Dynamics of Leukemia Stem-like Cell Extinction in Acute Promyelocytic Leukemia

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Abstract

Many tumors are believed to be maintained by a small number of cancer stem–like cells, where cure is thought to require eradication of this cell population. In this study, we investigated the dynamics of acute promyelocytic leukemia (APL) before and during therapy with regard to disease initiation, progression, and therapeutic response. This investigation used a mathematical model of hematopoiesis and a dataset derived from the North American Intergroup Study INT0129. The known phenotypic constraints of APL could be explained by a combination of differentiation blockade of PML–RARα–positive cells and suppression of normal hematopoiesis. All-trans retinoic acid (ATRA) neutralizes the differentiation block and decreases the proliferation rate of leukemic stem cells in vivo. Prolonged ATRA treatment after chemotherapy can cure patients with APL by eliminating the stem-like cell population over the course of approximately one year. To our knowledge, this study offers the first estimate of the average duration of therapy that is required to eliminate stem-like cancer cells from a human tumor, with the potential for the refinement of treatment strategies to better manage human malignancy. Cancer Res; 74(19): 5386–96. ©2014 AACR.

Major Finding

By combining a mathematical model of hematopoiesis with data from a large randomized trial of acute promyelocytic leukemia, this study offers the first determination of the average duration of therapy required to eliminate all stem-like cells in a human tumor.

Introduction

The cancer stem cell (CSC) hypothesis states that at the root of most (perhaps all) tumors, there is a population of cancer stem cells that is not only able to renew itself but also gives rise to the bulk of the tumor cell population (26–29). These CSCs are essential for the origin and the continued growth and maintenance of the tumor. As a consequence, these cells are an important target of therapy, as it is thought that eradication of such cells is necessary for a potential cure of the tumor. Various models have been developed that address this hypothesis from a theoretical perspective and there is increasing evidence for its support from animal models of cancer. Although CSCs were initially isolated from patients with acute leukemia (26–29), they have now been identified in virtually all types of tumors. It is therefore important to understand the dynamics of these cells under therapy and whether they can be eradicated, leading to cure of the disease and long-term survival of patients. Here, we utilize data from a clinical trial of therapy for APL to understand the dynamics of leukemic stem cells under therapy. We use a mathematical/computational model of hematopoiesis together with quantitative data from the North American Intergroup Study INT0129 (30, 31) to determine the probability that the leukemic stem cells are eradicated at a certain time under this treatment regimen. APL was chosen for a number of reasons: (i) the disease is well defined with most patients having the translocation t(15q22;17q12), leading to PML–RARα oncogene activation (14–16), (ii) the tumor burden can be quantitated using quantitative real-time PCR (qRT-PCR; refs. 31, 32), (iii) targeted therapy in the form of all-trans retinoic acid (ATRA) is available and highly effective (33, 34), (iv) the availability of serial quantitative data on disease burden from a large randomized clinical trial that allows us to investigate the effects of ATRA treatment and chemotherapy separately (31, 32, 35), and (v) the availability of a mathematical/computational model of hematopoiesis that has already been utilized to understand the dynamics of...
Dynamics of Acute Promyelocytic Leukemia

Quick Guide to Equations and Assumptions

Our multicompartment model of hematopoiesis has been described elsewhere, but here we describe it briefly for clarity (Fig. 1; refs. 1–6). Cells in a given compartment \( i \) with probability \( \epsilon_i \) differentiate and produce two cells that migrate to the next downstream compartment \( (i + 1) \) or self-renew and increase compartment \( i \) by one cell with probability \( 1 - \epsilon_i \). Here, “compartments” are not understood as physical spaces but as an accounting tool to keep track of the replication and differentiation of each cell. Proliferation rates \( r_i \) are intrinsic for each compartment and with \( r_i < r_{i+1} \). Parameters describing cells in non–stem cell compartments \( i > 0 \) are fixed, by allometric scaling arguments (1) and data from human hematopoiesis and are given by \( \epsilon_i^h = 0.85 \), \( r_i^h = (\epsilon_i^h)^2 r_i^l \) with \( y^h = 1.26 \). Thus, the rate of proliferation increases exponentially with the compartment [subscripts refer to the compartment, whereas superscripts refer to healthy (h) or cancerous (c) cells]. In the stem cell compartment \( i = 0 \), \( N_0 = 400, \epsilon_0^h = 0.5 \), and \( r_0^h = 1/365 \) days (7–10). Undisturbed, the above system reaches a steady state, where cell numbers fluctuate around an average cell count. In the absence of disease, the steady state for each compartment is given by

