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REVIEW ARTICLE

Mathematical Models of Cancer Stem Cells

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ABSTRACT

Human cancers are thought to be sustained in their growth by a pathologic counterpart of normal adult stem cells: cancer stem cells. This concept was first developed in human myeloid leukemias and is today being extended to solid tumors such as breast and brain cancers. A quantitative understanding of cancer stem cells requires a mathematical framework to describe the dynamics of cancer initiation and progression, the response to treatment, and the evolution of resistance. In this review, I use chronic myeloid leukemia as an example to discuss how mathematical and computational techniques have been used to gain insights into the biology of cancer stem cells.

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INTRODUCTION

The field of stem-cell biology was initiated in 1917 when Artur Pappenheim postulated the concept of hematopoietic stem cells.[1] Their existence was later demonstrated with experiments showing that leukemia could be transmitted with a single cell[2] and bone-marrow reconstitution experiments after lethal irradiation in mice.[3] In the years since, many more tissue-specific stem cells have been isolated.[4-6] At approximately the same time that stem cells were discovered, cells from both solid tumors and leukemias were reported to vary in their ability to form colonies in vitro and in vivo.[7,8] This and other observations led to the cancer stem-cell hypothesis, suggesting that the entire tumor cell mass arises from a small number of cancer stem cells that, like normal stem cells, have the ability to indefinitely self-renew while repopulating the distinct cell types found in the tumor.[8,9] Cancer stem cells, too, were first described in the hematopoietic system, with the identification of acute myeloid leukemia stem cells in 1994[10] and of acute lymphocytic leukemia stem cells soon thereafter.[11] The existence of cancer stem cells has since been demonstrated for solid tumors such as breast and brain cancers.[12,13]

The mathematical exploration of cancer was initiated in the 1950s with a study of the age-dependent incidence curves of human cancers. Nordling,[14] Armitage and Doll,[15] and Fisher[16] noticed that on a doubly logarithmic plane, the incidence data of most cancers is a straight line whose slope may be used to estimate the number of mutations necessary to drive tumorigenesis. Their finding that the data could be explained by the requirement of several probabilistic events for cancer evolution became known as the multistep theory of carcinogenesis.[17] In the early 1970s, Knudson conducted a statistical analysis of retinoblastoma incidence in children and proposed the two-hit hypothesis, suggesting that two hits in the *RB1* gene are the rate-limiting steps of retinoblastoma[18] and leading to the concept of a tumor suppressor gene.[19] These studies sparked the interest in a mathematical approach to cancer, and much subsequent work was produced.[20-25] In recent years, cancer stem cells have become the subject of theoretical investigations as well, and studies were performed to elucidate the biology and dynamics of colorectal cancer stem cells,[26-30] breast cancer stem cells,[31] hematologic malignancies,[32-35] and the role of stem cells in the evolution of drug resistance.[35-38] In this review, I discuss mathematical models that explore stem-cell dynamics in cancer initiation and progression as well as treatment response and resistance, and use chronic myeloid leukemia (CML) as a specific example.

CML represents the first human cancer in which molecularly targeted therapy leads to a dramatic clinical response.[39] Imatinib mesylate is a potent inhibitor of the *BCR-ABL* fusion oncogene that drives the leukemia, and induces remission in all stages of the disease.[40] Although CML represents one of the most well-studied cancers, several critical questions remain: (1) CML is associated with the *BCR-ABL* oncogene, but the total number of mutations necessary to initiate the disease is unknown. Is the *BCR-ABL* oncogene sufficient to cause chronic-phase CML? (2) In most patients, imatinib fails to eliminate residual disease, which has been shown to be part of the stem-cell compartment.[41] How do leukemic stem cells respond to

imatinib therapy? (3) A substantial fraction of patients evolves point mutations in the ABL kinase domain leading to treatment failure.[42] What are the dynamics of resistance? (4) Disease progression has been reported to correlate with an expansion of leukemic stem cells[43] or progenitors.[44] In which cellular compartment do mutations that drive progression to blast crisis arise? In the following sections, I will discuss how these topics have successfully been addressed with mathematical and computational techniques.

HOW MANY MUTATIONS ARE NEEDED TO CAUSE CHRONIC-PHASE CML?

The *BCR-ABL* fusion oncogene is the hallmark of CML, but it is unknown whether any other mutations are needed to cause the chronic phase of the disease. So far, experimental evidence has not been able to show conclusively how many mutations are necessary to initiate CML. Approximately 30% of healthy individuals express *BCR-ABL* at low levels.[45] This could mean that they have not yet evolved a second, disease-causing mutation, or that the Philadelphia chromosome has arisen in a differentiated cell not capable of self-renewal. In the latter case, the continuous production of healthy hematopoietic cells would eventually replace the *BCR-ABL*–positive clone. Mouse models reproduce a CML-like disease when expressing the *BCR-ABL* oncogene alone[46] or in combination with v-abl.[47] Finally, exposure to ionizing irradiation increases the risk of CML only after a prolonged latent period,[48] suggesting either that further mutations need to accumulate, or that the mutant clone has a slow rate of expansion.

