Evolution of ibrutinib resistance in chronic lymphocytic leukemia (CLL)

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The Bruton tyrosine kinase inhibitor (BTKi) ibrutinib is a new targeted therapy for patients with chronic lymphocytic leukemia (CLL). Ibrutinib is given orally on a continuous schedule and induces durable remissions in the majority of CLL patients. However, a small proportion of patients initially responds to the BTKi and then develops resistance. Estimating the frequency, timing, and individual risk of developing resistance to ibrutinib, therefore, would be valuable for long-term management of patients. Computational evolutionary models, based on measured kinetic parameters of patients, allow us to approach these questions and to develop a roadmap for personalized prognosis and treatment management.

Our kinetic models predict that BTKi-resistant mutants exist before initiation of ibrutinib therapy, although they only comprise a minority of the overall tumor burden. Furthermore, we can estimate the time required for resistant cells to grow to detectable levels. We predict that this can be highly variable, depending mostly on growth and death rates of the individual CLL cell clone. For a specific patient, this time can be predicted with a high degree of certainty. Our model can thus be used to predict for how long ibrutinib can suppress the disease in individual patients. Furthermore, the model can suggest whether prior debulking of the tumor with chemo-immunotherapy can prolong progression-free survival under ibrutinib. Finally, by applying the models to data that document progression during ibrutinib therapy, we estimated that resistant mutants might have a small (~2%) mean fitness advantage in the absence of treatment, compared with sensitive cells.

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hronic lymphocytic leukemia (CLL), the most common adult leukemia in the Western hemisphere, is characterized by the expansion of CD5+CD23+ mature monoclonal B cells in the peripheral blood, as well as in lymph nodes and bone marrow. B-cell receptor (BCR) signaling plays a central pathogenic role in CLL, based on structural restrictions of the BCR and BCR-dependent survival and growth of the malignant B cells (1, 2). Bruton tyrosine kinase (BTK), a nonreceptor tyrosine kinase of the Tec kinase family, is essential for BCR signaling. Ibrutinib (previously called PCI-32765) is a potent (IC50, 0.5 nM) BTK inhibitor that inactivates BTK through irreversible covalent bonding to Cys-481 in the ATP-binding domain of BTK (3).

In CLL, ibrutinib characteristically causes an early redistribution of tissue-resident CLL cells into the peripheral blood, with rapid resolution of enlarged lymph nodes, along with a surge in lymphocytosis (7, 12). This lymphocytosis is asymptomatic, transient, and resolves in most patients during the first few months of therapy. However, the majority of ibrutinib-treated patients do not achieve complete remission (7), and instead the lymphocyte counts stabilize in the long term in many patients at levels that are significantly lower than before treatment, but higher than normal.

Although the clinical data so far demonstrate extremely encouraging responses in patients, the question arises as to how long the control of the disease can be maintained during continuous ibrutinib therapy. In particular, drug-resistant mutants can arise that can initiate renewed growth. Indeed, in a minority of patients the growth of drug-resistant mutants has already been documented (13–15). Resistance has been found to be caused by point mutations, and a number of different mutants have been documented. These mutants have been shown to have a mutation in the BTK binding site of ibrutinib or gain-of-function mutations in PLCγ2, which lead to autonomous BCR activity.

The aim of our study was to determine whether, based on previously measured kinetic parameters of the disease in the absence of treatment (16) and during ibrutinib therapy (17), it is possible to use mathematical models to predict the evolutionary dynamics of ibrutinib-resistant mutants, and to predict how long control of CLL with single-agent ibrutinib treatment can be maintained.

Results

BTK Inhibitor-Resistant CLL Cells Are Present Before the Start of Treatment. An important question that is currently debated in the context of targeted treatments of cancer is whether resistant subclones are present before the start of therapy, or whether they...
evolve during the treatment phase. Here, this question is analyzed in the context of ibrutinib treatment of CLL.

Using our computational modeling approach, we assume that a colony of cancer cells grows stochastically from a single cell, and at each division there is a small probability of creating a resistant mutant. In the simplest scenario, we assume that: (i) the mutants are neutral in the absence of treatment (that is, they grow at the same rate as the wild-type CLL cells); (ii) CLL cells acquire drug-resistant mutations at the physiological mutation rate of human cells, and thus do not display elevated mutation rates (e.g., caused by genetic instability); and (iii) mutant generation requires one genetic hit.