\[
N_i^h = \frac{N_0^h}{y^h} \left( \frac{2}{2 - y^h} \right)^{i-1}.
\]

Under this parameter setting, we need 32 compartments to represent hematopoiesis with a daily output of approximately 3.5 \times 10^{11} \text{ cells} (1–6). This can be viewed as an almost continuous differentiation process of cells (Fig. 1).

Because of the high cell turnover rates, the dynamics of normal cells in such hierarchies can be captured by a linear system of differential equations (5, 6). An arbitrary number of mutations can be described analytically by similar equations if mutated cells proliferate independently (5, 6). We model the dynamics of normal and leukemic cell lineages by such systems of differential equations (5, 6). Our multicompartment model of hematopoiesis has been described elsewhere, but here we describe it briefly for clarity (Fig. 1; refs. 1–6). Cells in compartment \( i \) increase in number due to self-renewal at rate \( (1 - \epsilon_i^h) r_i^h N_i^h \) and decrease due to differentiation of cells into compartment \( i + 1 \) at rate \( \epsilon_i^h r_i^h N_i^h \), leading to a change in number rising from processes within the compartment of \( (1 - 2 \epsilon_i^h) r_i^h N_i^h \). In addition, there is an influx from upstream compartment \( i - 1 \) at rate \( +2 \epsilon_i^h r_i^h N_i^h \), see Fig. 1 for a graphical representation. Collecting all terms gives

\[
\frac{dN_i^h}{dt} = \begin{cases} 
(1 - \epsilon_i^h) r_i^h N_i^h + 2 \epsilon_i^h \epsilon_{i-1}^h r_{i-1}^h N_{i-1}^h & i < l \\
(1 - \epsilon_i^h) r_i^h \frac{N_i^h}{N_i^h + N_l^h} + 2 \epsilon_i^h \epsilon_{i-1}^h r_{i-1}^h N_{i-1}^h & i = l \\
(1 - \epsilon_i^h) r_i^h \frac{N_i^h}{N_i^h + N_{l+1}^h} + 2 \epsilon_i^h \epsilon_{i-1}^h r_{i-1}^h \frac{(N_i^h)^2}{N_i^h + N_{l+1}^h} & i > l.
\end{cases}
\]

Here, \( l \) denotes the compartment where the cancer-driving mutation occurs, i.e., the leukemic stem-like cells. Compartments upstream of \( l \) are not affected by this mutation and proliferate independently (Fig. 1). However, the proliferation of healthy cells, \( N_l^h \), in and downstream of compartment \( l \) is potentially inhibited by the leukemic cells, \( N_l^c \). This interference is modeled by Hill functions (11). In the absence of malignant cells, homeostasis is normal, but proliferation of healthy cells is suppressed with increasing numbers of leukemic cells (12).

The dynamics of leukemic cells can be described by the following set of equations

\[
\frac{dN_i^c}{dt} = \begin{cases} 
0 & i < l \\
(1 - \epsilon_i^c) r_i^c N_i^c & i = l \\
(1 - \epsilon_i^c) r_i^c N_i^c + 2 \epsilon_i^c \epsilon_{i-1}^c r_{i-1}^c N_{i-1}^c & i > l.
\end{cases}
\]

In the absence of therapy, the number of leukemic cells in the compartment of origin grows exponentially for \( \epsilon^c < 0.5 \), leading to an even faster growth in downstream compartments. Leukemic cells proliferate independently of healthy cells as evidenced by observations in animal models of disease (13). The leukemia-driving cells occupy compartment \( l \) (Fig. 1, bottom). Thus, leukemic cells can only be found in and downstream of compartment \( l \). In addition, the leukemic cell proliferation properties differ significantly from those of healthy cells.