The age-specific incidence data of CML increases with a slope of 2.86 on a doubly logarithmic plane. A slope of almost 3 could indicate that there are two mutations, in addition to the *BCR-ABL* oncogene, that have not yet been discovered. Indeed, the incidence data was used to calibrate a multistage model of carcinogenesis predicting that the chronic phase of the disease is caused by three mutations accumulating in one stem cell.[49] However, the model neglects the population genetics of stem cells (such as the number of susceptible cells and the fitness effects of mutations), which are indispensable for drawing a meaningful conclusion.

Let us discuss a population genetics model of CML initiation and its epidemiologic consequences.[50] Initially, there is a population of wild-type hematopoietic stem cells. During each cell division, a cell carrying the Philadelphia chromosome arises with a certain probability, and such a cell has a fitness advantage (larger net growth rate) compared with wild-type cells (Fig 1A). Assume that the probability to diagnose the disease is linearly proportional to the number of leukemic stem cells present. This stochastic process is characterized by three waiting times, or the time needed for rate-limiting steps: (1) the waiting time until the production of the first surviving leukemic stem cell, (2) the time for clonal expansion of its lineage, and (3) the time until detection of the disease. Under particular circumstances, for instance when the time for clonal expansion is sufficiently long and the rate of diagnosis is small, this simple one-mutation model can give rise to incidence curves with a slope of up to 3. The age-specific incidence data for CML is obtained from the SEER registry (www.seer.cancer.gov), which covers approximately 10% of the US population, and is adjusted to obtain the probability to be diagnosed with CML per year (Table 1). The resulting incidence curve is a nearly straight line on a doubly logarithmic plot with slope 2.86. The one-mutation model is found to fit to the incidence data for plausible parameter