Under the scenario just described, Fig. 1 plots the probability of creating at least one resistant mutant for different colony sizes and different mutation rates. The vertical axis shows the log10 colony size upon detection, and the horizontal axis shows the range of log10 mutation rates. The numbers marked next to individual contours on the graph denote the probability of existence of resistant mutants. For example, we can see that for small mutation rates and for very small colony sizes (the lower left corner of the diagram) the probability of resistance is negligible. This probability grows with the mutation rate and with the colony size.

For CLL, the relevant parameter regime is shown by a rectangle in the upper left part of the diagram in Fig. 1. We assume that the number of CLL cells in tissue upon start of treatment ranges between 10^13 and 10^15 (as demonstrated in ref. 17). The range of possible mutation rates is taken between 10^{-9} and 10^{-7}. If resistance can only be induced by a single point mutation, then the mutation rate would be 10^{-9} (18–22). In contrast, if multiple different point mutations independently induce resistance, then the mutation rate is higher. It is reasonable to assume an upper limit for the mutation rate of 10^{-7}, which means that hundreds of different point mutations can independently cause resistance. As we can see, for this region of the diagram in Fig. 1, the probability of resistance generation is very close to 1. Calculations show that this probability is always greater than 0.995; in other words, the chance of not finding resistance is smaller than 0.5%. This lowest bound corresponds to the colony size of 10^{12} and the mutation rate of 10^{-7}. We note that this finding holds true for any division and death rates. We have illustrated our cases assuming that the death rate is 10% of the division rate. The lowest chance to generate resistance during growth occurs when the expanding cell population does not die (23). Even under this extreme assumption, the probability that resistant mutants exist upon treatment initiation is still practically certain.

Now, suppose that assumption (i) above does not hold. In the next section we present indication that ibrutinib-resistant CLL cells may have a certain fitness advantage, even in the absence of treatment. In this case, our conclusions definitely hold, and the presence of resistance before treatment is a certainty. Next, let us assume that resistant mutants are characterized by a certain selective disadvantage compared with wild-type (although this has not been observed for ibrutinib treatment of CLL, it has been observed in the context of other targeted cancer therapies (24)). In this case, it turns out that the estimates for the probability of resistance are very nearly the same as shown above. Regardless of the properties of the mutants, the probability of mutant generation is extremely high. The reason that it does not depend on whether the mutants are advantageous or disadvantageous is because the main contribution to mutant generation comes from a very large (compared with the inverse mutation rate) number of cell divisions. In other words, it is most likely that the resistant mutants are generated relatively late in the colony’s history, where the population is so large that a new mutant is very likely to be produced in each generation. This finding is in contrast to another possible mechanism whereby a mutant is generated early in the colony’s history and grows to large numbers. The latter scenario, which is strongly dependent on the mutant properties of growth, does not contribute significantly here.

If assumption (ii) above does not hold, that is, if ibrutinib-resistant mutants are generated with an elevated mutation rate, the chances of having resistance are even higher. In other words, this assumption does not influence our conclusion.

The only circumstance under which we can expect the presence of resistance with less certainty is if assumption (iii) is violated; that is, if mutant generation requires more than one mutational hit (for example, if gene amplification is required for resistance) (25). The higher the number of mutational steps required, the less the chance of generating a resistant cell by the time of detection. Because the only documented ibrutinib-resistant mutants have been shown to be caused by point mutations, however, we will focus on this scenario (14, 15).

Resistant Mutants Can Have a Selective Advantage in the Absence of Treatment. We know that resistant mutants enjoy a significant advantage during treatment as they continue to grow, whereas wild-type cells decline under ibrutinib. What can we say about growth of mutants in the absence of treatment? For PLCγ2 mutations, calcium flux in DT40 cells after stimulation with anti-IgM antibody was enhanced compared with nonmutant PLCγ2, even in the absence of ibrutinib (15). This result indicates enhanced pathway activation in the context of the PLCγ2 mutation, which could theoretically translate into a survival and fitness advantage of mutant cells, although this has so far not been demonstrated. For the mutation in the ibrutinib-binding site in BTK, there is so far no indication for a mutant fitness advantage (15).