Model Constraints

1. Phenotype: Because PML–RARα expression reduces differentiation and enhances self-renewal of cells (13–17), we supposed that \( \epsilon^c < \epsilon^h = 0.85 \). Generally, APL is associated with pancytopenia due to a reduction in bone marrow output. Therefore, we adjusted our parameters to reduce the cells in compartment 31 to 10% to 20% of normal while ensuring that the
intramedullary compartments were hypercellular. ATRA reverses the differentiation block and, therefore, $e^t \rightarrow 0.85$. *In vitro*, ATRA slows down the rate of replication of leukemic cells by at least a factor of 0.61 (18). While chemotherapy kills the majority of cancer cells, the proliferation properties of surviving cancer cells remain unchanged.

2. **Time to diagnosis and origin of the disease:** The disease must start from a single leukemic stem cell in keeping with the clonal origin of cancer. Using data from Guibal and colleagues (13), we inferred that the minimum time for diagnosis in mice is approximately 120 days. Using our previously described allometric scaling relationship (19, 20) comparing timescales between mice and men $\frac{T_{mi}}{T_{mu}} = \left(\frac{M_{mi}}{M_{mu}}\right)^{1/4}$, where $T$ and $M$ refer to the time to diagnosis and the species-specific adult mass, respectively, we could determine that the minimum time from the appearance of the first leukemic stem cell in humans to diagnosis is more than 872 days. An animal model of APL suggests that the disease may originate in a colony-forming unit, granulocyte-macrophage (CFU-GM cell; ref. 13), which in our model would reside in compartments 13 to 15. A limited study of three patients also suggests that CD34+CD38− cells do not have the t(15;22;17q12), typical of the disease (21).

3. **Clonal burden in bone marrow:** The average tumor burden of leukemic stem cells at the time of diagnosis is approximately 1% of the tumor population present in the bone marrow (22), and the leukemic cells represent 65% to 98% of the marrow cellularity (23).

4. **The average time for the bone marrow to appear normal after therapy with ATRA is 38 days (range, 25–90 days; ref. 23).**

5. **Patients treated with ATRA alone for induction often have an increase in their leukocyte count that peaks between 12 and 14 days after initiation of therapy (23).**

6. **Time to relapse:** If patients are treated with ATRA alone until they have a morphologic remission, they relapse on average after approximately 110 days (45–300; ref. 24, 25).

7. **ATRA does not alter the dynamic properties of normal hematopoietic cells.**

mutations in other hematologic disorders (1–6). In the following, we provide a brief summary of the clinical trial, a description of the mathematical model together with the justification of the constraints used to determine the model parameters, followed by a description of the data fitting and presentation of results. Details of the mathematical model as well as the clinical trial are provided in Materials and Methods. In summary, our mathematical model contains a fixed number of compartments that represent different stages of cell differentiation. At each stage, cells proliferate with a fixed rate $r_i$ and differentiate into the next downstream compartment with probability $e_i$. This general framework allows us to describe normal hematopoiesis as well as the initiation and progression of different types of leukemia, defined by changes in proliferation and differentiation parameters of malignant cells. As a result, we provide an estimate of the timescale with which a leukemic stem cell population is eliminated in humans.