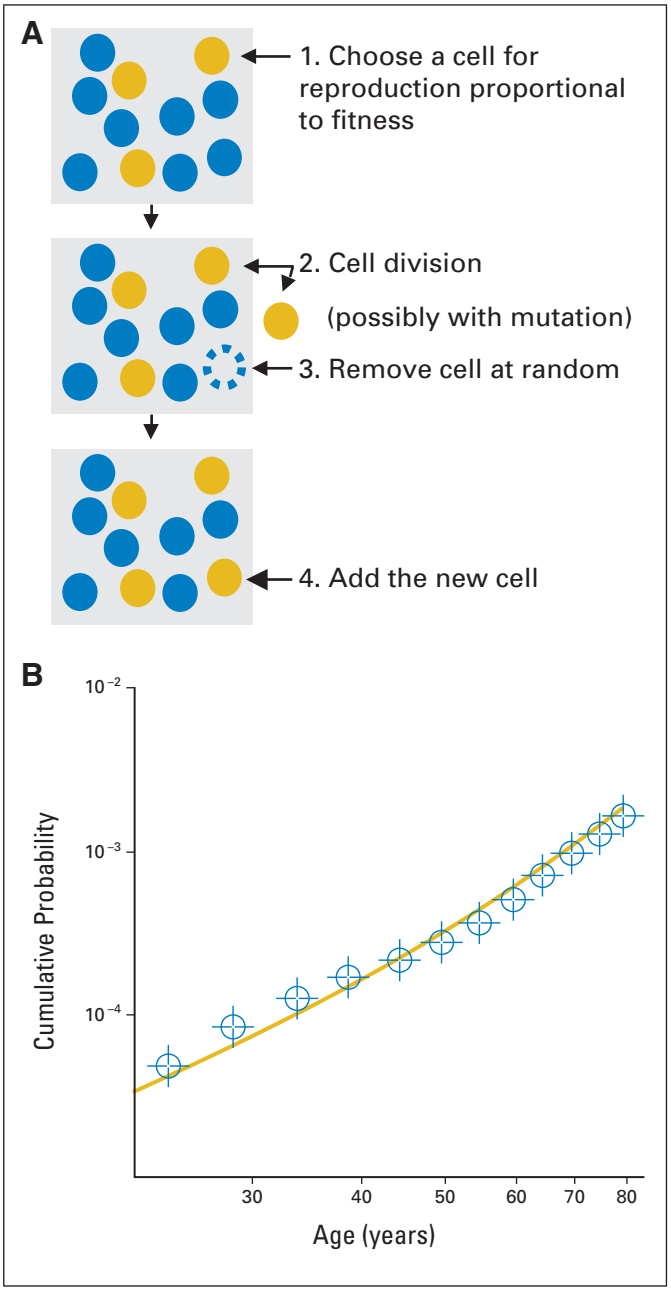

Fig 1. Chronic myeloid leukemia (CML) incidence. (A) The model. Initially, all stem cells are wild type. At each time step, a cell is chosen for reproduction proportional to fitness, and its offspring replaces another randomly chosen cell. Leukemic stem cells arise at a certain rate per cell division and have a fitness advantage. The rate of CML diagnosis is proportional to the number of leukemic stem cells. This model allows us to study the evolutionary dynamics of CML initiation. (B) The graph shows the numerical simulation of the probability to be diagnosed with CML (equation 1 in Michor, Iwasa, and Nowak[50]; line) and the adjusted cumulative CML incidence data from Table 1 (circles). Parameter values are mutation rate 3×10^{-8} per cell division, population size 10^5, relative fitness of mutated cells 1.01 corresponding to a 1% fitness advantage, average cell cycle time 60 days, and probability of diagnosis 10^{-3}. Figure adapted from Michor, Iwasa, and Nowak.[50]

Table 1. Chronic Myeloid Leukemia Incidence Data

Age Class (years)	Cases in SEER (1973-2002)	Cases per Year	US Standard Population	Adjusted Cumulative Probability
1-4	20	17	15,191,619	.0000055
5-9	22	18	19,919,840	.0000102
10-14	27	23	20,056,779	.0000159
15-19	50	42	19,819,518	.0000264
20-24	92	77	18,257,225	.0000475
25-29	147	123	17,722,067	.0000823
30-34	189	158	19,511,370	.0001228
35-39	225	188	22,179,956	.0001653
40-44	241	202	22,479,229	.0002103
45-49	281	235	19,805,793	.0002698
50-54	351	294	17,224,359	.0003552
55-59	436	365	13,307,234	.0004925
60-64	508	425	10,654,272	.0006922
65-69	569	476	9,409,940	.0009455
70-74	611	511	8,725,574	.0012388
75-79	635	532	7,414,559	.0015973
80-84	465	389	4,900,234	.0019944
85+	379	317	4,259,173	.0023666

NOTE. The table shows the number of chronic myeloid leukemia (CML) cases in the Surveillance, Epidemiology, and End Results (SEER) registry (1973-2002) for age classes of 5 years (columns 1 and 2), cases per year (column 3; there were 4,400 CML cases in 2000,[51] so each SEER entry is multiplied with 4,400/5,256), the US Census data from 2000 (column 4), and the adjusted cumulative probability to be diagnosed with CML before a certain age (column 5; the cases per year are divided by the census data to get the probabilities $p(i)$ to be diagnosed with CML per year of age, which are used to calculate the probabilities $q(k)$ to be diagnosed with CML anytime before age k: $q(k) = 1 - \Pi_{i=1}^{k}[1 - p(i)]$). Table adapted from Michor, Iwasa, and Nowak.[50]

choices (Fig 1B). Therefore, the hypothesis that the Philadelphia chromosome alone is sufficient to initiate chronic-phase CML is consistent with the observed incidence curve.[52] This mathematical model does not serve as a proof that no further mutations are necessary, but can be used as supportive evidence that the *BCR-ABL* oncogene may be enough to cause the chronic phase. A firm establishment that *BCR-ABL* is sufficient requires further experimental investigations.

HOW DO LEUKEMIC STEM CELLS RESPOND TO IMATINIB THERAPY?

The hypothesis that leukemic stem cells cannot be depleted by imatinib therapy is supported by several experimental in vitro studies,[53-55] but an in vivo demonstration is complicated by the fact that a direct measurement of stem-cell abundance requires frequent bone marrow aspirates. However, *BCR-ABL* transcript levels in peripheral blood can readily be determined by a quantitative real-time polymerase chain reaction assay. *BCR-ABL* values are expressed as a percentage of BCR transcript levels, and give an estimate of the fraction of terminally differentiated leukemic cells, because the blood predominantly contains terminally differentiated cells. These data have been used in a series of theoretical investigations to infer the behavior of leukemic stem cells during imatinib therapy.[32,33,35]

Successful therapy leads to a biphasic exponential decline of leukemic cells in peripheral blood (Fig 2A).[32] The first slope, determined by calculating the exponential decline between 0 and 3 months after initiation of imatinib therapy, has a mean of 0.05 ($\pm$ 0.02); this corresponds to a 5% depletion of leukemic cells per day. The second slope, determined by calculating the exponential decline between 3 and 12 months, has a mean of 0.008 ($\pm$ 0.004) which corresponds to a 0.8% depletion per day. Some patients discontinue imatinib as a result of complications or adverse effects (Fig 2B). In those patients, the number of leukemic cells rises within weeks to levels at or beyond pretreatment baseline despite continuous treatment for up to 3 years.

