Intermittent targeted therapies and stochastic evolution in patients affected by chronic myeloid leukemia

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Abstract. Front line therapy for the treatment of patients affected by chronic myeloid leukemia (CML) is based on the administration of tyrosine kinase inhibitors, namely imatinib or, more recently, axitinib. Although imatinib is highly effective and represents an example of a successful molecular targeted therapy, the appearance of resistance is observed in a proportion of patients, especially those in advanced stages. In this work, we investigate the appearance of resistance in patients affected by CML, by modeling the evolutionary dynamics of cancerous cell populations in a simulated patient treated by an intermittent targeted therapy. We simulate, with the Monte Carlo method, the stochastic evolution of initially healthy cells to leukemic clones, due to genetic mutations and changes in their reproductive behavior. We first present the model and its validation with experimental data by considering a continuous therapy. Then, we investigate how fluctuations in the number of leukemic cells affect patient response to the therapy when the drug is administered with an intermittent time scheduling. Here we show that an intermittent therapy (IT) represents a valid choice in patients with high risk of toxicity, despite an associated delay to the complete restoration of healthy cells. Moreover, a suitably tuned IT can reduce the probability of developing resistance.
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**Keywords:** classical Monte Carlo simulations, models for evolution (theory), mutational and evolutionary processes (theory), computational biology

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### 1. Introduction

Although cancer is a leading cause of death in the world, little is still known about the mechanism of its growth and destruction [1, 2]. In fact, cancer growth dynamics is a typical example of complex dynamics in which the role of fluctuations can be relevant, as in biological systems [3–18].

Chronic myeloid leukemia (CML) is a blood cancer causing 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. This generally leads to an increased risk of infection and strongly limits the production of healthy red cells and platelets. The chromosome abnormality that causes the development of CML is the Philadelphia (Ph) chromosome and consists of a reciprocal translocation occurring in both stem cells and progenitors, giving rise to the BCR–ABL gene. BCR is the breakpoint cluster region, and ABL stands for Abelson, the name of a leukemia virus carrying a similar protein [19].

Nowadays, the front line therapy for the treatment of CML is based on the administration of highly effective tyrosine kinase inhibitors (TKIs) which are able to turn off the signal produced by the Ph chromosome and inhibit the proliferation of leukemic hematopoietic cells. The administration of TKIs represents the first example of a successful molecular targeted therapy. These inhibitors greatly reduce the growth of the tumor clone but can cause toxicity in fully treated patients [20]. Moreover, a great number of genetic alterations due to a chromosomal instability affects immature leukemic cells. Specifically, the basis for the development of acquired resistance to imatinib-like drugs in a relevant fraction of patients is point mutations [21, 22]. In patients harboring such mutations, novel second line TKIs have demonstrated encouraging efficacy, obtaining hematologic and cytogenetic remission in the majority of patients.
with acquired resistance in chronic phase disease. Unfortunately, in advanced stages of the disease, responses are more limited and relapse is common.

Recently, in clinical studies devoted to the reduction of hematologic toxicity in patients affected by CML, a new therapeutic approach, based on the intermittent dosage of TKIs, has been investigated [20, 23, 24]. In fact, it was shown that an intermittent therapy (IT) can be considered a good alternative to standard daily dosing in patients with persistent signs of myelotoxicity, without compromising the patient cytogenetic response. Moreover, in recent clinical studies on the treatment of advanced prostate cancer and breast cancer, the introduction of breaks in long-term anticancer treatments can prolong tumor response to the therapy [25–27]. In other words, IT can give rise to inhibition and disruption of the most resistant or dependent cancer clones. In particular in CML, a temporary interruption of imatinib-like therapy has significantly reduced the presence of leukemic cells showing the malignant mutations in a resistant patient [28, 29].

Along these lines, and driven by patient necessity to effectively reduce drug toxicity during a continuous TKIs-based treatment of CML, and the need to 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 IT. In particular in this work, we study the fluctuations in cancer growth dynamics in patients affected by CML and developing resistance to the standard therapy by the Monte Carlo (MC) method. The evolutionary dynamics of cancerous cell populations is modeled in numerically simulated patients treated by a continuous or an intermittent targeted therapy. In our model, initially healthy cells can experience genetic mutations and change their reproductive behavior, becoming leukemic clones. We simulate a TKIs-based treatment of CML by modifying the fitness and death rate of cancerous cells.