### Patients and Methods

#### Clinical trial INT0129

The North American Intergroup trial of ATRA in APL was initially reported in 1997 with subsequent updates (30, 36, 37). Patients were recruited from centers affiliated with the Cancer and Leukemia Group B, the Southwest Oncology Group, and the Eastern Cooperative Oncology Group. Briefly, patients with newly diagnosed APL were randomly assigned to induction therapy either with combination chemotherapy: daunorubicin (45 mg/m² daily on days 1–3) and cytosine arabinoside (100 mg/m² by continuous infusion for 7 days; $N = 191$) or ATRA alone (45 mg/m² orally in two divided doses daily) that could be given for up to 90 days ($N = 188$). Pediatric patients less than 3 years of age were treated with the same protocol, but with appropriate dose modifications. Subsequently, patients who achieved a complete remission with induction therapy received two cycles of consolidation: the first cycle was identical to the initial induction chemotherapy regimen while the second cycle consisted of high dose cytosine arabinoside (2 g/m² every 12 hours for 4 days) with daunorubicin 45 mg/m² daily on days 1 and 2. The patients were subsequently randomized to observation or maintenance therapy with ATRA (45 mg/m² orally in two divided doses daily) for up to 1 year. Fifty-four patients who received induction with ATRA were randomized to maintenance with ATRA, whereas 56 patients initially treated with chemotherapy went on ATRA maintenance after the second randomization. Patients had serial measurement of the PML–RARα oncogene as previously described (31, 32, 35). Our mathematical/computational model was fitted to this serial data, after normalization of PML–RARα to GAPDH with the pretreatment copy number value of PML–RARα/GAPDH normalized to 1. Serial collection of blood samples for qRT-PCR quantification was not mandatory for the trial and as a result the data set is incomplete in this respect.

#### Parameter estimation

We use the initial condition of 1 leukemic stem cell in compartment $l$ with the described constraints to numerically solve the deterministic equations from above for each compartment using standard numerical procedures implemented in *Mathematica*. Throughout this process, we assume that the effect of ATRA on leukemic cells is constant. Each numerical solution gives the disease scenario arising from a given set of model parameters. In our case, we had to determine four key parameters: (i) the proliferation rate and (ii) differentiation probability in the compartment of the cancer-initiating cell
Therapy.

Indirectly altered by the response of the leukemic cells to ATRA therapy and thus their dynamic properties are only indirectly altered by the response of the leukemic cells to therapy. Healthy cells are not affected by ATRA therapy. Chemotherapy was implemented as a single catastrophe event, in which the majority of cancer and healthy cells were killed, but the proliferation properties of the surviving cancer cells remain unchanged. Healthy cells are not affected by ATRA therapy and thus their dynamic properties are only indirectly altered by the response of the leukemic cells to therapy.

Stochastic simulations

We inferred the distribution of extinction times of leukemic stem–like cells by performing stochastic simulations implemented by a Gillespie algorithm (38), based on an agent-based representation of our hierarchical organization (5). We used parameter estimations from our deterministic fitting procedure, and therefore parameters were fixed during all stochastic simulations. We performed in total $10^7$ independent stochastic simulations and recorded the time until all cancer stem–like cells went extinct, leading to a distribution of cancer stem–like extinction times.

Results

Dynamics of untreated APL

Initially, we had to determine the time course and dynamic properties of the leukemic stem and progenitor cells taking into account the constraints that we identified from the literature, in particular the known biology of the disease (Fig. 1; refs. 13–17, 21, 23). Given that PML–RARα expression leads to a block in differentiation, we had to impose a lower probability for the differentiation of leukemic cells compared with healthy cells $c_i < c_h = 0.85$ in our model. The leukemic stem cell in APL may

Figure 1. Schematics of our hierarchical hematopoiesis model. Top, undisturbed healthy hematopoiesis. At the root of our hierarchical structure (compartment 0) is the stem cell compartment with a few slowly dividing stem cells (light red, proliferation rate $r_0$) that can self-renew with probability $1 - c_{15}^0$ and differentiate with probability $c_{15}^0$. Cells undergo several differentiations with probabilities $c_{16}^i$ and proliferate with rates $r_i$ until they reach the mature cell state (compartment 31 in our case, dark red). Bottom, hematopoiesis is disturbed by leukemic cells (dark yellow). The leukemia-driving mutation occurs in compartment 15. The self-renewal capacity $(1 - c_{15}^0)$ of the leukemic cells is significantly increased and malignant cells accumulate in bone marrow compartments. Concomitantly, the proliferation of healthy cells (red) is suppressed by cancer cells and thus the healthy cell count decreases in compartments downstream of compartment 15.
arise in a CFU-GM cell (13, 21), and therefore $l = 15$ was chosen as the founding compartment of APL in our hierarchical model, based on our prior results (1, 39). We estimated that the minimum time between the onset of the first leukemic stem cell and disease was 872 days (13, 19, 20), where disease was defined as a reduction in bone marrow output to approximately 20% of normal, leading to cytopenias that are typical for this leukemia. At the same time, the bone marrow compartments will be found in the bloodstream. The parameter estimates that led to the best fit to the data are presented in Table 1.