A mathematical model describing four layers of the differentiation hierarchy of leukemic cells (leukemic stem cells, progenitors, differentiated, and terminally differentiated cells) is fit to the data and suggests that the first slope represents the depletion of differentiated leukemic cells.[32] These cells have an average life span of 20 days during therapy and, on reaching a steady-state with leukemic progenitors, decline at the latter cells' turnover rate. The second slope represents the depletion of leukemic progenitors, which have an average life span of 125 days during therapy. Imatinib therapy leads to an at least 5,000-fold decrease in the production of terminally differentiated leukemic cells from leukemic stem cells and therefore, discontinuation of imatinib leads to a sudden 5,000-fold increase in their production. The levels the cell count reaches after discontinuation of the drug informs about the dynamics of the cell population that is driving the disease: the leukemic stem cells. Resurgence to levels beyond pretreatment baseline signifies that leukemic stem cells are not depleted by imatinib therapy.

Quiescence of leukemic stem cells has been investigated as one explanation for the lack of their depletion by imatinib.[33,35,54] Leukemic stem cells are thought to switch between a dormant, imatinib-insensitive state and a proliferating, imatinib-susceptible state. The propensity of cells to be in either state depends on a cell-specific affinity, which they lose while proliferating and regain while dormant.[33] In the context of these models, the first slope is interpreted as a depletion of cycling leukemic stem cells by imatinib, whereas the second slope represents the depletion of dormant cells as they re-enter the cell cycle.[33,35] The models predict that imatinib can deplete (cycling) leukemic stem cells and that prolonged therapy may cure the disease, particularly if combined with a proliferation-stimulating agent that pushes dormant leukemic stem cells into the cell cycle and makes them susceptible to imatinib therapy.

To distinguish between the predictions of the models, more clinical and experimental work is warranted. The alternative interpretations of the biphasic decline could be tested by measuring average life spans of leukemic differentiated cells, progenitors, and cycling stem cells, as well as the relative abundance of quiescent cells. The long-term response to imatinib can inform about the behavior of leukemic stem cells during imatinib therapy: If the leukemic burden settles around a constant value or slowly increases, then leukemic stem cells survive or expand during therapy, whereas they are depleted if the cell count continues to decrease.[56] A clinical trial exploring the effects of combination therapy of imatinib and a proliferation-stimulating agent can help shed lights onto these issues. The number of circulating leukemic stem cells could also be measured and may be interpreted as a proxy for the abundance of leukemic stem cells in the marrow. Additionally, other possibilities of imatinib insensitivity, such as drug export by