Here we show that IT could represent a valid alternative in patients with high risk of toxicity, being a suitably tuned pulsed therapy more effective in reducing the probability of developing resistance. We start out, in section 2, with a description of the model, giving the details of the simulation process. Results are reported in section 3 and conclusions are drawn in section 4.

### 2. Model of CML evolution

In this section we describe the model used to reproduce the dynamics of leukemic cells in patients affected by CML. The time evolution of CML has been analyzed using deterministic approaches [30–33] as much as models based on stochastic approaches [2, 5, 8, 10, 14, 34–38]. According to clinical studies on cancer genetics [39, 40], which analyze the time behaviour of the level of BCR–ABL positive cells in patients treated with imatinib, both deterministic and stochastic models consider that leukemia initiation coincides with the occurrence of multiple mutations in a single non-differentiated blood cell. In our model, the blood is composed of four cell populations: healthy cells (type-0), first-mutated cells (type-1), double-mutated leukemic cells (type-2) and resistant leukemic clones (type-3). Moreover, each population consists of the sum of stem cells, progenitors, differentiated and terminally differentiated cells.
Stem cells, which possess an indefinite potential for self-renewal [32], or progenitors [19] can experience mutations which cause the appearance of leukemic differentiated and terminally differentiated cells. Therefore, we model mutation rates and fitness by considering the averages over the sub-populations of stem cells and progenitors. Conversely, death rates are obtained as averages over the values of all four sub-populations.

To simulate the cancer dynamics we study the time evolution of \( N = 10^4 \) cells, a value much smaller than the typical number of blood cells contained in a human organism, but large enough to permit a statistical analysis of the cancer evolution in a given blood ‘region’. The cancer dynamics is obtained by keeping constant the total number of blood cells, which is a reasonable assumption for the cells in a single blood compartment. An event of reproduction, mutation or death occurs as a random outcome of a Monte Carlo simulation, according to the procedure already applied in numerous theoretical investigations [8, 33–36]. In particular, cell reproduction, mutation and death are assumed to occur asynchronously. In other words, the cell dynamics is simulated by a stochastic process consisting, at each time step, of a birth and a death event i.e. a Moran process [41]. The logical steps for a single reproduction and death event are schematically shown in figure 1. The birth process is simulated by randomly choosing one of the \( N \) cells with a probability which is proportional to its reproductive rate. This is set equal to 1 for cells of type-0 and type-1. The fitness of leukemic cells is assumed to be 10 times that of normal cells, when no therapy is present. In the presence of imatinib-based treatment this value becomes lower for the cells sensitive to the drug, while resistant leukemic cells maintain unaltered fitness.

In this work, in the presence of imatinib, the reproductive capability of the double-mutated leukemic cells (type-2) is assumed to decrease to a specific value, which depends on the number of type-2 leukemic cells present at that time, according to the following relation

\[
F_2(t) = \frac{(C_1 - C_2)}{(0.5^{C_3})} \cdot \left(0.5^{C_3} - \frac{N_2(t)}{(N_2(t) + N))^{C_3}}\right) + C_2
\]

with \( C_1 = 0.985, \ C_2 = 0.01, \ C_3 = 0.5 \) and \( N \) the total number of cells. The values of such parameters 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 [2, 32]. In figure 2(a) we show the behavior of the fitness of type-2 leukemic cells as a function of the number of leukemic cells sensitive to the therapy given by equation (1). The parameters have been selected in order to leave the modeled percentage as an upper level of the clinical data (conservative approach).
Mutations from healthy (type-0) to first-mutated (type-1) cells and from type-1 to double-mutated (type-2) cells, i.e. leukemic cells sensitive to the therapy, occur with rates $M_{01} = 0.0005$ and $M_{12} = 0.002$, respectively. These values are in a good agreement with those used in previous theoretical works [8, 32]. Simulations performed with different values of $M_{01}$ and $M_{12}$ showed differences only in the waiting time before the first leukemic cell appears, but no significant modifications occur in the successive development of leukemia. In our model, taking into account the apoptosis that tyrosine kinase induces in BCR–ABL expressing cells [42, 43], the death rate of the type-2 leukemic cells can be enhanced by acting on the drug being administered, while the net reproductive capability of the resistant leukemic cells (type-3) is not influenced by the imatinib-based treatment. Finally, back mutations and direct transitions from healthy (type-0) to leukemic cells (type-2) or to resistant clones (type-3) are neglected.