Our fits suggest that the leukemic stem cells replicate faster and self-renew with a higher probability than normal CFU-GM cells in the same compartment. Even based on their higher self-renewal capacity alone, they have a considerable fitness advantage compared with the normal progenitor cells. If we count the number of offspring cells produced by a mutant cell in a given compartment and compare it with the number of offspring of a normal cell, we obtain for relative fitness in our mathematical model ($f_i$; ref. 40).

\[
f_j = \frac{1 - x_j^\text{ATRA}}{1 - x_j^\text{normal}}.
\]

This translates into a relative fitness advantage of 6.9 compared with normal CFU-GM (17). Moreover, our fitting suggests that the leukemic progenitor cells (downstream of the leukemic stem cells) have an extremely high fitness advantage (based on the virtual absence of normal cells in the circulation), estimated at 75 compared with their normal counterparts (normalized to 1) due to the block in differentiation (and enhanced self-renewal). These estimates provide a vivid explanation of the rapid disease progression and early high lethality associated with this disease before the advent of ATRA therapy.

**Dynamics of disease under chemotherapy treatment**

We implement chemotherapy treatment as a single catastrophic event, in which the majority of both leukemic and healthy cells are killed instantly. The proliferation parameters of surviving cells remain unchanged after chemotherapy treatment. Therefore, we only need to infer a single parameter (the fraction of killed cells under chemotherapy) to determine the dynamics of patients with APL under chemotherapy from our mathematical model. We vary the fraction of killed cells and fit the resulting dynamics to the serial qRT-PCR data of the fraction of patients treated with chemotherapy in the INT0129 trial. Our best parameter estimates (see Figs. 2 and 3) suggest that only 0.3% of all cancer cells survived, compatible with more than 2 log kill of leukemic cells. However, this also implies that approximately $10^5$ leukemic stem cells survive and relapse is to be expected. This prediction is confirmed by observations from the follow up of the INT0129 trial were the risk of relapse of chemotherapy-treated patients (without ATRA maintenance) was high. Note, that the characteristic peak of bone marrow output, 10 to 12 days after ATRA treatment, does not occur with chemotherapy (Fig. 2A vs. C).

### Table 1. Parameter estimate for APL stem and progenitor cells before therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$i = 15$</th>
<th>$16 \leq i \leq 25$</th>
<th>$26 \leq i \leq 31$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma_i^c$</td>
<td>1.34</td>
<td>1.12</td>
<td>1.12</td>
</tr>
<tr>
<td>$\gamma_i^h$</td>
<td>0.45</td>
<td>0.07</td>
<td>0.85</td>
</tr>
</tbody>
</table>

NOTE: Here, $i = 15$ represents the compartment of the founding APL cancer stem-like cell. Compartments $16 \leq i \leq 25$ represent the bone marrow cell load and compartments $26 \leq i \leq 31$ differentiated cells that can be found in the bloodstream. The parameters $\gamma_i^c$ denote the differentiation probabilities of cancerous cells in compartment $i$ and the parameters $\gamma_i^h$ the increase in proliferation rate of cancerous cells per compartment.

