



Interplay of mutations, alternate mechanisms, and treatment breaks in leukaemia: Understanding and implications studied with stochastic models

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ABSTRACT

Bcr-Abl1 kinase domain mutations are the most prevalent cause of treatment resistance in chronic myeloid leukaemia (CML). Alternate resistance pathways nevertheless exist, and cell line experiments show certain patterns in the gain, and loss, of some of these alternate adaptations. These adaptations have clinical consequences when the tumour develops mechanisms that are beneficial to its growth under treatment, but slow down its growth when not treated. The results of temporarily halting treatment in CML have not been widely discussed in the clinic and there is no robust theoretical model that could suggest when such a pause in therapy can be tolerated. We constructed a dynamic model of how mechanisms such as Bcr-Abl1 overexpression and drug transporter upregulation evolve to produce resistance in cell lines, and investigate its behaviour subject to different treatment schedules, in particular when the treatment is paused ('drug holiday'). Our study results suggest that the presence of additional resistance mechanisms creates an environment which favours mutations that are either preexisting or occur late during treatment. Importantly, the results suggest the existence of tumour drug addiction, where cancer cells become dependent on the drug for (optimal) survival, which could be exploited through a treatment holiday. All simulation code is available at <https://github.com/Sandalmoth/dual-adaptation>.

1. Introduction

Chronic myeloid leukaemia (CML) is a myeloproliferative malignancy driven by signalling from the overactive fusion tyrosine kinase Bcr-Abl1. The modern treatment regimen is targeted therapy with tyrosine kinase inhibitors (TKIs) designed to specifically bind Bcr-Abl1, and it has significantly improved patient outcomes since its introduction. Despite this success, resistance to these drugs is observed in around 20% of the patients. The development of drug-resistant tumours occurs through a variety of mechanisms. The most common mechanism of resistance (estimated to be responsible to 60% to 80% of the cases [1]) is the development of Bcr-Abl1 kinase domain (KD) mutations that limit the efficacy of the inhibitor. Other significant mechanisms include: Bcr-Abl1 overexpression such that achievable TKI concentrations are not enough to inhibit signalling, drug transporter upregulation which lowers the intracellular concentration of the TKIs, and alternate pathway activation where the oncogenic signalling from Bcr-Abl1 is replaced by other pathways [2,3]. In general, mutations involve a distinct discrete change, i.e., a cell is either mutated or not. The effect of resistance mutations tends to be more abrupt once they take over enough cells

in a population [4]. Resistance mechanisms other than mutations can have a more gradual effect.

The emergence of resistance mutations in CML has been widely studied using experiment and theory [5–7], and further efforts to optimize treatment protocols with the intent of minimizing resistance or prolonging survival have been made. In a common modelling approach, a population of cancer cells is simulated as a stochastic process when the survival of particular cells is of interest [8]. Such strategy has been used, for instance, in studies showing that drug rotations as well as drug combinations may decrease the risk of resistance in CML [9–11]. Similar results have been obtained for other kinase-driven malignancies [12–18].

The behaviour of a large number of cancer cells can often be treated with deterministic models. A hallmark of CML is the presence of a slowly growing pool of cancer stem cells (CSCs), which produces more differentiated and more rapidly growing cells. Production and differentiation of cells proceeds thereafter in a multi-stage hierarchy. Thus, the small pool of CSCs generate very many (dysfunctional) differentiated cells [19,20]. According to the cancer stem cell theory mutations occur

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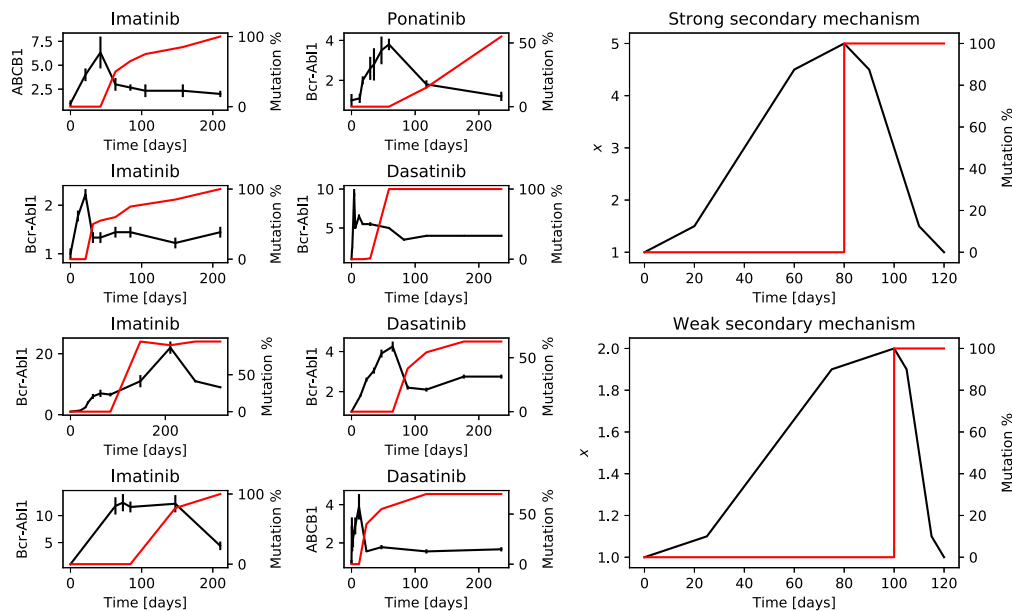


Fig. 1. A Comparison the acquisition and loss of mutations and alternate resistance mechanisms from reported cell line experiments lines [22–25] and our two reference timelines. Black lines show adaptation, red lines show prevalence of cells with resistance mutations. X-axis shows the time. Y-axis shows adaptation (left, corresponding to the black curves) and mutation prevalence in percent (right, corresponding to the red curves). Experimental data is reproduced on the left-hand side, with the expression of either Bcr-Abl1 or ABCB1 representing two separate adaptation mechanisms. The two larger frames on the right show our reference timelines, where the axis on the left is the schematic parameter of degree of non-mutation resistance driving phenomena x (explain in the Methods section). We treat adaptation by two different mechanisms, ‘strong’ and ‘weak’ as explained in the text, which is manifested by the shape of the black curves on the right-hand side.

in the context of this cellular hierarchy, and have to happen in the stem cell pool or they will eventually die out [21]. The question of when a mutation can be detected is effectively answered by looking at how its more differentiated progeny increase in number. It is however not commonplace in either type of model to include any non-mutation resistance mechanism when models of tumour growth are considered.