To give a more realistic description of the effects produced by drug administration on the simulated cancer evolution, in our model the response of the cells to the therapy can occur through multiple genetic changes, which cause an enhancement of the mutation rates. This idea agrees with experimental findings in which, due to certain mutation events, increased rates are observed in successive mutations [44, 45]. Specifically, in an increasing number of patients clinical studies evidenced the onset of resistance to imatinib within a period of two years after the beginning of the treatment.

The appearance of resistance determines a worsening of the disease with a passage to the chronic, accelerated and blast crisis phases [29]. In these conditions the number of immature leukemic cells (myeloblasts) in the blood or bone marrow increases [46]. We take into account this behaviour by assuming that the type-2 cells undergo a mutation, becoming type-3 cells at a rate $M_{23}$ whose value is not constant. In particular, $M_{23}$ increases as a function of the number $N_2$ of leukemic double-mutated cells. The dependence of $M_{23}$ on $N_2$ has been analyzed by using clinical data showing the frequency of acquired hematologic resistance with respect to the number of myeloblasts in the blood.

Figure 2. (a) Fitness of type-2 leukemic cells as a function of the number of leukemic cells sensitive to the therapy, calculated by equation (1) and reproducing the experimental behavior, of the frequency of deleterious mutations associated with the appearance of resistance as a function of disease progression [29]. (b) Mutation rate $M_{23}$ calculated by equation (2) as a function of type-2 leukemic cells.
On this basis, adopting the standard definition of CML phases cited above [29], the following equation has been obtained

\[ M_{23} = a + (b \cdot (1 - e^{-N_2/c})/d). \] (2)

Here \( a = 2 \cdot 10^{-4} \), \( b = 8.5 \cdot 10^3 \), \( c = 2.5 \cdot 10^3 \) and \( d = 4 \cdot 10^7 \) are the values that allow us to obtain from equation (2) theoretical values in the best agreement with experimental findings [29]. The latency value of the mutation rate from type-2 to type-3 cells is \( M_{23} = 2 \cdot 10^{-4} \). This increases, doubling in value for increasing values of the number \( N_2 \) of immature cells. In figure 2(b) we show the behavior of the mutation rate \( M_{23} \) as a function of the number of leukemic cells sensitive to the therapy according to equation (2).

In figure 3 we show the dead-event normalized space as a function of the number \( N \) of the type-2 leukemic cells. In particular, this picture represents the probability space for all four cell populations to experience a death event, when a random number in the Monte Carlo procedure is extracted between 0 and 1. These probabilities are identical (25%) at low numbers of type-2 leukemic cells. When this number increases, the probability associated with a death event for a type-2 leukemic cell also increases, while remaining equally distributed among the other three cell populations.

For continuous drug therapy the simulation results of the percentage of leukemic cells as a function of time, compared with the BCR–ABL percentage values, observed in clinical investigations and reported in [32] (red squares) and [2] (blue squares) are shown in figure 4. The functional behavior of the number of leukemic cells with time is decreasing during the efficacy phase of the therapy and subsequently increases because of the appearance of resistance. Our theoretical results (continuous green line) are in good agreement with the experimental ones, thus validating our model.

The appearance of drug resistance within two years after the beginning of the therapy suggests that the mutation from drug-sensitive (type-2) to drug-resistant (type-3) leukemic cells can occur as an evolutionary adaptation of the cancerous cells to the drug. It is therefore reasonable to hypothesize that, when the therapy is interrupted,
the mutation rate $M_{23}$ decreases, with a strong reduction of the risk that the patient develops resistance to the drug.

In our numerical analysis the time is measured in units of cell divisions. Assuming that 500 cell divisions occur in one day, the time necessary to completely restore healthy cells is almost 200 d. This result agrees with experimental data observed in clinical studies with an optimal response to the therapy [2, 32].

3. Results and discussion

In this section we analyze the fluctuations in the patient response to TKIs-based therapies, characterized by different drug administration schedules. With the proportion of malignant cells close to 99% at the time of diagnosis, the initial condition in our simulations is that all the patient cells are leukemic type-2. Nevertheless, healthy cells and first-mutated cells participate in the reproduction process, because we are simulating the evolutionary dynamics of all four subpopulations of cells and stem cells 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. Therefore, type-3 cells acquire the chance to reproduce themselves only after the first resistant cell is generated.

With the standard daily dosage of TKIs sufficiently high to persist in patient plasma for about 24 h [20, 47], standard CML treatment is achieved with continuous therapy. In order to lower the number of leukemic cells as rapidly as possible (figure 3), during the first 200 d of treatment our patients are simulated as receiving imatinib with the standard daily dosage, corresponding to a fully continuous modeled therapy. This choice has been made firstly because, for patient health, it is necessary to restore a suitable level of red cells and platelets and, secondly, to reduce the probability of developing resistance, which is greater when the number of immature leukemic cells is higher.

