

DOSE DEPENDENT SURVIVAL RESPONSE IN CHRONIC MYELOID LEUKEMIA UNDER CONTINUOUS AND PULSED TARGETED THERAPY*

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A simulative study of cancer growth dynamics in patients affected by Chronic Myeloid Leukemia (CML), under the effect of a targeted dose-dependent continuous or pulsed therapy, is presented. We have developed a model for the dynamics of CML in which the stochastic evolution of white blood cell populations are simulated by adopting a Monte Carlo approach. Several scenarios in the evolutionary dynamics of white blood cells, as a consequence of the efficacy of the different modelled therapies, pulsed or continuous, are described. The best results, in terms of a permanent disappearance of the leukemic phenotype, are achieved with a continuous therapy and higher dosage. However, our findings demonstrate that an intermittent therapy could represent a valid choice in patients with high risk of toxicity, when a long-term therapy is considered. A suitably tuned pulsed therapy can enhance the treatment efficacy and reduce the percentage of patients developing resistance.

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1. Introduction

Chronic Myeloid Leukemia (CML) is a cancer of the blood cells characterized by an overproduction of myeloid cells (one of the main types of white blood cells) which are released into the blood when they are immature and unable to work properly, leading to an increased risk of infection

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and strongly limiting the production of healthy red cells and platelets. The molecular hallmark of CML is a specific chromosomal abnormality, named Philadelphia (Ph) chromosome, caused by a reciprocal translocation: part of the BCR (“breakpoint cluster region”) gene from chromosome 22 is fused with part of the ABL gene on chromosome 9 (ABL stands for “Abelson”, the name of a leukemia virus which carries a similar protein). BCR–ABL fusion gene activates a number of cell cycle controlling proteins and tyrosine kinase enzymes, speeding up cell division and causing genomic instability.

Front line therapy in CML is represented by tyrosine kinase inhibitors that turn off the signal produced by the Ph chromosome, limiting the proliferation of BCR–ABL expressing hematopoietic cells. The standard dosage of imatinib drug administration is 400 mg/d for patients in chronic phase (CP), but this value is often increased to 600 mg/d or even 800 mg/d in patients in accelerated phase (AP). These inhibitors greatly reduce the growth of the tumor clone but can cause toxicity on full-treated patients [1]. Moreover, a great number of genetic alterations are produced as a consequence of the chromosomal instability that characterizes immature leukemic cells. In particular, point mutations within the protein tyrosine kinase domain in the BCR–ABL gene itself [2–4] are the basis for the development of acquired resistance to imatinib-like drugs in a relevant fraction of patients.

In order to reduce the impact of hematologic toxicity in patients affected by CML, a new therapeutic approach, based on the intermittent dosage of tyrosine kinase inhibitors, has been recently investigated on clinical studies [1, 5, 6]. These works have demonstrated that an intermittent therapy (IT) can be considered a good alternative to the standard daily dosing in patients with persistent signs of myelotoxicity, without compromising the patient cytogenetic response. Moreover, recent clinical studies on the treatment of hormone-sensitive advanced prostate cancer have demonstrated that the introduction of breaks in long-term anticancer treatments can prolong positively the tumor response to the therapy [7, 8]. A similar result is also obtained in a clinical study on the breast cancer [9]. A “drug-driven dependence deprivation effect” might be able to inhibit and destroy the most resistant or dependent cancer clones. In the context of the CML, a temporary interruption of imatinib therapy was found to significantly reduce the presence of a cell clone showing a BCR–ABL Y252H (P-loop) mutation in a resistant patient [10, 11].

Following these ideas and driven by the effective patient necessity to reduce drug toxicity during the imatinib-based treatment of CML and design a strategy to stave off resistance, we have developed a model of leukemic cell evolutionary dynamics to investigate the response of simulated patients treated by an imatinib-like therapy. The aim of this work is to find the optimal therapeutic approach that minimize imatinib side-effects, maximizing

the clinical benefits of this treatment and ameliorating as much as possible the quality of life of the patient. In Section 2 we describe the model and give the details of the simulation process. Results are reported in Section 3 and conclusions are drawn in Section 4.