In cell-line experiments with cells derived from CML-patients a peculiar phenomenon can be observed where the expression of Bcr-Abl1 or the drug transporter ABCB1 gradually increases in response to TKI exposure, only to sharply drop when a resistance mutation occurs (Fig. 1) [22–25]. The increased expression is in both cases suggested to be mediated by double minutes, i.e., small extrachromosomal circular fragments of DNA which can be present in varying numbers, though other mechanisms may be present [23,26]. This results in a gradual increase in the prevalence of cells that carry the Bcr-Abl1-mutation-independent resistance phenotype. However, upon the appearance of a sub-clone with mutated Abl1, there is a sharp decrease in the prevalence of these non-Bcr-Abl1-mutated cells, as the mutation alone sufficiently protects the cells from TKIs. Adaptation mechanisms such as increased expression of certain genes are not without cost, so that in mutated cells such mechanisms are selected against, leading to their rapid decline in the proportion of adapted cells. In particular, Bcr-Abl1 overexpression may owe some of its detrimental effect to increased reactive oxygen species [27], which has weak effect unless Bcr-Abl1 is overexpressed.

We suggest that overexpression of Bcr-Abl1 can be the basis for drug addiction phenomena, i.e., that the cells adapt to the drug in a way that becomes destructive if the drug is removed. When cancer cells become addicted to the drug that is used to treat them, positive effects are observed as a consequence of removing treatment [28,29]. Clearly, ceasing treatment with Abl1 inhibitors is not a good strategy in the long run though, since tumour cells strongly depend on Bcr-Abl1 for survival, and this study does not advocate for this. Rather, we consider cases in which treatment is withdrawn for a predefined period due to toxicities or special circumstances, such as pregnancy. The need for understanding the consequences of such a break is our motivation to model drug holidays.

We model the adaptation from increased expression of Bcr-Abl1 or ABCB1 by introducing a per-cell *expression parameter* into a birth-death process. Each individual cell grows at a rate determined by its expression parameter and by whether or not it has a Bcr-Abl1 KD resistance mutation, the effects of which are summarized and used to guide the birth-death process. The expression parameter is inherited with variation on a continuous scale, whereas a mutation is a discrete event. Experiments in cell lines show that adaptation mechanisms that are different from Bcr-Abl1 KD resistance mutations can increase the growth rate upon treatment [22–25]. The parametrized model can reproduce such effects. From that model, we simulated how expression increased and decreased in cells, and the fate of a mutation depending on when it occurs. Furthermore, we explicitly examined tumour drug addiction effects by simulating drug holidays, i.e. periods without treatment. We argue that mutations and increased protein expression are different enough in their behaviour to warrant being modelled separately, as the former is rare and discrete, and in the latter small changes occur often on a continuous scale. To the best of our knowledge, this is the first model in which resistance mutations and adaptation through increased expression are considered as two different, yet simultaneous, resistance mechanisms in cancer.

While we are not aware of mathematical modelling approach used to study the effect of drug holidays in the context of CML, other modelling approaches should be mentioned that might also be useful to this aim. The effect of ceasing treatment completely after a designated period is debated in the literature, and models that are based on the kinetics of Bcr-Abl1 measurements in patients can be used to predict the outcome of such treatment stop (reviewed in [30]). In the context of cytotoxic drugs (chemotherapy), it is common to apply cycles of therapy and stops in between. The effect of a weak resistance mechanism in breast cancer, through the contribution from the stroma, has been recently studied in such context using agent based modelling [31]. The approach is different to the one we apply here, as it employs a two dimensional grid on which the cells grow. In contrast, a stochastic, multi-branching type model that explicitly considers transient drug resistance was developed and applied to tumour growth. In this case, the authors considered also a therapeutic approach aimed at inhibiting

such mode of resistance under different schedules that also involve conventional therapy and treatment cycles [32].

This article is organized as follows. In Section 2 we detail the theoretical approach. Details about the implementation and parametrization are given in Section 3. This section can be used by interested readers who wish to expand on our approach or re-parametrize based on other experiments. The Results section (Section 4) starts with a summary on the results of the fitting of the growth rate function (Section 4.1), which can be skipped by readers who wish to concentrate on the biomedical outcomes of the study. The consequences of adaptation to treatment and resistance are presented in Section 4.2. The Results section ends with our application of the model to cases where patients need to halt their treatment (Section 4.3) and it is examined when it is best to do so in this case. Readers who are mostly interested in the medical aspects of this study should concentrate their attention to this section. The article ends with Discussion (Section 5) and Conclusions (Section 6).

2. Approach

Each cell is characterized by a continuous parameter x which represents the degree of non-mutation resistance driving phenomena in the cell. x could represent Bcr-Abl1 expression, drug efflux transporter activity, or a combination of effects. The growth rate of any single cell is given by $\lambda(x)$. When a cell is generated following parent cell division, it is given a new value of x , which is determined from its parent's x and some random variation. In simple terms, cells which grow fast produce more offspring similar to themselves.

In cells without resistance mutations, treatment is assumed to change $\lambda(x)$ in two ways. First, the function is shifted right (i.e. towards higher expression) to model that an increased expression of the tumour promoting gene, higher expression of efflux channels or a combination thereof is needed to compensate for the drug. This could be explained by apoptosis induced compensatory proliferation [4]. Second, the maximum growth rate is altered to model that these adaptations may not be able to restore full function. In other words, an increased expression of one of the aforementioned factors may not be able to fully compensate the effects of the inhibitors, and will never result in exceeding the original non-treated growth rate in our model. To model TKI treatment and mutant cells, $\lambda(x)$ is modified. For simplicity, we model mutant cells as totally resistant, i.e., assuming that their growth is not affected by the presence of the drug. There is some evidence that Bcr-Abl1 KD mutations may alter the enzyme's function [33–35]. This may result in a modification in the basal, untreated, growth rates of cells carrying that mutation. Such modifications to the growth rate are not modelled here since the effects vary between different mutations in both direction and magnitude, not always significantly. Our earlier study suggested that any effect of a mutation on cell proliferation is marginal when compared to the effect on drug resistance [11]. Furthermore, we did not observe any marked changes in the growth rate of a nilotinib resistant cell line (reported in [36]) compared with that of the parental cell line. The death rate is also assumed to be unaltered by the mutation since it represents the demise of a cell, which is a natural process. In contrast, drugs affect the growth of a tumour in our model and do so in a different way for the native and mutant cells.