These data cannot tell us whether resistant mutants indeed enjoy a fitness advantage compared with the wild-type when they grow in vivo in the absence of treatment. Therefore, we will use an epidemiological approach to investigate this question. In particular, we will use the data collected so far on ibrutinib treatment response in CLL patients to see what level of mutant fitness is consistent with the observations. Using the literature, we compiled the information on nine different cohorts of CLL patients who were treated with ibrutinib for different median time durations (6, 7, 13, 26–29) (see SI Appendix for details). The number of patients showing progressive disease was reported in each case. Using computer simulations, we determined what level of mutant fitness in the absence of treatment is most consistent with these data. Although the exact numbers depend somewhat on the assumed level of mutant detection (SI Appendix), one overall conclusion is clear: if the resistant mutants have a fitness advantage in the absence of treatment, this advantage is not very large, about 1.5%. This figure refers to the mean fitness advantage. In other words, if all resistant
mutants have the same fitness in the absence of treatment, then this fitness is 1.5% larger than that of the wild-type. If, on the other hand, only 10% of all ibrutinib-resistant mutations confer fitness advantage and the rest 90% are neutral in the absence of treatment, then the advantageous mutants must be about 15% fitter than the wild-type, thus yielding the same 1.5% mean fitness advantage.

The Number of Resistant Cells at Detection Is Very Low. By using our model, we were able to predict the expected population size of resistant cells at the time of tumor detection. Using the parameter values from Messmer et al. (16) and Wodarz et al. (17), we find that the number of mutants in patients at the start of treatment typically has the order-of-magnitude of $10^6$ to $10^8$ cells. In Fig. 2A we present a histogram of numerically predicted mutant population sizes that were obtained in the following way. We randomly picked division and death rates chosen inside the bounds given by Messmer et al. (16) and paired them with randomly selected population sizes at treatment start chosen between the minimum and the maximum values measured in Wodarz et al. (17). A population of 1,000 artificial "patients" was created in this way, and then the expected number of mutants at start of treatment was calculated. We performed this procedure assuming that resistant mutants are neutral at the start of treatment (the blue histogram in Fig. 2A), and then repeated it with the assumption that resistant mutants have a 1.5% fitness advantage in the absence of treatment (the red histogram Fig. 2A). We can see that the median values for the mutant population are very similar in the two cases (and given by $1.1 \times 10^7$ and $1.9 \times 10^7$ cells). The main difference manifests itself in a thicker "tail" of the distribution: if the mutants have a selective advantage before treatment, there will be a very small percentage of patients with a significant population of resistant mutants at the start of treatment. About 0.1% of the artificial "patients" had a population of mutants greater than 1% of the total CLL cells.

The question arises as to whether these mutants can be responsible for the long-term dynamics of CLL cells observed during a population of mutants greater than 1% of the total CLL cells.

To test this hypothesis we need to compare the predicted sizes of the mutant colonies with the number of CLL cells in tissue during the plateau phase. The number of CLL cells in the blood has been measured during this time frame, but the great majority of the disease resides in the tissue. Based on the blood measurements and on volumetric analysis in the tissue, the number of CLL cells in the tissue has been estimated in ref. 17. During the plateau phase, the obtained values of CLL cells in tissue were rather varied, with the median of $1.25 \times 10^{11}$ and the minimum value given by $4 \times 10^8$.

Our computations show that the presence of resistant mutants in CLL is unlikely to explain the long-term stabilization of the lymphocyte counts in the patients. In Fig. 2B we present a histogram of projected mutant population sizes after 300 d of treatment. We can see that the majority of patients will have a mutant colony sizes that are about $10^5$-times smaller than the typical plateau value for CLL cells. The median size of a mutant colony after 300 d is $4.0 \times 10^7$ and $9.7 \times 10^7$ cells, for neutral and advantageous (1.5%) mutants, respectively. This number is much smaller than the median of $1.25 \times 10^{11}$ for estimated numbers of tissue CLL cells during the plateau phase. Another reason for the observed plateau to be of a different nature compared with a resistant clone is that the former is relatively stable, whereas the latter is predicted to expand exponentially, and can thus show different overall dynamics, depending on the rate at which resistant mutants grow.

To conclude, our analysis shows that at the time of treatment initiation, and even after 300 d of treatment with ibrutinib, resistant mutant colonies are most likely to be very small, well below the detection limit. This finding is in agreement with the currently available data (14, 15). Resistance mutations were detected when progressive disease was first observed, but not before treatment or during the initial response to treatment (including lymphocytosis) (14, 15). In addition, the CLL cells found during lymphocytosis in nonprogressing patients have been found to be quiescent rather than carrying mutations that confer ibrutinib resistance (30, 31).

When Can Resistance Be Detected? We have shown that typically, we do not expect resistance to be observable for the time frames of treatment considered so far (300 d after therapy initiation). It is inevitable, however, that the resistant colony will grow and eventually become sizable. The question is how long this process may take.