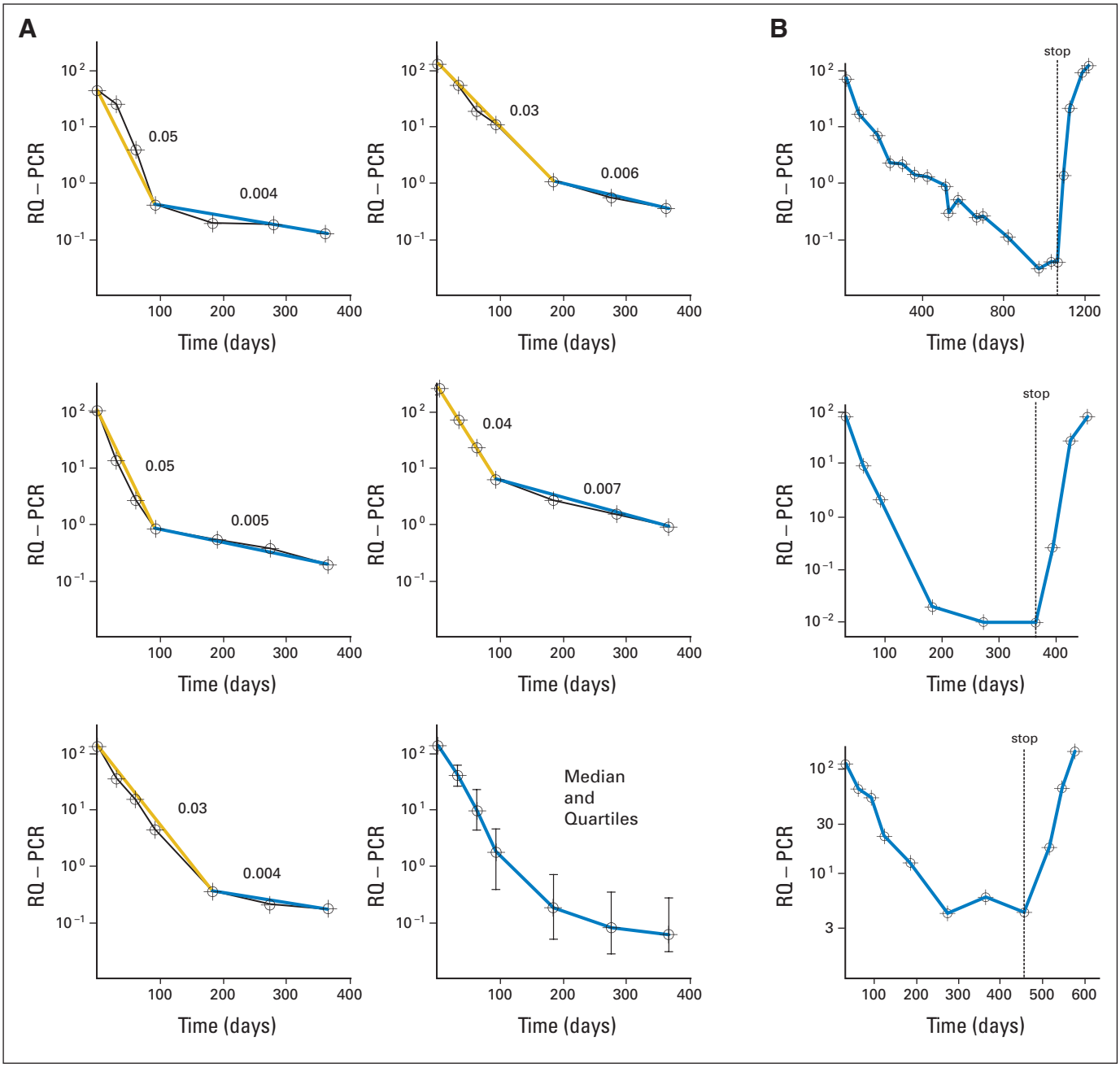

Fig 2. Chronic myeloid leukemia response to imatinib therapy. (A) Imatinib leads to a biphasic decline of leukemic cells. The first five panels show the *BCR-ABL* transcript levels in the blood of five patients. The sixth panel shows the median with quartiles taken over all patients who do not evolve resistance mutations. Imatinib therapy starts on day 0. (B) Discontinuation of imatinib therapy in three patients after 1 to 3 years lead to a rapid increase of leukemic cells to levels at or beyond pretreatment baseline. Therefore, leukemic stem cells are not depleted by imatinib during this time period. Figure adapted from Michor et al.[32] RQ-PCR, real-time quantitative polymerase chain reaction.

multidrug-resistance efflux pumps[53] and *BCR-ABL* independence of leukemic stem cells,[57] should be investigated.

WHAT ARE THE DYNAMICS OF IMATINIB RESISTANCE?

A substantial fraction of patients develops acquired resistance to imatinib. Mutations in the ABL kinase domain are the main mechanism for resistance and account for 70% to 80% of cases with treatment failure.[42,43,58] Sometimes resistance can already be detected at the time of diagnosis of CML.[59] Resistant leukemic cells emerge after an initially successful response to imatinib therapy and lead to a relapse of the disease (Fig 3). The average slope was determined by calculating the exponential increase after the first appearance of resistance mutations in thirty patients[32]; a mean value of 0.02 ($\pm$ 0.01) per day was obtained. Of those patients who start imatinib in the early