A detailed description of the simulation process is given in the Methods section (Section 3). Simulations were carried out using two different approaches. For fewer than 1000 cells, simulations were carried out using a modified version of Gillespie's algorithm [37]. However, when the number of cells becomes large, such simulations become too computationally expensive. Hence, when simulating a large number of cells, a different protocol is used which is based on time-dependent distributions of x , which are evolved using a set of partial differential equations (PDEs, Eq. (3)). We then do not explicitly simulate individual mutations, but the average number of mutations can then be obtained from the number of newly generated cells. This can be calculated more directly and thus greatly reduces the number of computations needed. The simulation algorithm is determined in the beginning of the run based on the initial number of cells.

3. Computational methods

3.1. Model details

We use a variation of a birth–death process to model the cell line culture. Each cell has a continuous parameter x which represents its expression of non-mutation resistance phenomena. The growth rate of any single cell is given by $\lambda(x)$, and the death rate is a constant μ so for the birth–death process with N cells the total intensities for birth and death (λ_n and μ_n respectively) are

$$\lambda_n = \sum_{i=1}^N \lambda(x_i) \quad (1)$$

and

$$\mu_n = N\mu$$

When a cell is born it is given a new value of $x_{\text{new}} = x_{\text{parent}} + Y$ where Y is a random variable and x_{parent} is chosen from the extant cells with probability

$$f_{X(\text{parent}),t}(x_{\text{parent}}) = \frac{\lambda(x_{\text{parent}})}{\sum_{i=1}^N \lambda(x_i)}. \quad (2)$$

Since all cells have the same death rate, the cells that die are chosen randomly.

For a large number of cells modelling is carried out using a time dependent distribution of x for the whole population ($f_{X,t}$), rather than considering individual cells. It can be shown that the time evolution of $f_{X,t}$ is described by Eq. (3) (derivation in S1 Appendix).

$$\begin{aligned} \frac{\partial f_{X,t}(x)}{\partial t} = & \int_{-\infty}^{\infty} f_{X,t}(\zeta) \lambda(\zeta) f_Y(x - \zeta) d\zeta \\ & - f_{X,t}(x) \int \lambda(\xi) f_{X,t}(\xi) d\xi \end{aligned} \quad (3)$$

This provides a good model of how the x probability density changes over time when compared to exact simulations (S1 Fig) using a logistic branching process model [38].

The steady state distribution $f_{X,\infty}$ can be found numerically either by simulating the above PDE until convergence, or, noting that the steady state distribution will satisfy

$$f_{X,t}(x) \propto f_Y * [\lambda(x) f_{X,t}(x)] \quad (4)$$

where $*$ is the convolution operator. We can find the stationary distribution numerically by iterating

$$f_{n+1}(x) = f_Y * (f_n(x) \lambda(x)) \quad (5)$$

starting from an arbitrary distribution. Eq. (5) converges faster than the PDE (Eq. (3)) and is faster to calculate.

3.2. ABC parameter fitting

The parametrizable function chosen to model $\lambda(x)$ was

$$\lambda(x) = \begin{cases} \frac{m}{R} K^a (1 - K)^b & \text{if } u - wc < x < u - w(c - 1) \\ 0 & \text{otherwise} \end{cases} \quad (6)$$

where

$$R = a^a b^b s^{-s} \quad (7)$$

$$K = \frac{x - u}{w} + c \quad (8)$$

$$a = sc \quad (9)$$

$$b = s - a \quad (10)$$

The parameters s defines how sharply peaked the curve is, w sets the width between the endpoints, m is the height of the maximum, u is the location of the maximum, and c defines the position of the maximum

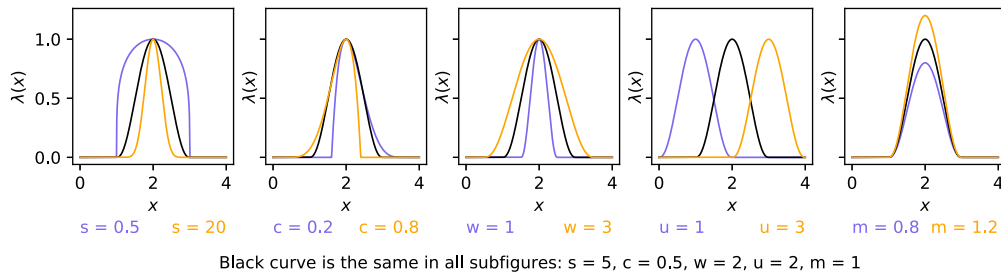


Fig. 2. Visualization of how the parameters influence the rate function $\lambda(x)$. s defines how sharply peaked the curve is (leftmost panel), c defines the position of the maximum relative to the range where it is non-zero (second left), w sets the width between the endpoints (middle), u is the location of the maximum (second right) and m is the height of the maximum (rightmost panel).

relative to the range where it is non-zero (Fig. 2). Note that in principle a and b could be used as parameters instead of s and c , however, the effects of the latter are more intuitive. In retrospect, a simpler bell shaped function might have been sufficient, especially if truncated to a finite range, as the posterior probabilities for most parameters were not particularly narrow (S1 Table).

Reference curves in the form of mean parameter over time for acquisition and loss of adaptation were constructed manually to qualitatively resemble available data [22–25]. Using pyABC [39] the parameters were fit using a sequential Monte Carlo approach with a dynamic population size. The standard deviation of a centred gaussian noise function was fit at the same time. Parameter priors are listed in S1 Table. The distance function used was the RMSD between the average x in the population over time calculated using the PDE, Eq. (3), and the reference timelines.