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 $5 \cdot 10^3$ times. This means that a total
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number of 5000 patients are simulated in this study. Our simulated patients, subjected to a continuous therapy for the first 200 d, are then treated by three different therapeutic strategies characterized by: (i) continuous drug administration (namely, ‘CT’), (ii) long interruptions (7 d of stop after 21 of continuous therapy, namely ‘IT21-7’) or (iii) short breaks (one day yes, one day no, namely ‘IT1’).

First of all we have estimated the mean waiting time for the development of resistance and the average increasing trend in the number of resistant cells. The mean waiting time before the first appearance of a resistant type-3 cell is calculated by averaging over all patients developing resistance. The results of our calculations are shown in figure 5. We see that a time delay is associated with different therapies. In particular the longest waiting time is achieved when an IT1 therapy is administrated (green dotted line in the figure).

In figure 6 we show the temporal evolution of the average numbers of the different cell populations. Specifically healthy and first-mutated cells (green lines), leukemic cells still sensitive to the therapy (red lines) and resistant leukemic clones (blue lines). Continuous lines refer to the continuous therapy. Dashed and dotted lines represent an IT21-7 and IT1 therapy with time breaks of 7 and 1 d, respectively.

In figure 7 we show how intrinsic fluctuations within the cancerous system drive the number of patients developing resistance as a function of the time $T_{\text{start}}$ at which the first resistant clone is generated, for the three therapeutic approaches here explored: continuous therapy (blue), IT1 (green), and IT21-7 (red). Data corresponding to the first 200 d of continuous treatment, being common among the three modeled therapies, are not considered in this study. After the first 200 d of continuous treatment, we recorded the number of patients having developed resistance, and detected the presence of the first type-3 leukemic cell, at a specific time $T_{\text{start}}$, by adopting a sampling rate of 7 d for a total duration of two years from the beginning of the treatment. Greater fluctuations in the number of patients developing resistance are observed in the presence of an IT21-7 drug administration strategy. This is partly due to the assumption of considering in the model the appearance of resistance as an evolutionary process of reaction against the presence of the drug in the patient’s body. As a matter of fact, this assumption is responsible for the periodical missing detection of patients developing resistance in IT21-7 therapy (missing

Figure 5. Average trend of resistance development under CT (solid line) and IT with breaks of 7 (dashed red line) and 1 (dotted green line) days. The mean waiting time before the first appearance of a resistant type-3 cell is calculated by averaging over all patients developing resistance.
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Figure 6. Temporal evolution of cell populations: healthy and first-mutated cells (green lines), leukemic cells still sensitive to the therapy (red lines) and resistant leukemic clones (blue lines). These values of the number of cells represent averages over 5000 simulated patients. Continuous lines refer to CT. Dashed and dotted lines represent an IT21-7 and IT1 therapy with time breaks of 7 and 1 d, respectively.

Figure 7. Number of patients developing resistance as a function of the time $T_{\text{start}}$ at which the first resistant clone is generated. The three therapeutic approaches are explored: CT (blue), IT1 (green) and IT21-7 (red). Inside the figure we also report the number of simulated patients who did not develop resistance after two years of treatment.

red lines in figure 7), but this interruption inevitably causes a drastic increase in the number of type-2 leukemic cells. In figure 7 we also report the number of simulated patients who did not develop resistance after two years of treatment. In this respect, we have found that lower numbers of patients developing resistant leukemic clones are achieved when an IT1 therapy is administered (green data in figure 7).

The crucial point for patient long-term survival to CML is avoiding those mutations causing a type-2 leukemic cell to become type-3 resistant to the therapy. In our model the occurrence probability for such harmful mutations depends on the presence of the drug, and increases with the number of type-2 leukemic cells. This is the reason why the study of fluctuations in the number of type-2 leukemic cells is so crucial for a clear estimate of the balance between the benefits of the therapy and the risk of developing cancer resistance.
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Figure 8. Number of type-2 leukemic cells for the 5000 simulated patients (left panels) and distributions of simulated patients among the type-2 leukemic cells (right panels). Blue symbols/curves refer to patients treated by CT. Red and green diamonds/curves are used for ITs with breaks of 7 d and 1 d, respectively. The time window starts at the 214th day, after 200 d of continuous drug administration, by adopting a sampling rate of two weeks.
In figure 8 (left panels) we show how the number of type-2 leukemic cells is distributed among all the simulated patients after the first 200 d, at fixed time steps of 14 d for 8 weeks. Continuations of figure 8 with the subsequent 8 and 16 weeks are shown.