2. The model

Studies on cancer genetics [12–14] confirmed that cancer arises when a single cell experiences multiple mutations. Following this basic idea, the evolutionary dynamics of cancer initiation and progression has been theoretically approached by mathematical deterministic equations [15–18] or stochastic models [19–28]. These works describe the temporal evolution of the concentration of BCR–ABL positive cells, experimentally observed in patients treated with imatinib, in terms of a partial or total failure of the drug efficacy on cancer cells.

In our model, we simulate the evolutionary dynamics of $N = 10^4$ replicating cells, distributed over four populations: healthy cells (type-0), first-mutated cells (type-1), double-mutated leukemic cells (type-2) and resistant leukemic clones (type-3). The total number of cells is kept constant during the time evolution, as it can be reasonably assumed for a blood cancer. The value $N = 10^4$ is several orders of magnitude lower than the typical total contents of blood cells in humans, but it is great enough for the statistical study of the cancer development in a single blood compartment.

We have adopted a Monte Carlo approach to simulate the random process of cell selection for reproduction, mutation and death, as already done by several authors in theoretical cancer studies (see [21, 27, 28], to cite a few). The stochastic dynamics of the cancer evolution is modelled by assuming that cells reproduce asynchronously. This means that each elementary step of the stochastic process consists of a birth and a death event, *i.e.* a Moran process [29]. For the birth, one of the N cells is randomly chosen proportionally to the fitness. In our simulations the fitness of type-0 and type-1 cells are set equal to 1 as already adopted in other theoretical studies [21, 28]. In the absence of any therapy, the reproductive rate of a leukemic cell is assumed to be 10 times that of a normal cell. The therapy lowers this value only for those cells which are sensitive to the drug; the resistant clone is unaffected by the therapy and its reproductive capability remains unchanged. In particular, we have assumed that imatinib reduces the fitness of type-2 leukemic cells to 0.04 or 0.02 if the drug is administrated with a dose of 400 mg/d or 800 mg/d, respectively. Fitness values have been chosen in order to match the response of type-2 leukemic cells to that experimentally observed in patients treated by an imatinib-based targeted therapy [15, 19].

Each cell can reproduce itself or mutate. Population of healthy cells mutates to cells of type-1 (first allele mutation) at a rate M_{01} equal to 0.0005; type-1 cells mutate to type-2, which are leukemic cells sensitive to the therapy, at a rate M_{12} equal to 0.002. These values, comparable with the mutation rates adopted in the models of Refs. [15, 21], guarantee a good agreement between our findings and clinical results. We performed simulations also for different values of M_{01} and M_{12} , finding that only the waiting time before the first appearance of a leukemic cell is sensitive to these values and they do not significantly affect the subsequent course of the disease, which is the main focus of this work.

For the death, since the tyrosine kinase inhibition by imatinib induces apoptosis in BCR-ABL expressing cells [30], we take into account this effect by increasing, during the therapy, the death rate of type-2 leukemic cells with respect to the remaining cell populations. Fig. 1 shows a logical map of the evolution of the blood cells from normal to cancerous types. We do not consider back mutations and neglect direct transitions from healthy (type-0) to leukemic cells (type-2) or to resistant clones (type-3). Genetics studies show the evidence that certain mutations increase the rate at which subsequent mutations occur [31, 32]. From the experimental point of view, clinical studies after two years of treatment show an increasing trend in the number of patients developing resistance to imatinib with the progression of the disease through the chronic, accelerated and blast crisis phases [11].

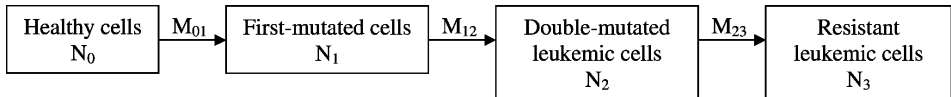


Fig. 1. Logical map of the evolution of the blood cells from healthy cells (type-0) to first-mutated (type-1), double-mutated leukemic cells (type-2) and leukemic resistant cells (type-3). The mutation rates are assumed as follows: $M_{01} = 0.0005$, $M_{12} = 0.002$, M_{23} depends on the number N_2 of type-2 leukemic cells, as shown in Fig. 2.