3.3. Evaluating the mutation risk

The offspring x density over time was calculated using a PDE simulation starting from a population of non-mutated cells adapted to a drug-free environment. The starting distribution was calculated using the iterative method described by Eq. (5). The child density at any particular time was calculated from the parameter density as

$$f_{X^{(new)},t}(x) = (f_{X,t}\lambda(x) * f_Y)(x) \quad (11)$$

and numerically normalized to an area under the curve of 1.

Subsequently, the survival chance of a mutant cell born at any time t was calculated by randomly generating a new mutant cell using the x distribution above and, using Gillespie's algorithm, simulating until its lineage either (a) died out or (b) reached a size threshold (1000 cells). Case (a) was counted as dying whereas case (b) was counted as surviving/escaping (resulting in a drug resistant phenotype in practical terms). The mutant cells were subject to a death rate equal to the growth rate of normal cells at that time, for reasons discussed below (Section 4.2). The simulation was repeated 100000 times per time point to generate adequate statistics.

The average growth of non-mutated cells at a particular time is

$$\bar{\lambda} = \int_{-\infty}^{\infty} f_{X,t}(x)\lambda(x)dx \quad (12)$$

where $\lambda(x)$ depends on whether the cells are exposed to treatment or not at that particular time. The mutation risk, i.e. the probability of a mutant cell which eventually reaches the threshold number of cells (1000) is born at a given time, per normal cell in the population is then

$$p_{\text{resistance}} = p_{\mu} p_{\text{surv},t} \int_{-\infty}^{\infty} f_{X,t}(x)\lambda(x)dx \quad (13)$$

with p_{μ} being the odds of a resistance mutation in each new cell, and $p_{\text{surv},t}$ being the survival chance of a mutant cell born at time t calculated numerically as described above. p_{μ} is not known to a very high accuracy, but is estimated to be on the order of 10^{-7} Bcr-Abl1 KD resistance mutations per cell division [19], although it may vary between cell lines, among patients and with disease progress.

3.4. Evaluating the effects of a drug holiday

The drug holiday simulations were carried out as above except that the child x density and normal cell growth rate were calculated including a drug holiday. A drug holiday x density timeline (from which child density and growth rate can be calculated) was calculated in four steps:

1. Calculate the starting x density assuming no treatment using the iterative method.
2. Simulate with PDE until start of drug holiday using non-mutated cells with treatment rate function.
3. Simulate with PDE for duration of drug holiday using non-mutated cells without treatment rate function, starting from the final x density from previous step.
4. Simulate with PDE until fixed total time using non-mutated cells with treatment rate function, starting from the final x density from the previous step.

From those x densities, the growth rate was calculated using Eq. (12) using the $\lambda(x)$ for normal cells with or without treatment depending on the segment. Cumulative growth was calculated as the integral of the growth rate curve. A total of 792 different holiday start/duration combinations were tested in a uniform grid; with start days 0–115 and 0–144 for the strong and weak secondary mechanism, respectively, and durations 0–49 days and 0–27 days (ditto). These ranges were chosen such that the holiday start occurs anywhere from immediately to after the x adaptation has reached a steady state, and that the holiday can be long enough that x can adapt back to baseline during the holiday. To evaluate mutation risk, the same procedure as before was applied to the drug holiday x density timelines. These simulations were repeated 10000 times per time point for the strong secondary mechanism scenario, and 1000 times per time point for the weak secondary mechanism.

4. Results

4.1. Fitting the growth rate function to experiments

A parametrizable function for $\lambda(x)$ was adopted with the requirement that it satisfies the following criteria (see the Methods section).

- $\lambda(x) = 0$ for low x , since some amount of activity (of Bcr-Abl1, transporters, etc.) is required for (malignant) function.
- $\lambda(x) = 0$ for high x , since these adaptations are costly and only so much can be tolerated.
- The function has a single peak that reflects an optimal balance between benefit and cost.
- The slopes to zero above and below the maximum can be adjusted individually, since the fitness cost does not have to be symmetric.
- The location and height of the maximum can be adjusted independently of other parameters, to model a change in treatment.

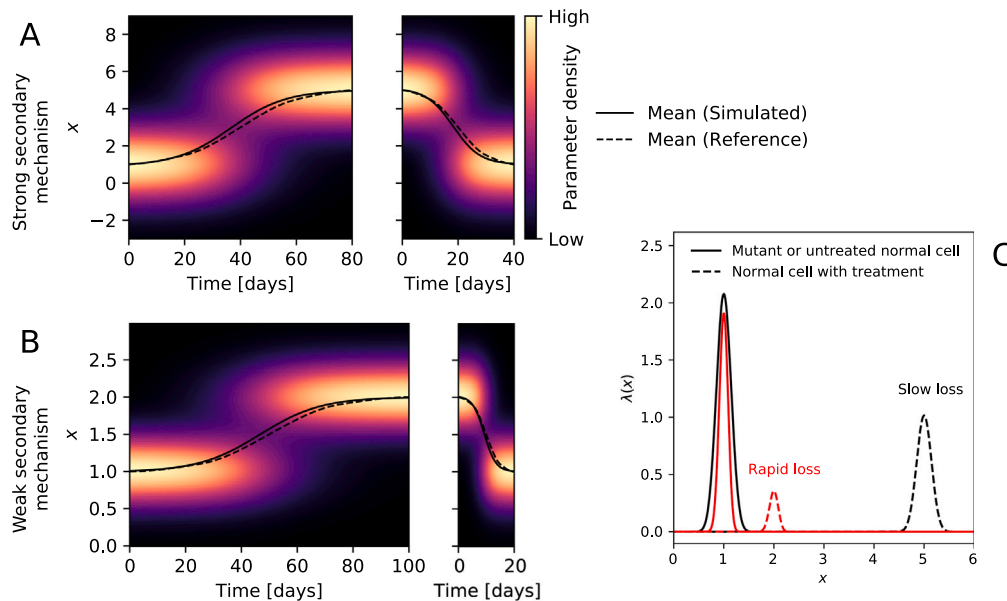


Fig. 3. Weak and strong secondary mechanisms. A. Time dependent probability density functions for the expression parameter x (Eq. (3)). $\lambda(x)$ (growth rate) parameters from the ABC fitting procedure (solid lines) reproduce the reference curves (dashed lines), as shown for the strong secondary mechanism. B. Same as (A), for the weak secondary mechanism. Note the different scales on the vertical axis between A and B. The strong and weak secondary mechanism cases are each composed of two phases: Gaining the adaptation during treatment (long time period, left in A and B) and losing it upon mutation or interrupted treatment (short time period, right in A and B). C: $\lambda(x)$ functions from ABC fitting. The solid lines refer to mutant cells or untreated cells, whereas the dashed lines refer to treated cells. The case for slow loss of adaptation is shown in black and rapid loss is shown in red.