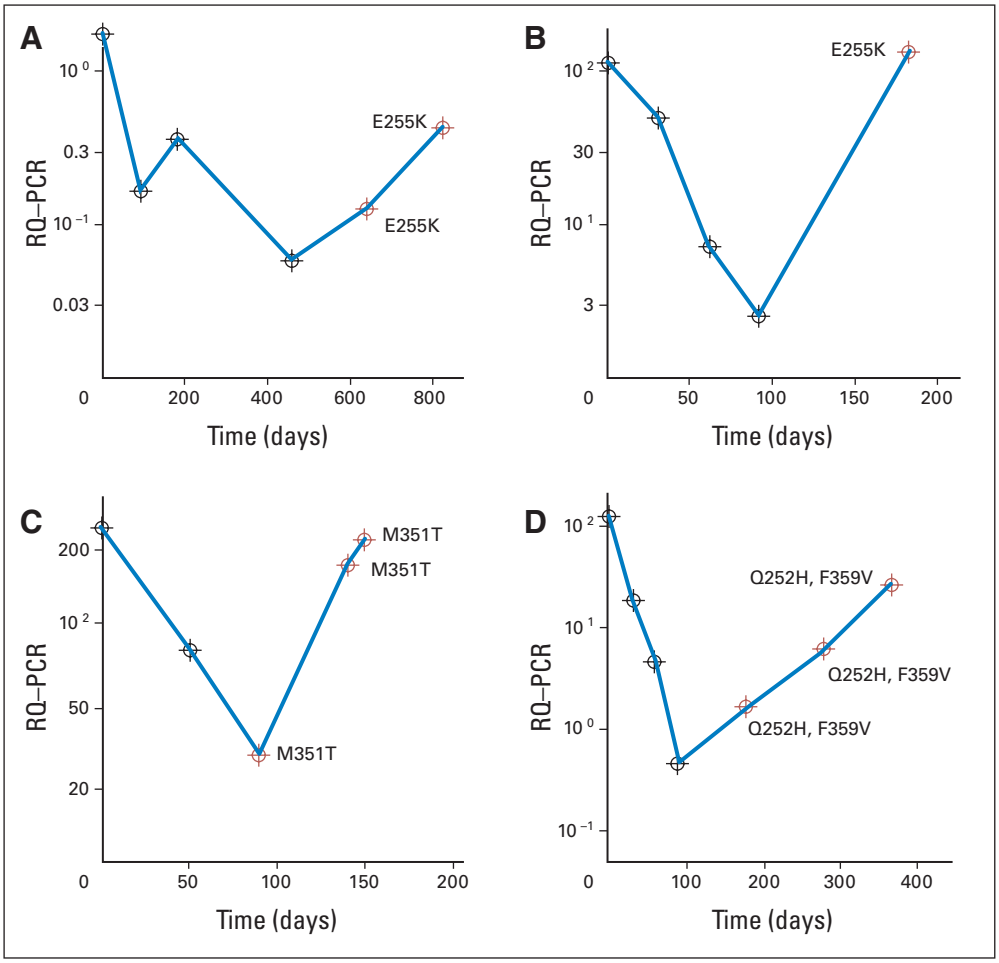

Fig 3. Acquired resistance to imatinib therapy. Approximately 40 different point mutations have been identified, each of which is sufficient to confer resistance to imatinib. The panels show the dynamics of resistance in four patients, with the labels denoting the individual mutations detected at various time points. Resistance mutations lead to a relapse of leukemic cells. Figure adapted from Michor et al.[32] RQ-PCR, real-time quantitative polymerase chain reaction.

chronic, late chronic and accelerated phase, respectively, 12%, 32%, and 62% develop detectable resistance mutations within 2 years of treatment (Table 2).[59]

A stochastic process model can be used to analyze the evolution of resistance and predict the fraction of patients harboring mutated cells at diagnosis.[36] The model considers an exponentially growing population of leukemic stem cells that may accumulate mutations conferring resistance to imatinib therapy, and is used to calculate the probability of resistance once the patient is diagnosed (Table 3). The higher incidence of resistance in patients in later stages of the disease can be explained by an increased leukemic stem-cell burden (or a larger number of cell divisions that have occurred until that time). Another mathematical model investigates how quiescence of leukemic stem cells affects the evolutionary dynamics of drug resistance.[35] If treatment consists of a single drug, then quiescence is found to have no effect on the probability that mutant cells exist before CML diagnosis; if treatment involves a combination of two or more drugs with different targets, however, then cellular quiescence does increase the chance of resistance. Although quiescence prolongs the time it takes to eradicate the tumor, the treatment phase is unimportant for the evolution of resistance because most mutations emerge before the start of therapy. Therefore, a reduction of the quiescent stem-cell population by therapy (eg, by combining imatinib with a proliferation-stimulating agent) will not reduce the risk of resistance.

Table 2. Observed Incidence of Imatinib Resistance

Year	%		
	Early Chronic Phase	Late Chronic Phase	Accelerated Phase
1	5.9	14	38
2	12	32	62

NOTE. Observed percentage of patients with acquired resistance mutations during the first and second year of treatment. Early chronic phase refers to patients who commenced imatinib within 1 year of diagnosis. Adapted from Michor, Hughes, and Iwasa.[32]

Table 3. Predicted Fraction of Patients With Resistance Mutations

Mutation Rate	No. of Leukemic Stem Cells (%)			
	10^5	10^6	10^7	10^8
4×10^{-7}	5.4	43	100	100
4×10^{-8}	0.6	5.4	43	100