### Dynamics of disease under therapy with ATRA

ATRA alters the behavior of leukemic cells by (i) inducing differentiation and (ii) slows down the rate of replication of leukemic stem cells. Fitting of our model to serial RT-PCR data from the INT0129 trial (Figs. 2 and 3A) provides an estimate for the leukemic cell parameters under therapy as reported in Table 2. We consider that therapy starts immediately after diagnosis or 870 days from the appearance of the first leukemic stem cell. We assume that the differentiation probabilities return to normal under ATRA therapy. The proliferation rates within the bone marrow, $\gamma_i^\text{ATRA}$, are fixed by the time until the bone marrow output peaks. The proliferation rate of cancer stem cells, $\gamma_i^\text{ATRA}$, has only a minor influence on this dynamics due to the exponential growth characteristics of hematopoiesis, and is thus difficult to estimate from the data. However, it is a crucial parameter to assess the probability of relapse. For the best parameter estimate, the rate of replication of the leukemic stem cells returns to normal compared with other CFU-GM cells ($\gamma_{15} = 1.34 \rightarrow \gamma_{\text{ATRA}}^{15} = 1.26$). At the same time, the differentiation block of the cells is removed ($\gamma_i^h = 0.85 \rightarrow \gamma_i^\text{ATRA}$). Zhu and colleagues showed that the doubling time of NB4 cells treated with ATRA increased in vitro from 25.2 hours to 41.26 hours (slowed the cells by a factor of 0.6x ref. 18). In our in vivo model, there is also such a slowdown effect. Our proliferation rates in compartment $i$ scale via $r_i = (\gamma_i^{\text{normal}})^{r_0}$.

Thus, a decrease in doubling times of cancer cells under ATRA treatment by a factor of 0.6 in the experiment corresponds to $\gamma_i^\text{ATRA}^{15} \approx 0.4$ in our theoretical model based on the scaling of replication rates. With this in mind, our relative reduction in leukemic stem cell replication ($\gamma_i^c = 1.34 \rightarrow \gamma_i^\text{ATRA} = 1.26$) is in qualitative agreement with the finding of Zhu and colleagues. We note that the replication rate in vivo would be expected to be slower than what is observed in vitro (41).

In addition, ATRA therapy affects more downstream progenitor cells correcting their differentiation block back to normal ($\gamma_i^{16-25} = 0.07 \rightarrow \gamma_i^{\text{ATRA}}^{16-25} = 0.85$). Fitting also suggests...
that ATRA increases the rate of replication of downstream leukemic progenitors compared with normal cells. The latter prediction is compatible with the observation of a rapid increase in the neutrophil count in patients treated with ATRA alone (Figs. 2 and 3C).

In Fig. 4, we provide a comparison of individual fits of the model to patient specific data for a patient induced with ATRA (Fig. 4A) and another patient randomized to chemotherapy only induction (Fig. 4B). The model fits especially well the ATRA-treated patient.

**Extinction time of leukemic stem cells**

Our model is in line with in vivo mouse experiments (13) that before the initiation of therapy, the leukemic stem cell population \( (i = 15) \), increases exponentially (Fig. 3B). Thus, at the time of diagnosis \( (T_{\text{diag}}) \), we expect to find \( N_{15}^{c}(T_{\text{diag}}) \) cells, where

\[
N_{15}^{c}(T_{\text{diag}}) = e^{(1-2c_i)T_{\text{diag}}} T_{\text{diag}}^{c_i}
\]

For our parameters, we obtain \( 4 \times 10^7 \) leukemic stem cells at the time of diagnosis in compartment 15 (CFU-GM), where the mutation originates (19, 20). Under ATRA therapy, the number of leukemic stem cells decreases exponentially such that

\[
N_{15}^{\text{ATRA}}(t) = N_{15}^{c}(T_{\text{diag}}) e^{(1-2c_i)T_{\text{diag}}} T_{\text{diag}}^{c_i}
\]

Therefore, the average extinction time for the leukemic stem–like cells (i.e., for the elimination of all leukemic stem–like cells) is given by:

\[
e_{\text{ext}} = -\frac{(1-2c_i)T_{\text{diag}}}{(1-e^{(1-2c_i)T_{\text{diag}}} T_{\text{diag}}^{c_i})}
\]

On average the time for clonal extinction under ATRA treatment is approximately 312 days (0.36 \( \times \) 870 days). However, this time increases exponentially with the number of cells at diagnosis, and, therefore, continued therapy with ATRA is a prerequisite to cure this disease. These results are compatible with clinical observations and justify the need for maintenance therapy for approximately 1 year that seems to lead to a cure in many patients as treated in the INT0129 trial (Fig. 3D).