Although available experiments demonstrate a certain pattern in the gain and loss of resistance adaptations, as discussed, the experimental data is too varied in their details to be used directly as targets for modelling. Hence, we constructed two reference timelines for the gain and loss of adaptations using data from [22–25] that cover much of the variation seen in the experimental data. The two timelines were: (I) A large increase in the expression parameter that was lost slowly in the absence of treatment or the development of a resistance mutation; this is phenomenologically tied to the idea of increased expression being capable of providing a relatively high degree of resistance and (II) a small increase in the expression parameter gained more slowly and lost more rapidly than in (I) under the same conditions, which corresponds to a weaker secondary resistance mechanism (Fig. 3). We then fitted the parameters of $\lambda(x)$ using Approximate Bayesian Computation (ABC) such that the mean x over time, calculated with the PDE (Eq. (3)), reproduced the knowledge-based timelines (Fig. 3, S1 Fig and S1 Table). The fitting suggested that only a narrow x range is permissible under a particular set of conditions (see the Methods section), which is a consequence of the tight homeostasis maintained in most cellular processes. The ratio between the peak height of $\lambda(x)$ in a resistance mutation free cell when exposed to treatment, and the peak height of $\lambda(x)$ in an untreated cell (or cell with resistance mutation), and the noise, i.e. how much a child cells x differs from its parent cells x , were the most important features in fitting the rate function(s) to the reference timeline. With the weak secondary mechanism, the maximum growth rate of non-mutated cells with treatment was much lower than with the strong secondary mechanism, hence their names. This difference relates to how potent the secondary effect is at generating resistance. As resistance mutations are a more common resistance mechanism than Bcr-Abl1 or ABCB1 overexpression in practice, it is likely that the latter two are also less potent, especially given that they do not rely on rare mutation events. Given that we do not know the extent of resistance that the latter two can provide, the strong and weak cases were both modelled as they cover two different possibilities.

4.2. The risk of acquiring a resistance mutation changes over time

We sought to examine the consequences of introducing a second, gradual, form of resistance adaptation on the risk of mutation-based resistance. From the PDE we can calculate the distribution of the expression parameter for new cells born at a particular time. The likelihood that a single mutant cell produced at a given time survives (leading to drug resistance further down the line) depends on two things. Firstly, the value of a newly born mutant's x parameter needs to coincide with the mutant $\lambda(x)$ -function (otherwise it will grow slowly or not at all, drastically reducing the probability that the cells descendants will take hold in the population). Secondly, as a single mutant (or eventually a small number of mutant cells) is located among a vastly larger number of non-mutated cells, it is exposed to some competition-induced death rate. Since the mutation does not fundamentally alter the function of the cell, we can assume that resistance-mutation-free and mutant cells are the same from a death perspective. As the effective population size is, to some extent, fixed at a steady state (of note, in cell experiments, part of the death chance is induced by discarding/diluting the cell culture rather than direct competitive pressure) the death rate of all cells is roughly equal to the non-mutated cell birth rate. As such, a tiny fraction of mutant cells will hardly affect the overall growth rate of the tumour. In vivo, if mutant cells are fairly rare they are not expected to modify the survival of the whole tumour initially, as they are subject to the same external effects (natural death, competition for nutrients, etc.) as the other cells.

The mutation risk, i.e. the risk of a mutation that leads to drug resistance occurring at a particular time, was evaluated taking both the increasing productions of mutants, and their diminishing fitness advantage, into account (Fig. 4). The rate of mutant cells being produced is proportional to the growth rate and number of non-mutated cells.

Notably, in both the strong and weak secondary mechanism scenarios, as the cells adapted to the treatment the division rate of normal cells increased and the fitness advantage of mutant cells decreased.

