukemia, and its elimination correlates with prolonged remissions in some patients, suggesting that the tumor stem cell pool has been eradicated [63]. Isolation of tumor stem cells using these markers will perhaps open the way for curative therapies specifically directed at this critical cell compartment.

Hematopoietic neoplasms have a distinct hierarchy with a small compartment of malignant stem cells that maintain the tumor. Successful therapy requires eradication of this pool of cells; otherwise, progressive disease is very likely. Successful therapeutic agents must enhance the death rate of this rare population of cells. Therapy that is designed to inhibit mitosis of malignant stem cells or to return their characteristics to those of normal stem cells cannot eradicate the disease quickly. The malignant stem cell is the ultimate therapeutic target.

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DISCLOSURES

The authors indicate no potential conflicts of interest.

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