We have run the mean course of resistant-mutant growth starting from the expected mutant numbers at the start of treatment. We assume that resistance becomes a problem and contributes to visible disease relapse when the size of the resistant colony reaches $10^{10}$ cells. Although we know the number of tumor cells in the blood at which the disease becomes detectable, most of the CLL cells reside in tissue. The population size threshold for detection of solid tumors is about $10^9$ cells (32), and for the CLL cells to accumulate at detectable levels in the blood, the tissue disease burden likely has to be larger, hence our threshold level of $10^{10}$ cells.

As before, we studied 1,000 parameter combinations randomly created using the bounds from Messmer et al. (16) and Wodarz et al. (17). Assuming the mutation rate of $10^{-8}$, the model shows that 6% (12%) of patients in our sample will develop resistance before 2 y after start of treatment, 46% (59%) of patients will develop resistance before 5 y, and 75% (86%) of patients will develop it before 10 y (the amount in parenthesis refers to resistant mutants having an average fitness advantage of 1.5%). For 5% (1%) of the patients, resistance will not come up for the first 30 y after treatment. The mean time of developing resistance is about 9 y (5) after start of treatment. A numerical probability distribution function for the time when resistance reaches detection level is presented in Fig. 3.

Despite the fact that resistance is present with certainty in all tumors upon detection, the dynamics of resistance growth is predicted to be very different for different patients. Without assuming any differences in the mechanisms of resistance, the only parameters that are varied across our set are (i) the size of tumor at start of treatment and (ii) the kinetic parameters of untreated CLL cells. It turns out that it is the net growth rate of mutants (parameter $l_m - d_m$, i.e., division minus death rate of cells) that defines the eventual fate of the treatment. Fig. 4 illustrates this statement. In Fig. 4A and B we plot the expected growth dynamics of mutant colonies for neutral mutants and mutants with a 1.5% fitness advantage before treatment, respectively. The most noticeable difference between the two cases is that slightly advantageous mutants are present above the detection level in a small percentage of patients at the start of treatment. In both
graphs we observe a wide range of dynamic behaviors, where in some patients resistance does not arise before 10 years, and in others it comes up very quickly. What parameter is mostly responsible for this difference? Mathematically, the time to resistance for each patient decays with the mutant growth rate and with the tumor burden at start of treatment. The former parameter, however, exhibits an overall stronger influence on the rise of resistance. In Fig. 4C we plot, for each patient, the inverse time it takes for mutants to rise above the detection level of $10^{10}$ cells as a function of the net growth rate of the cells. We can see a strong positive correlation. The much weaker dependence on the population size at the start of treatment is masked by the growth rate correlation, as shown in Fig. 4D.

We conclude that the measured difference in the net growth rates of CLL cells alone (without evoking the differences in the mechanisms of resistance) can give rise to a significant heterogeneity of resistance growth dynamics. Furthermore, it is the net growth rates of CLL, rather than the size of the tumor upon initiation of therapy, which are predicted to be the strongest correlates of resistance.

**Average Behavior and Stochastic Variations.** So far, our results on the dynamics of mutant growth are based on averages derived from deterministic models. We have observed significant heterogeneity in the average time until resistant mutants grow to significant levels, based on differences in the parameters by which the patients are characterized, most importantly the rate of cell division and the rate of cell death. However, even if these parameters are identical, variation in the time until resistance rises to higher levels can come about through stochasticity that is inherent in the dynamics of cell growth, especially during phases when mutants grow from low numbers. Therefore, we performed numerical analysis of the stochastic model to study the variance in outcomes (SI Appendix).

Two measures were considered: (i) The number of mutants when treatment was initiated. Treatment was assumed to start when the number of tumor cells reached $8 \times 10^9$, which is the average estimated tumor size at the start of ibrutinib treatment in our previous study (17). (ii) The time it takes for resistant mutants to grow to significant levels. In these computations, we defined the time when the number of resistant tumor cells in the tissue compartments reached $10^9$ cells.

The algorithm was used for two parameter combinations, one characterized by faster growth (slow turnover of cells, where division rate of cells is much larger than death rate), and one characterized by slower growth (fast turnover, where division rate of cells is close to death rate). The results are similar for both scenarios and shown in Fig. 5. First consider the number of cells at start of therapy (Fig. 5A). We can see that there is a large degree of variation in this number, with a SD that is an order-of-magnitude higher than the mean (please note the logarithmic scale on the horizontal axis). This phenomenon has been previously described (33). On the other hand, the time until the resistant mutant reaches the threshold population size shows a much more narrow distribution and only little variation (Fig. 5B and C). The SD for the timing of mutant growth is an order-of-magnitude lower than the mean. The reason is that the time until the resistant mutant population rises to a threshold size is determined by the logarithm of the mean population size at the start of treatment (SI Appendix), which logarithmically reduces the spread in the results.