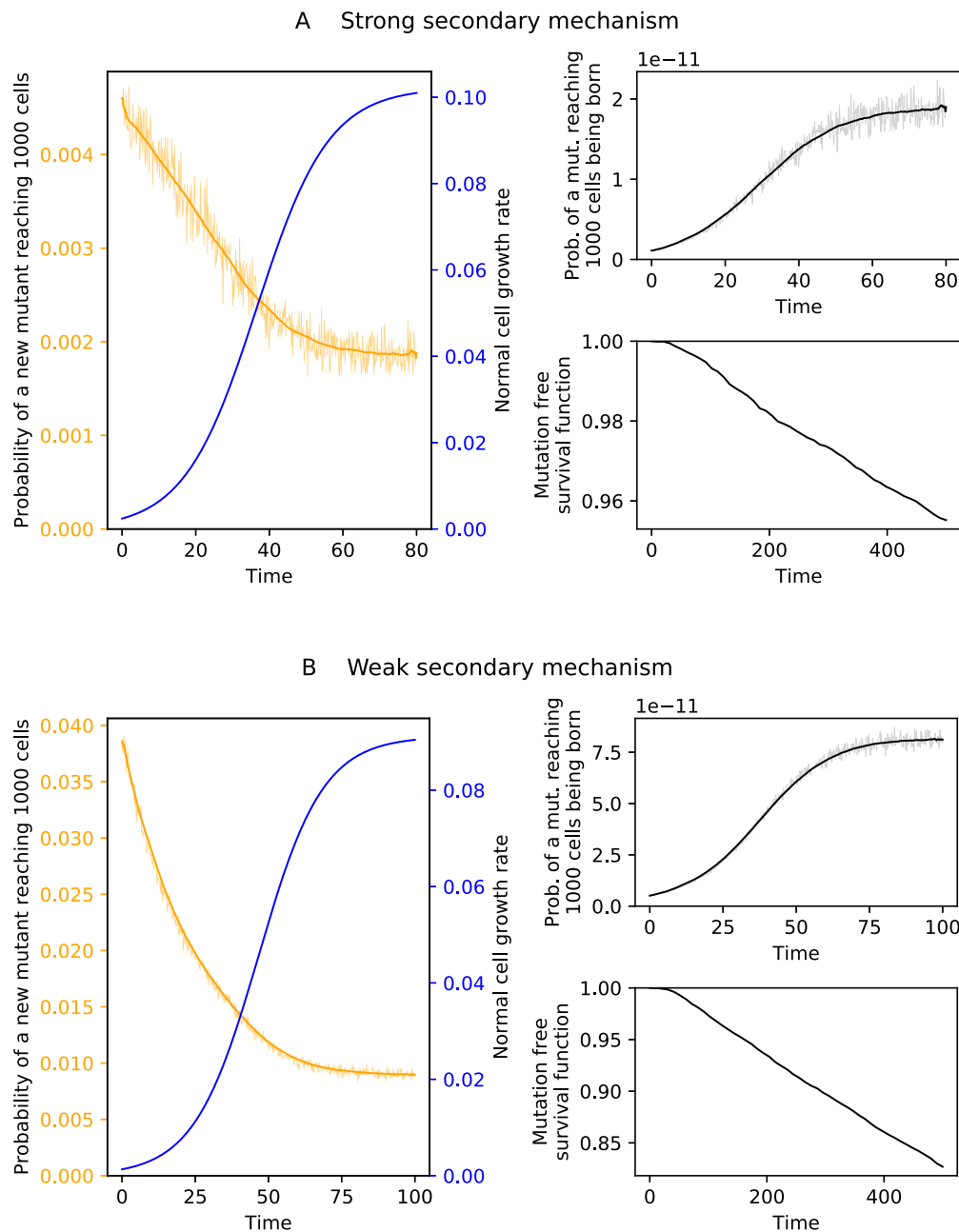


Fig. 4. Mutation risk (i.e. product of normal cell division rate and estimated mutant fixation chance) increases over time (top right panel in A, B) and plateaus after the x adaptation reaches equilibrium. Although the probability of a new mutant reaching 1000 cells decreases (orange lines) the division rate increases faster and to a greater degree (blue line), leading to an overall increased risk of resistance mutation. The thick orange and black lines are 101 point moving averages of their respective source data. The left-hand side panels were calculated using the PDE for the growth rate, and Gillespie's algorithm for the probability of reaching 1000 cells (Section 2: Approach). The survival functions for non-mutated populations are shown on the bottom right panels in A and B. The right-hand side panels were calculated assuming a mutation rate of 10^{-7} and a population size of $5 \cdot 10^6$ cells, which is an estimate of cell line experimental conditions.

These two phenomena have opposite effects on the risk of resistance mutations, but the division rate increased more than the fitness advantage decreased, thus the probability of mutant cells surviving still increased over time. Effectively, the dividing normal cells give rise to more mutants, which increases the mutants' probability to survive in the tumour even if they are individually less likely to yield a mutant lineage.

Another consequence of this adaptation is that preexisting mutations have an increased chance of causing resistance. At the very least, they have the same effect as like mutations that happen just at the start of treatment; it is also possible that they have grown to a greater number before the start of treatment. The mutants have the biggest fitness advantage as the therapy starts, and thus the probability that

they reach 1000 cells is highest early on (orange line, Fig. 4), for two reasons. First the non-mutated cells have yet to adapt by increasing Bcr-Abl1 expression or by other means, whereas the growth advantage of mutants is more pronounced. Second, mutants that occur early are more likely to inherit a (for the mutant) favourable expression parameter x . Consequently, one might infer that resistance mutations either happen very early, due to preexisting resistance mutations, or with a significant delay, as non-preexisting mutants are more likely when mutation risk has increased due to greater proliferation among normal cells. The latter effect can be seen as the initial flat and uneventful period in the survivor function, which is the probability that a cancer remains resistance-mutation free over time (Fig. 4). The strong and weak secondary mechanism case are qualitatively similar; the main

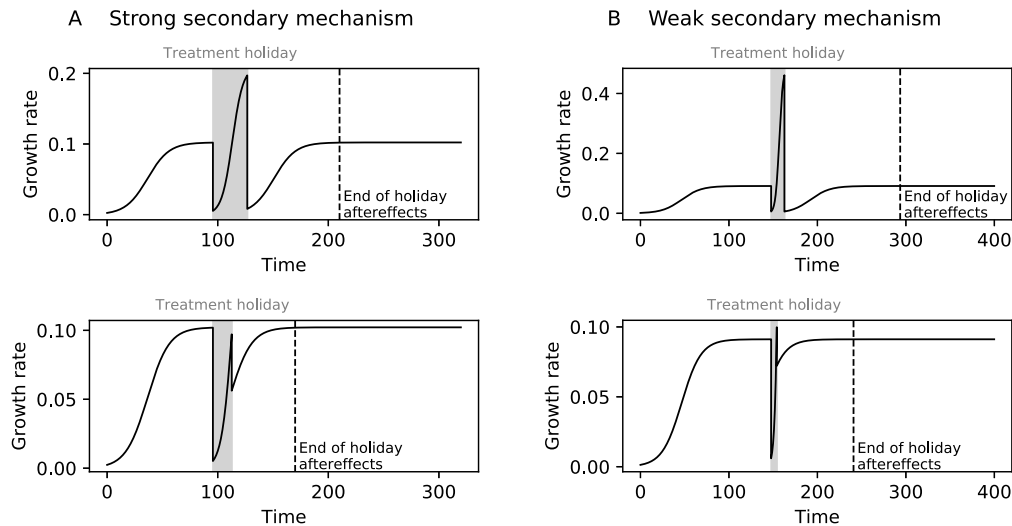


Fig. 5. Growth rate as a function of time. A drug holiday can reduce overall growth, especially after an initial period of adaptation. On top are the holidays with the biggest effect. At the bottom are drug holidays with the same start day as on top but with their durations shortened such that the growth rate never greatly exceeds non-treatment growth rates. Note the difference in scale between the different conditions, i.e., strong and weak secondary mechanisms.

difference between them is in the probability of a new mutant reaching 1000 cells.