**Relapse**

Relapse of seemingly successful treated patients is a common phenomenon in acute leukemia. Presumably, this relapse...
is caused by the remaining cancer stem and progenitor cells after therapy as well as the selection of mutant cells resistant to therapy through a variety of mechanisms, including mutations in the LBD domain of RARα, increased ATRA catabolism, abnormal trafficking of ATRA to the nucleus, the presence of cytoplasmic retinoic acid binding protein, and overexpression of BP1 (42, 43). Thus, the question of whether and when treatment eradicates all cancer stem cells is critical. As intrinsic cell properties, such as the exact time of cell proliferation or differentiation, are stochastic, one naturally expects the actual extinction time of the leukemic stem cell pool to differ in patients (44, 45). To obtain the distribution of these extinction times under ATRA treatment, we implemented a computational representation of the mathematical model (see Materials and Methods for details; parameters as in A). The black dot corresponds to the deterministic extinction time (here after 312 days) of the leukemic cancer stem–like cells given by equation (3). Because of the inherent stochastic nature of the proliferation properties of the cancer stem–like cells, only 42% of patients are cured after 312 days of ATRA treatment and we expect 58% of the patients with remaining APL stem–like cells in the bone marrow. After one year of ATRA treatment (gray dot), approximately 92% of patients had no remaining cancer stem–like cells left.

Discussion

If we hope to better treat cancer, with emerging therapeutic approaches, a detailed understanding of cancer initiation, cancer progression, and response to treatment is essential. Here, we combine a mathematical/computational approach that
The initial response to ATRA treatment is well described by our model prediction (blue line). However, this patient did not receive maintenance therapy and finally relapsed 861 days after the initial treatment. In contrast, the patient treated with chemotherapy had an initial response to therapy, but progressed in between cycles of chemotherapy. This variation could be caused by stochastic effects of cell proliferation together with a varying number of surviving cancer stem-like cells per treatment.

with clinical trial data of treatment response of patients with APL to ATRA therapy and/or chemotherapy. This approach allows us to model leukemia progression from the occurrence of the first leukemic stem cell until the potential elimination of the last cancer stem cell under treatment and thus provides a detailed understanding of all phases of APL under two different treatment regimes. At least one murine model of APL suggests that the disease originates in a CFU-GM cell (13, 22) and thus within the lower half (here compartment 15) of our hierarchical model. We acknowledge that there is still some disagreement on the true origin of the leukemic stem cell in APL (21, 46, 47), partly based on the animal model used (46, 48) and it is likely that other mutations in addition to the t(15q22;17q12) are required for APL to develop (49). Although the cancer stem cell hypothesis is increasingly accepted, and cancer-initiating (stem) cells have been isolated from many tumors, the field is still somewhat controversial (27–29). Several possible explanations exist for the divergent results observed vis-à-vis the presence, frequency, surface marker expression, and functional properties of these putative cells, including (i) the animal model used for engraftment that provides the complex microenvironment for cells to survive and grow, (ii) genetic/epigenetic heterogeneity between tumors, (iii) stage of the tumor, and others (47–49). However, the presence of cellular hierarchies within acute leukemia is less controversial and a critical component of our modeling approach.

We find that an interaction of leukemic and healthy cells is sufficient to explain the known phenotypic constraints of APL. The bone marrow in APL usually appears hypercellular at diagnosis, but a fraction of patients may have a hypocellular bone marrow at diagnosis. This can be explained by the suppression of healthy cells combined with a differentiation block of APL cells in the bone marrow.