Being these phases characterized by the presence of an increasing number of immature leukemic cells (myeloblasts) in the blood or bone marrow [33], we have assumed in our model that type-2 cells become resistant type-3 by mutating at a rate M_{23} which has not a constant value, but increases with the number N_2 of leukemic cells double-mutated (Fig. 2). The theoretical trend of M_{23} vs. N_2 has been extrapolated from the behavior of the frequency of acquired hematologic resistance as a function of the number of myeloblasts in the blood, accordingly with the standard definition of CML phases cited above [11], and calculated by the equation

$$M_{23} = a + \left(\frac{b(1 - e^{-N_2/c})}{d} \right), \tag{1}$$

where $a = 2 \times 10^{-4}$, $b = 8.5 \times 10^3$, $c = 2.5 \times 10^3$ and $d = 4 \times 10^7$ are the best parameters for which the Eq. (1) reproduces the experimental behaviour [11]. In this way the mutation rate M_{23} has a latency value of 2×10^{-4} , while it progressively reaches the double of its value in the presence of an increase of immature cells N_2 .

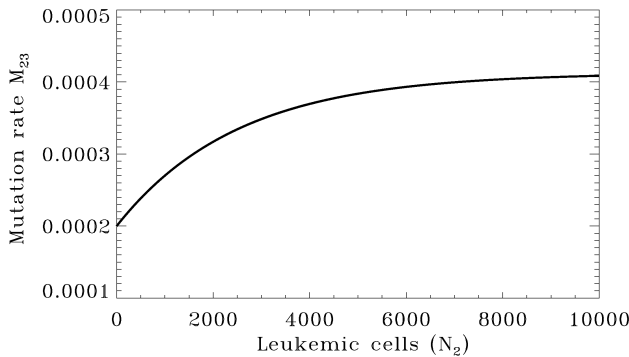


Fig. 2. Mutation rate M_{23} as a function of the number of leukemic cells sensitive to the therapy, calculated by Eq. (1) and reproducing the experimental behavior, reported in [11], of the frequency of deleterious mutations associated to the appearance of resistance as a function of disease progression.

Since the specific chromosomal mutation (or set of mutations) that causes the cancerous cell to become resistant and insensitive to the therapy can be considered an evolutionary reaction of the cell against the drug, we assume that, during the periods of absence of therapy, the mutation rate M_{23} is reduced to such very low values that we reasonable keep it equal to zero. In our simulations time is measured in units of cell divisions. A time scaling from cell division to days is performed by assuming a complete restore of healthy cells in almost 100 days, as experimentally observed in clinical cases of optimal therapy response [15,19].

3. Results and discussion

Our simulations start with all the patient cells of leukemic type still sensitive to the therapy (type-2). Healthy cells and first-mutated cells participate to the reproduction process although their initial value is set to zero. This is because we are simulating the evolutionary dynamics of differentiated cells; stem cells, that we do not take into account in our model, are always able

to produce them. In this context, the resistant clone may originate only by a stochastic process of mutation from the leukemic phenotype. Type-3 cells acquire the chance to reproduce themselves only after the first resistant cell is generated.

In order to lower the number of leukemic cells as rapidly as possible, the first 100 days of therapy are devoted to a continuous therapy. This choice has been made firstly because, for the patient health, it is necessary to restore a suitable level of red cells and platelets, secondly to reduce the probability of developing resistance, which is greater when the number of immature leukemic cells is higher. We have investigated two cases of continuous therapy: with (i) the standard daily dosage of 400 mg/d and (ii) a doubled dosage of 800 mg/d. For the subsequent 300 days we have simulated both a continuous therapy and an intermittent therapy, characterized by dose durations of 0.9 days separated by breaks of 0.4 days.