NOTE. Predicted percentage of patients that harbor resistance mutations depending on the abundance of leukemic stem cells and the mutation rate conferring resistance. Resistant leukemic cells can be below detection limit in some patients. Adapted from Michor, Hughes, and Iwasa.[32]

Table 4. Annual Rate of Disease Progression During Imatinib Therapy

Year	% Progression	% Accelerated Phase/Blast Crisis
1	3.4	1.5
2	7.5	2.8
3	4.8	1.6
4	1.5	0.9

NOTE. Progression and evolution to accelerated phase/blast crisis in patients treated with imatinib. Progression is defined as loss of complete hematologic response of major cytogenetic response. Adapted from Michor.[61]

Further theoretical investigations will inform about the efficacy of the use of multiple drugs, such as imatinib, dasatinib, and nilotinib, and predict the chance that resistance evolves during such treatment strategies. Also, calculations of the probability of resistance and treatment outcome can be customized to individual patients such that their disease is optimally managed. The development of new compounds that inhibit cells carrying resistance mutations against currently available drugs is an experimental priority, and mathematical analyses can help understand their efficacy and impact on cancer stem cells.

WHERE DO MUTATIONS DRIVING PROGRESSION TO CML BLAST CRISIS ARISE?

CML progresses through three distinct clinical stages: chronic phase, accelerated phase, and blast crisis. Progression to blast crisis is supported by self-renewing blast-crisis stem cells. These cells drive blast crisis like leukemic stem cells drive the chronic phase, and arise as a result of genetic and/or epigenetic events such as duplication of the Philadelphia chromosome, trisomy 8, and inactivation of p16 and p53.[60] The cell of origin of blast-crisis stem cells is a subject of controversy. Many experimental findings support that blasts arise by mutation of leukemic stem cells,[43] but new evidence suggests that blasts may evolve from leukemic progenitors instead: leukemic progenitors isolated from blast-crisis patients are found to have self-renewal capacities and increased β-catenin and *BCR-ABL* expression, and to expand during disease progression.[44] Knowledge of the cell type and mutations driving blast crisis would increase the understanding of the natural history of CML, and may suggest new treatment strategies for blast-crisis patients.

A mathematical model of CML progression can be used to investigate the cell of origin and the dynamics of blast crisis.[61] Blast-crisis stem cells could, in principle, arise by (epi)genetic changes accumulating in leukemic stem cells or in leukemic progenitors. The mutations arising in stem cells may be activating oncogenes and inactivating tumor suppressor genes, whereas the mutations arising in progenitors can additionally include changes facilitating self-renewal. In the model, the evolutionary process of mutation is encapsulated in a parameter describing the rate at which blasts arise and survive. The observed probability of progression to blast crisis is 1% to 2% per year for patients receiving imatinib therapy (Table 4) and 10% to 20% per year for patients receiving previous therapies such as α-interferon plus cytarabine.[62] Hence, imatinib reduces the progression rate 10-fold compared with previous (ineffective) therapies.

Imatinib seems to be incapable of depleting leukemic stem cells by considerable amounts (Fig 3). Therefore, the abundance of leukemic stem cells should not differ substantially between imatinib-treated and -untreated patients, if imatinib is administered for short periods. If blasts arise by mutation from leukemic stem cells, then the probabilities of progression to blast crisis with and without imatinib should be the same: treatment would not attenuate blast crisis if it did not change the abundance of the target cell population. Conversely, if blast crisis is driven by leukemic progenitors, then the rates of progression are expected to differ because imatinib does deplete leukemic stem cells (Fig 4). The latter pattern is seen in CML patients. Hence, CML blast-crisis mutations are likely to arise in leukemic progenitors.

There are two caveats to this conclusion. First, imatinib may be able to reduce the increased mutation rates brought about by the *BCR-ABL* oncogene. A CML mouse model suggests that *BCR-ABL* increases the point mutation rate two- to three-fold, and that this effect can be reversed by imatinib therapy.[63] However, an increase in the mutation rate of this order of magnitude does not change the conclusion of the mathematical model. Second, imatinib might reduce the expansion of leukemic stem cells without depleting them. This expansion occurs slowly (with a net growth rate of about 0.5%