Figure 9. Continuation of figure 8. Blue symbols/curves refer to patients treated by CT. Red and green diamonds/curves are used for ITs with breaks of 7 d and 1 d, respectively.

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in figures 9 and 10, respectively. In all three figures, the blue colour is used for patients treated by CT, red for IT21-7 and green for IT1. Figures 8–10 (right panels) also show the distribution of patients with a given number of type-2 leukemic cells. Of course
the best results, in terms of lower levels of type-2 leukemic cells, are achieved with a continuous therapy. However, the results shown in figures 8–10 demonstrate that IT could also represent a valid choice in patients who cannot be given the drug continuously due, for example, to a problem of toxicity. In particular, we find that the number of type-2 leukemic cells in patients treated by IT21-7 shows a trend similar to that of CT for the first three weeks, while a characteristic peak (red in figure 8) is instead detected during the subsequent seven days without treatment. The number of type-2 leukemic cells is lowered during the subsequent 21 d of drug administration, keeping a fluctuating but still acceptable overall level of leukemic cells. In fact, even if an increase of the average number of type-2 leukemic cells is observed during an IT, this effect is partially counterbalanced by a reduction in the probability of developing resistance. In the case of IT1 therapy, the peak of type-2 leukemic cells shows an increasing trend reaching a saturation plateau at about 25% of the total number of leukemic cells. This stabilization appears to be sufficient to lower the number of patients developing resistance with respect to the other two therapeutic approaches here investigated.

In figures 8–10 we also highlight the presence of multiple states of dynamical equilibrium in the number of type-2 leukemic cells in the cases of intermittent drug administration. In particular, this effect is more evident in the IT21-7 (red symbols/curves) case, where lower populated bands (left panels) and peaks (right panels) can be easily detected with some periodicity. This finding is also present in the IT1 case, but with lower population densities.

Finally, in figure 11 we show how intrinsic fluctuations affect the percentage of patients developing resistance as a function of time, following the aforementioned therapeutic strategies. The statistical error associated with these values, calculated by repeating our set of simulations 10 times, is less than 0.5%. System fluctuations do not significantly affect the average number of patients developing resistance within the first 200 d of continuous drug administration, corresponding to essentially the same (continuous) therapeutic approach. After differentiation of the therapy, we find an advantage of IT over continuous drug administration. Over the whole duration of 2 years, IT characterized by therapy breaks of 7 d (IT21-7) or 1 d (IT1) shows a reduction.
in the average number of patients developing resistance. In particular, a significant improvement (a reduction of about 5%) is found for IT1 with respect to the traditional continuous drug administration.

4. Conclusions

Recent clinical oncology investigations indicate that an IT may be effective in reducing both the incidence of toxicity and the complications that arise from side effects due to long-term continuous treatment with TKIs. In this work, by using a computational approach, we have studied the effects of fluctuations in a complex cancer system of multiple-mutated leukemic cells with the aim of exploring the consequences of an intermittent TKI-based therapy on the evolutionary dynamics of normal and cancerous cells in patients affected by CML, with respect to the standard treatment based on continuous drug administration. We have studied the evolutionary dynamics of healthy and leukemic cells of 5000 simulated patients in the presence of three types of therapies: standard daily dose administration (continuous), or delayed by 7 or 1 d time lags (intermittent).

We have found that an IT may cause a slight increase of the average number of leukemic cells with respect to continuous drug administration, with CML being still sensitive to TKIs. Even considering this negative effect, we have found that an IT may represent a valid choice in patients with high risk of toxicity. In fact, the presence of a slight increase in the average number of leukemic cells is counterbalanced by a reduction in the average number of resistant clones. These findings are in agreement with recent clinical oncology trials [23, 48]. Our results show that an IT, appropriately tuned to specific patient necessities, should be preferred to the continuous one because of the lower risk of toxicity [24], also causing a reduction in the number of patients developing resistance to the therapy after two years of treatment. Finally, our IT results clearly show the presence of multiple states of dynamical equilibrium in the number of type-2 leukemic cells. But, unfortunately, the most populated equilibrium state always has the highest number of leukemic cells. The possibility of achieving a population reverse in CML progression is still an unsolved problem.

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