The occurrence of a genetic alteration that leads a leukemic cell to become resistant to the therapy is a stochastic process. Hence, in order to give a statistical significance to our descriptions, we have repeated every simulation 5×10^3 times. In Fig. 3 we show the temporal evolution of the number of healthy and first-mutated cells ((a), top panel), cancerous cells still sensitive to the therapy ((b), central panel) and resistant clones ((c), bottom panel), averaged over the whole set of simulated patients. A full continuous therapy throughout all 400 days is described by solid lines: plus and cross symbols are used to describe the patient response under a therapy with doses of 400 or 800 mg/d, respectively. The evolution of the same system of cells is shown, after 100 days of continuous treatment, in the presence of an intermittent therapy characterized by the same dose duration and short breaks of 0.4 days between two consecutive doses of drug.

Of course the best results, in terms of a permanent disappearance of the leukemic type-2 cells, is achieved with a continuous therapy with a dosage of 800 mg/d. This therapeutic approach is the most efficient in the first 100 days of the treatment because of the faster reduction of the number N_2 of leukemic cells, with respect to the case of the standard dosage of 400 mg/d. The faster reduction of N_2 brings to higher levels of healthy cells (Fig. 3 (a)), lower probabilities of developing resistant clones and, consequently, a lower number of N_3 within the first 100 days (Fig. 3 (c)). For the subsequent 300 days, the results shown in Fig. 3 demonstrate that an intermittent therapy could also represent a valid choice in patients with high risk of toxicity. In fact, even if an increase of the average number of leukemic cells sensitive to the therapy is observed during an intermittent therapy, this effect is not significant for breaks of short duration and it is also partially counterbalanced by a reduction on the average number of resistant cells. In a long term strategy, the IT with breaks of 0.4 days appears to be more effective with respect to both the continuous therapies adopted in the first 100 days. In

this context, it is important to note that an IT with a doubled dosage with respect to the standard dose does not bring to any significant improvement in terms of a reduction on the number of leukemic cells.

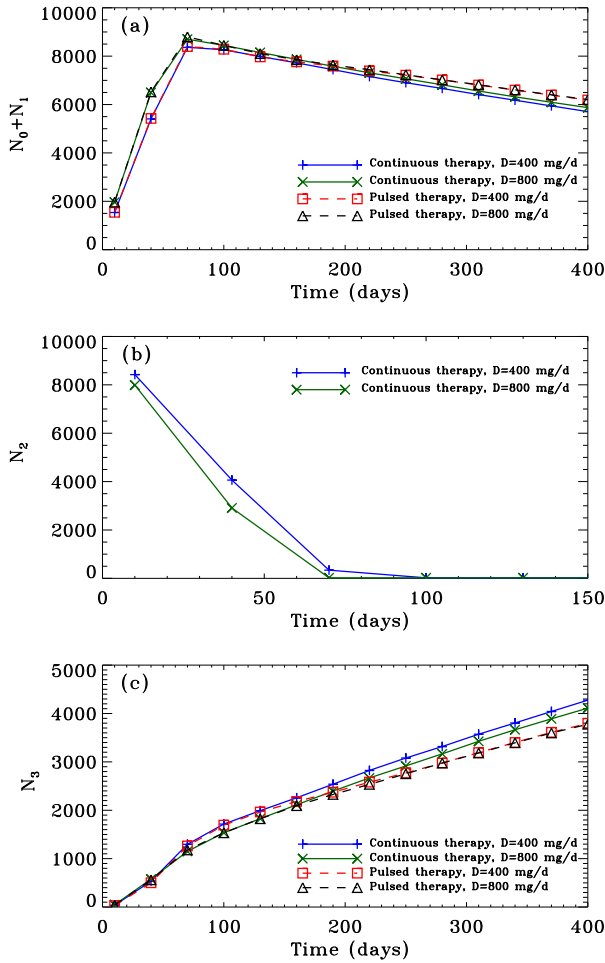


Fig. 3. Temporal evolution of cell populations: (a) Healthy and first-mutated cells, (b) leukemic cells still sensitive to the therapy in the first 100 days and (c) resistant leukemic clones. These values of the number of cells represent averages over 5000 simulated patients. Solid blue line (with pluses) is referred to a continuous therapy with the standard dose of 400 mg/d; solid green line (with crosses) is referred to a continuous therapy with the dose of 800 mg/d. Dashed lines show the dynamics of our system of cells in the presence of an IT with breaks of 0.4 days, after 100 days of continuous treatment. Dashed red line (with squares) is adopted for a pulsed therapy with doses of 400 mg/d; dashed black line (with triangles) is used for a pulsed therapy with doses of 800 mg/d.