4.3. Optimal timing of treatment holiday

As the tumour cells adapt to treatment by increasing Bcr-Abl1 expression (or another similarly behaved resistance adaptation) they simultaneously become increasingly maladapted to life without treatment. It follows that removing treatment once cells are sufficiently adapted could be beneficial for some period as the tumour cells would be forced to re-adapt. To examine this, we used the model to examine the risk of developing a resistance mutation over a range of treatment holidays, focussing on two aspects: First, whether a drug holiday can reduce the overall growth of cells which do not carry resistance mutations (assuming that they make up the majority of the cancer burden). This can be evaluated directly from simulations based on the PDE. Second, whether the growth can be reduced enough to also lower the risk of developing resistance mutations, as slower growth implies that fewer mutants are produced. This is not a guarantee, as without treatment the cells that reproduce will come from the x distribution where the mutant cells grow optimally, thus a mutant cell produced without treatment pressure is more likely to have a well adapted x parameter and thus is less likely to die.

Drug holidays are most simply defined by the time when they start and their duration. We ran simulations over a range of both, making sure that the start date range was long enough to cover the initial adaptation. The simulations revealed that overall growth could be reduced by introducing drug holidays (Figs. 5 and 6). From the heatmaps in Fig. 6 we selected the drug holidays with the biggest positive effect. In the strong secondary mechanism case, under our assumptions, an approximately 31 days drug holiday, 83 days drug treatment cycle could be repeated indefinitely after an initial adaptation period to reduce overall cancer cell growth. However, a 17 days of holiday, 57 days of treatment cycle may be preferable, as it prevents the growth from ever exceeding with-treatment rates. For the weak secondary mechanism case, the numbers are 16 days of holiday, 134 days of treatment and 6 days of holiday, 86 days of treatment respectively (Fig. 5). Not much is known about the adaptation in vivo, but if it behaves similarly the strong secondary mechanism case, this could imply an advantage for using a periodic treatment on people in long-term remission.

In both the strong and weak secondary mechanism scenarios, the cumulative mutation risk always increased with a treatment holiday,

though the increase was not linear and very small for short holiday durations (Fig. 7), whereas the effect on cumulative growth was more or less linear for short drug holidays. Thus, the best balance between treatment effect and mutations risk was struck for short drug holidays. In either case, it seems that a drug holiday is only warranted after an initial treatment period, which creates the exploitable drug addiction effect.

5. Discussion

CML is a fairly rare disease and not enough data from clinical samples exists to allow modelling based on such samples. In particular, there are risks and difficulties with bone marrow extraction that do not enable clinicians to perform such tests often enough to allow quantitative modelling. Thus, we have modelled the evolution of non-mutation based resistance mechanisms in CML cell lines. We view this as a necessary limitation of the study. Cell lines are a valid experimental model in this context as it has been shown in a cancer model that cell growth in cell lines has characteristics that are similar to that of cell growth in a tumour [40]. Another limitation of the study is that specific non-mutational resistance mechanisms were not explicitly considered in the present work. Rather, we discussed weak and strong resistance mechanisms. This matches the clinical situation where resistance is attributed to either (1) resistance mutations or (2) any other mechanism, with the former diagnosis implying a change to another Abl1 inhibitor if available, and the latter indicating that such a change would not be useful (with some exceptions, e.g., inhibitors that also block efflux pumps [41]).

An interesting aspect worth considering is that some mutations might slow down the growth rate of tumour cells [40] for example by making the enzyme drug target less efficient [35]. Moreover, different mutations confer varying levels of resistance. These rarely get fixed in the tumour cell population, although a single mutation can become dominant owing to a combined effect of direct resistance (minimizing drug efficacy), influence on the cellular growth rate and genetic or evolutionary aspects. Such aspects describe how likely it is that a certain variation occurs on the background of the gene in a specific cancer [42]. Including such features inevitably complicates the model, and is relied on the availability of high quality clinical or experimental data that is often non-existent or incomplete. For this reason, such effects have not been considered in our model. This is not expected to modify the qualitative findings, but should be considered for development of treatment regimens in patients. Drug dosage might also influence the

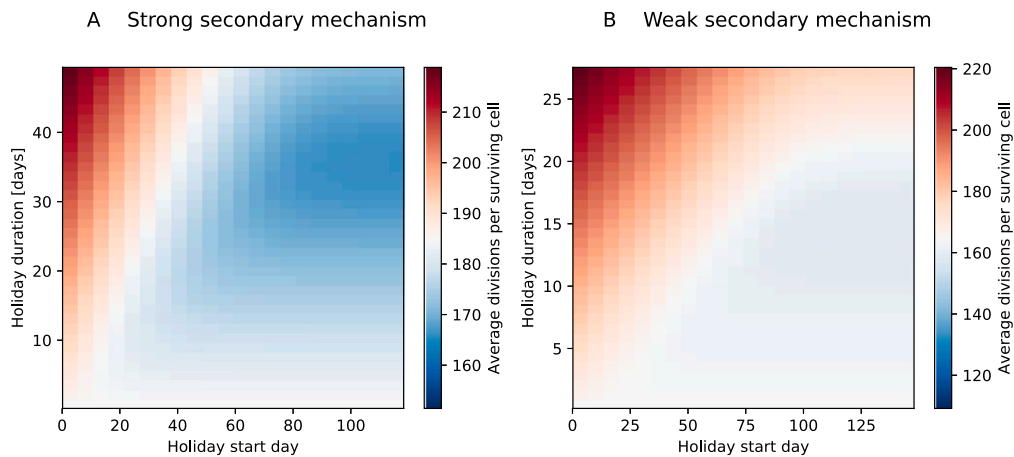


Fig. 6. Holiday effect on cumulative growth over a range of parameters. The colour scale has been centred such that white regions are equivalent to no holiday, blue and red represent fewer and higher average number of cell divisions, respectively. Two mechanisms are considered: strong (left) and weak (right). Early start or long duration are not optimal in terms of cell division. There is a region of optimal start day and duration with the strong mechanism (left, dark blue). With the weak mechanism, there is a smaller benefit for drug holidays in terms of cell divisions.