Our model of acute promyelocytic leukemia also reveals that bone marrow failure syndromes are not necessarily due to failures of the normal hematopoietic stem cell pool. They can also occur by suppression of the proliferation of normal cells by leukemic cells, for example, due to competition for cytokines. This also implies that hematopoiesis returns to normal after the eradication of the malignant cells as is typical for many acute leukemias and is in line with our model as well as recent observations in vivo (12).

Our model suggests that, in addition to the block of differentiation, APL stem–like cells have an increased proliferation rate, and thus they have a significant fitness advantage (6.9 to 1.0) compared with normal cells. Despite this fitness advantage, the disease progresses slowly

### Table 2. Parameters estimates for APL cells in response to ATRA therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$i = 15$</th>
<th>$16 \leq i \leq 25$</th>
<th>$26 \leq i \leq 31$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma_1^{i}$</td>
<td>1.26</td>
<td>1.26</td>
<td>1.26</td>
</tr>
<tr>
<td>$\gamma_2^{i,TRA}$</td>
<td>1.26</td>
<td>1.24</td>
<td>1.44</td>
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<tr>
<td>$\delta_i$</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>$\epsilon_i^{TRA}$</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
</tbody>
</table>

NOTE: Here, $\gamma_i$ and $\epsilon_i^{TRA}$ denote the differentiation probabilities in compartment $i$ of healthy cells and cancerous cells under ATRA treatment, respectively. The parameters $\gamma_1$ and $\gamma_2^{TRA}$ represent the relative increase of the proliferation rate per compartment for healthy cells and cancer cells under ATRA treatment.
initially, as cells accumulate in the bone marrow, but only weakly affect the output of normal hematopoietic cells (17). The output of fully differentiated healthy cells only starts to decrease slowly approximately 60 days after the occurrence of the first leukemic stem cell and diagnosis typically occurs after approximately 870 days (2.4 years). This is substantially shorter than the timescale for the clinical development of chronic myeloid leukemia, multiple myeloma or solid malignancies such as colon cancer (3, 50).

We find that chemotherapy provides a significant initial response but is unlikely to cure the disease, as a substantial fraction of leukemic stem–like cells is expected to survive treatment and leads to relapse of the disease. Indeed, we find several patients in the INT0129 trial that underwent three cycles of chemotherapy (per protocol) but relapsed in time intervals of approximately 100 to 300 days.

The model suggests that ATRA has differential effects on APL stem–like cells compared with leukemic cells further downstream within its hierarchy. ATRA removes the differentiation block and increases the proliferation rate of APL cells in the bone marrow. This leads to a rapid expansion of most APL progenitor cells and causes the typical bone marrow peak after 10 to 13 days of ATRA treatment. The bone marrow will appear free of APL blasts after approximately 20 days. However, cure requires the extinction of all APL stem–like cells. We find, in line with in vitro studies, that ATRA decreases the proliferation rate of leukemic stem–like cells to 0.4 compared with untreated leukemic stem–like cells and thus slows down the eradication of APL stem–like cells. Thus, despite a fast initial response, continued ATRA therapy is necessary to reduce the risk of relapse.

Our model suggests that, in the absence of additional mutations, ATRA therapy for one year would result in a high likelihood of eradication of all leukemic stem–like cells and thus a cure in many patients. This is compatible with long-term follow-up of patients on the INT0129 trial, where only 3.3% of patients who achieved complete remission experienced a late relapse (defined as occurring >3 years after diagnosis; ref. 37). Our model in its current form does not consider the intrinsic heterogeneity present in most leukemias or the emergence of mutant subclones that may lead to relapse of the disease. Such extensions of the model may be possible in the future but require more detailed knowledge about the structure of the tumor. Regardless, our modeling also illustrates the impact of stochastic effects on the response to treatment, and in part explains why outcomes can be vastly different between patients with similar disease status at diagnosis, even without considerations of tumor evolution.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute.

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References


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