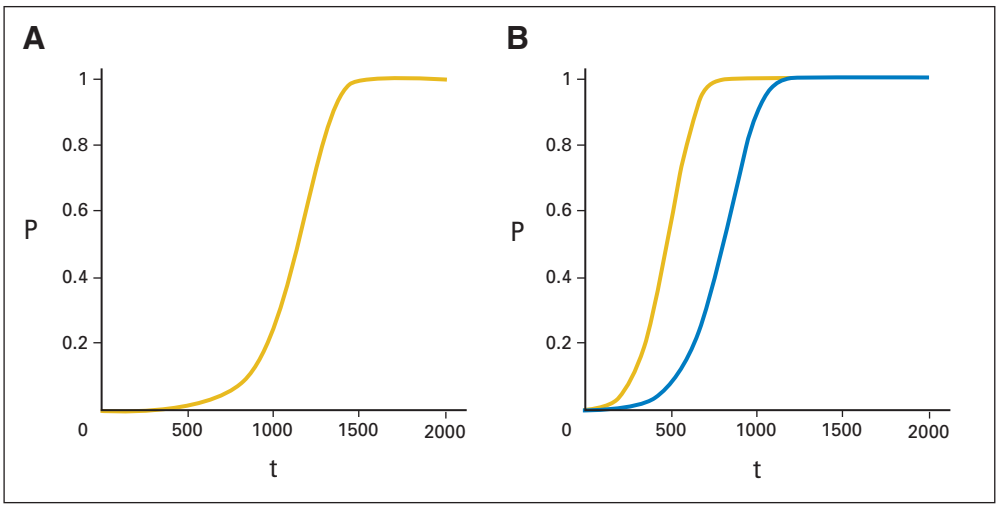

Fig 4. Evolution of blast crisis stem cells. The figure shows the probability of blast crisis over time if blast crisis stem cells arise by mutations accumulating in (A) leukemic stem cells (equations 2 and 4 in Michor[62]) or (B) leukemic progenitors (equations 3 and 4 in Iwasa, Nowak, Michor[60]). (A) The curve is the same for patients receiving imatinib therapy and for untreated patients, because imatinib cannot deplete leukemic stem cells. (B) The right curve shows the probability of blast crisis when the patient is treated with imatinib, and the left curve shows the probability of blast crisis when there is no treatment. The rate of progression with imatinib therapy is 10-fold lower than the rate of progression without imatinib therapy because imatinib depletes leukemic progenitors. Figure adapted from Michor.[62]

per day), and it is unlikely that robust clonal expansion can be maintained if the growth rate is decreased considerably. Robust expansion, though, is necessary to explain the relapse kinetics in patients who discontinue therapy. Hence, this explanation seems ungeneric, and blasts are likely to arise from leukemic progenitors. An identification of the genetic changes driving blast crisis, as well as a firm establishment that those changes arise in progenitors, requires further experimental investigation.

DISCUSSION AND OUTLOOK

In this review, I have presented examples for how mathematical and computational techniques can contribute to the understanding of cancer stem cells. I have used CML as an example and have discussed approaches to answer questions about the number of mutations necessary to cause chronic-phase CML, the treatment response of leukemic stem cells, the evolution of resistance to imatinib therapy, and the dynamics of progression to blast crisis. Although experimental validation remains necessary to demonstrate the molecular mechanisms, quantitative methods can help to distinguish between hypotheses and further the understanding of cancer stem cells.

The models discussed in this review represent only a small part of the literature on theory and cancer stem cells. Other fields of investigation include the dynamics of stem cells driving particular cancers such as colon or breast cancer,[26-31] as well as the role of symmetric and asymmetric stem-cell division in carcinogenesis.[64,65] In the latter case, mathematical models have been used to study the impact of changes in the probability for symmetric versus asymmetric replication on tumor dynamics. An increase in the probability of stem-cell self-renewal can lead to a rapid cancer stem-cell expansion even in the absence of a selective fitness advantage (as conferred by for instance an activated oncogene).[65] Mutations in several genes, such as *PINS*, *LGL*, and *HUGL-1*, can lead to this process and may be at the root of tumor development.

The mathematical approaches outlined in this review are not limited to the study of leukemic stem cells. Although CML appears to be a relatively simple malignancy driven by a single genetic aberration, it serves as an example of a disease managed by molecularly targeted therapy, and insights gained concerning its treatment response and dynamics of resistance are applicable to other (solid) cancers, too. The existence of cancer stem cells has been conjectured for most (if not all) types of tumors, but a demonstration of their presence, as well as an elucidation of their biologic characteristics, are still lacking for many cancers. Theoretical techniques similar to the ones outlined in this review can contribute to that goal; particularly, the question of whether targeted or general cytotoxic drugs can deplete cancer stem cells is amenable to mathematical investigation. The evolution of resistance represents a challenge for most cancer types and treatment options, and a quantitative understanding of its dynamics helps to determine how to optimize treatment options for individual patients. Also, the question of how many mutations are needed to cause a particular type of cancer and its progression, as well as the target cell population in which those mutations arise, can be investigated with computational and mathematical tools. The study of cancer stem cells is an exciting and important topic in cancer research and will profit considerably from theoretical input.

AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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