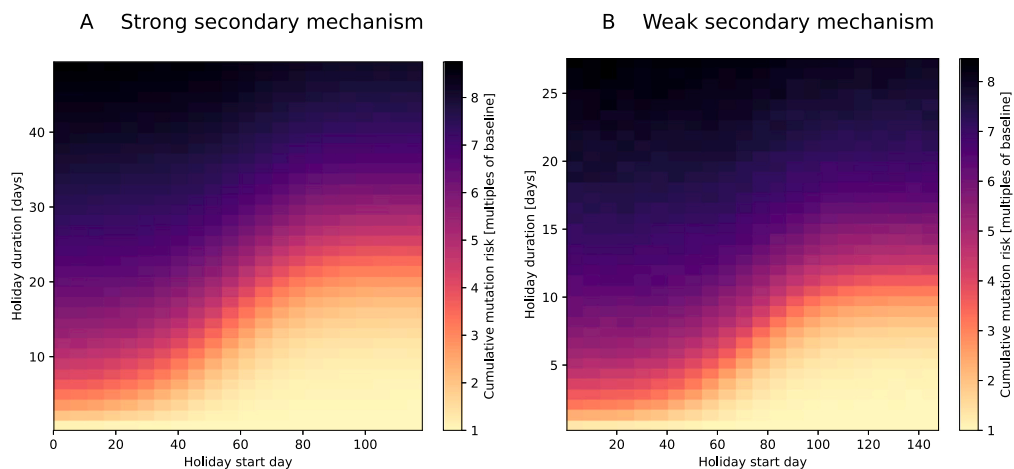


Fig. 7. Cumulative mutation risk as a function of drug holiday length and start time. The mutation risk always increases with a drug holiday. Irrespective of the type of resistance mechanism (strong or weak), longer duration increases the risk for mutations and the same is true when the drug holiday starts too early. The optimum duration of treatment before pause is somewhat different depending on the mechanism. It is 85–90 days with the strong secondary mechanism and 105–120 days in the weak one. In both models, the increase in risk with the duration of the therapy is slower than the decrease between days 40 and 80 (strong mechanism) or 60 and 100 (weak mechanism). The optimal times for starting a drug holiday where both effect and mutation risk are considered are shown in Fig. 5.

balance between the modes of resistance, with higher dosage driving the tumour towards heterogeneity [43].

The application itself is available on GitHub, <https://github.com/Sandalmoth/dual-adaptation>. The input is given in a text file (simulation-config.toml). Due to the heavy computational cost, the calculations cannot be run on personal computers at present but require a parallel machine with an MPI interface. This limits the use to computational biologists. On the other hand, the input is flexible enough to explore systems with additional properties. The GitHub package includes several input examples that could be used to study other systems using the parameters explained in sections 2 and 3. We envision that, using parameters that are derived directly from patients when such become available, it will be possible to apply the model to treatment as recently suggested in a policy article [44].

An important finding that arose from this study is that resistance mutations were more likely to be either preexisting, or occur after

a delay (Fig. 4). It would be beneficial to continuously test patients for resistance mutations with sensitive next generation sequencing methods in an effort to identify such mutations before a return of symptoms and a significant regression in treatment status, but the cost is limiting. From a cost minimizing perspective, based on our simulations it appears most rational to examine patients very soon after treatment starts, to test for preexisting mutants, and after they have been in remission for a longer time. We cannot predict the optimal interval with this model, since it is only based on cell line data.

Short treatment holidays were shown to have a positive effect on overall growth in our model (Fig. 6). Unfortunately, that positive effect was paired with an increased chance for resistance mutations (Fig. 7). The risk increases slowly at first, implying that short drug holidays are likely more viable in this aspect. The effects of a drug holiday on drug resistance adaptation are temporary, but it might be possible to identify optimal repeated drug holiday protocols (Fig. 5).

Different medical conditions lead to that TKI treatment needs to be paused to limit side effects; we have shown that it could contribute to an improved treatment effect as well. It has also been suggested that patients could discontinue TKI treatment after long remission [45]. This seems somewhat inadvisable given our results, as we predict only a temporary effect coupled with an increased risk of mutations down the line (unless the cancer is completely eradicated including cancer stem cells, which are much less sensitive to therapy [46]). Thus, patients are faced with a higher risk of resistance if the disease returns. For a young patient long term mutation risk, control might be a higher priority, thus drug holidays may be inadvisable. However, for an older patient, quality of life improvement from decreased cancer growth and a lower drug burden are coupled to a lower projected time when mutations could develop. It thus seems that a drug holiday could be a useful tool. It has been suggested that adaptive therapy, aimed at stability of the tumour population not at eradicating (only) sensitive cells, could be beneficial for preventing the proliferation of resistant cells [47]. Considering different mechanisms of resistance and also side effects, it might be possible to devise another form of adaptive therapy. In such therapy, a pause in treatment could be made after achieving a clear and durable clinical response. In this case, the risk for developing resistance mutations will be lower if such a treatment holiday can be pre-planned (for example, for planned pregnancy or medical treatments that are incompatible with Abl1 inhibitors). Drug holidays should not be planned too long after the start of the therapy to minimize the risk for resistance mutations.

6. Conclusions

We examined how resistance develops in cells that adapt both via highly potent resistance mutations, and gradual overexpression of proteins, using a model based on experimental data. Preexisting resistance mutations were favoured in this model, but the risk of resistance mutations also increased later on as adaptation through overexpression increased proliferation and thus the number of mutations produced. The adaptation through expression generated drug-addiction effects, hence some reduction of the overall cancer growth could be achieved by temporarily removing treatment. However, drug holidays always increased the risk of resistance mutations. Overall, treatment-free periods may be advised after an initial treatment period, especially if treatment breaks are also indicated by other circumstances.

Author contributions

The study was directed by RF with the help of AdW. HJGL performed the experiments and analysis, and wrote the manuscript with in cooperation with RF. All authors have read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.compbiomed.2023.107826>.

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