The observed reduction on the average number of resistant cells during IT with breaks of 0.4 days is essentially due to a lower number of patients developing resistance. To make clearer this point, in Fig. 4 we show the percentage of patients developing resistance as a function of time, following the aforementioned therapeutic strategies. An advantage of the IT with breaks of 0.4 days over the continuous therapy approach is evident for the whole duration of 400 days. A good agreement is observed between the theoretical percentages obtained in our simulations and the experimental ones. Specifically, the nearly linear trend observed in Fig. 4 between 80 and 400 days, can be reasonably extrapolated up to 800 days, finding that our theoretical results on the percentages of resistant patients account quite well for those observed in clinical experiments where patients are screened up to 2 years [11]. We also performed simulations with therapy breaks of 0.6 and 0.7 days. The results, not shown here, indicate that ITs characterized by breaks longer than 0.6 days, after an initial positive response, do not determine a significant improvement in terms of a reduction in the percentage of patients developing resistance.

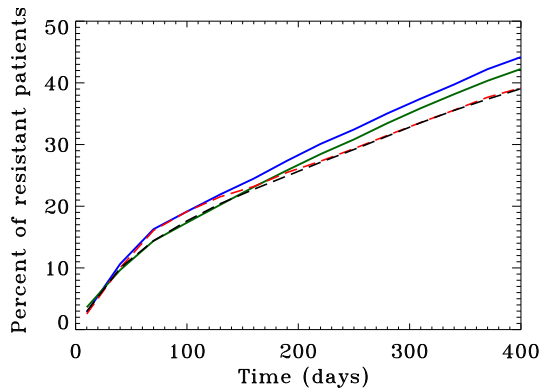


Fig. 4. Percentage of patients developing resistance as a function of time. Solid lines represent the cases of a continuous therapy with a dose of 400 mg/d upper line (blue) and 800 mg/d middle line (green). Dashed lines represent an intermittent therapy with dose of 400 mg/d (red line) and 800 mg/d (black line).

4. Conclusions

In this work, we have analyzed the effects of an intermittent targeted therapy on the evolutionary dynamics of normal and cancerous cell populations in patients affected by Chronic Myeloid Leukemia (CML), with the aim of finding a less aggressive but still effective therapy. Very recent clinical oncology investigations indicate that an intermittent therapy (IT) might reduce the incidence of toxicity of imatinib and the complications that arise

from the side effects of a long-term continuous treatment. Furthermore, the introduction of breaks in anti-cancer therapy could prevent the treatment failure due to drug resistance.

Using computational models, we have shown that an intermittent therapy can represent a valid choice in patients with high risk of toxicity, without compromising the chances of a sustained tumor suppression. By averaging over 5000 simulated patients, we have found that, in IT with dose durations of 0.9 days and therapy breaks shorter than 0.6 days, the presence of a slight increase on the average number of leukemic cells is partially counterbalanced by a reduction on the average number of resistant clones. Furthermore, we have shown that an intermittent therapy with dose durations of 0.9 days and breaks of 0.4 days results more effective of a continuous therapy with standard or doubled dosages. Besides, an IT with a break of 0.4 days reduces the number of patients developing resistance to the drug after 400 days of treatment.

Going behind the numerical details of the simulations, our findings show that an intermittent therapy, appropriately tuned on specific patient necessities, should be preferred to the continuous one. The introduction of short breaks in long-term imatinib based therapies can potentially enhance the treatment efficacy and considerably improve the quality of life of CML patients.

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