The insight that there is only a small amount of stochastic variation in the time until the resistant mutant reaches its threshold size for a given parameter set has important practical implications: it enables us to predict the long-term treatment outcome with ibrutinib, and to tailor treatment strategies individually to avoid complications from resistance. The time until resistant mutants emerge to significant levels is rather accurately predicted by the average. Hence, measurements of the cell division and death rates by heavy water labeling, and estimations of total tissue tumor burden from radiological data, can enable us to predict for how long continuous ibrutinib treatment can control CLL. If control is predicted to be maintained for decades, no special strategies to combat resistance are likely needed. In contrast, if tumor relapse because of resistance is predicted to occur within a few months, treatment schedules will have to be designed to prevent this relapse, as described next.

**Strategies to Overcome Resistance.** The above analysis indicates that drug-resistant mutants arise during the growth phase of CLL before the onset of treatment, and continue to grow during treatment. If resistant cells grow sufficiently to contribute to relapse during the life span of the patient, measures have to be implemented to combat this resistance against ibrutinib.

The first question to examine is whether early treatment, immediately upon detection, would significantly lower the probability that resistant mutants exist at the start of therapy. Let us assume that resistance is generated with a mutation rate of $10^{-6}$ and that the lowest detectable tumor tissue burden is approximately $10^9$ cells. Fig. 6A shows the probability that mutants resistant against ibrutinib exist as a function of the tissue tumor burden. This is plotted for different tumor cell turnover rates [i.e., ratios of cell division and death, as estimated in Messmer et al. (16)]. Fig. 6A shows that once the number of tissue tumor cells rises beyond $10^9$ cells, the probability for resistant mutants to exist is very high, about 93% for the lowest turnover rates and higher values for higher turnover rates. This finding is also shown in Fig. 6B. In this figure, we assumed that resistant mutants do not have a fitness advantage in the absence of resistance.
initiation of treatment (when tissue disease burden is at $8 \times 10^8$ cells). The probability of two-drug resistance increases with the turnover rate of the tumor, and hence a combination of two drugs could work. This is, however, not the case for high-turnover rates. For such tumors, more drugs would have to be combined to prevent resistance-induced disease progression. This theory can be explored further mathematically with the framework provided by Komarova and Wodarz (23), although it remains to be explored whether such combination treatments would be clinically practical considering the side effects.

**Discussion**

We used previously estimated key parameters of CLL dynamics in the absence of treatment and in the presence of ibrutinib therapy, as well as patient follow-up data, to analyze the evolutionary dynamics of drug resistant cell clones. The following key insights were obtained by using methods of evolutionary and computational biology:

First, drug-resistant CLL cells are almost certain to exist by the time of ibrutinib treatment initiation. Therefore, it is the preexistence of resistant cells that is important. The treatment phase of one-drug resistance for different values of the turnover (horizontal axis) is predicted to progress in 6.9 to 12.8 y. Fewer than 1% of patients (rather than 6%) are predicted to progress in $<2y$, 24% (rather than 46%) in $<5y$, 55% (rather than 75%) in $<10y$, and 14% (rather than 5%) of patients are predicted to remain progression-free for $>30y$.

A currently more hypothetical strategy could be to combine different kinase inhibitors [such as idelalisib (34)] with ibrutinib to prevent resistance-induced disease progression. If mutations that confer resistance against ibrutinib do not simultaneously confer resistance to the other inhibitors, then Fig. 6C shows that for low-turnover treatment, the probability for two-drug resistance is relatively low, and hence a combination of two drugs could work. This is, however, not the case for high-turnover rates. For such tumors, more drugs would have to be combined to prevent resistance-induced disease progression. This theory can be explored further mathematically with the framework provided by Komarova and Wodarz (23), although it remains to be explored whether such combination treatments would be clinically practical considering the side effects.

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debuling, which could lead to a longer duration for which the patient remains progression-free during ibrutinib therapy (Fig. 6D). Assume that chemo-immunotherapy reduces the tumor burden by two orders-of-magnitude. For the same parameters as above, the average time until progression now increases from 9 to 12.8 y. Fewer than 1% of patients (rather than 6%) are predicted to progress in $<2y$, 24% (rather than 46%) in $<5y$, 55% (rather than 75%) in $<10y$, and 14% (rather than 5%) of patients are predicted to remain progression-free for $>30y$.

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First, drug-resistant CLL cells are almost certain to exist by the time of ibrutinib treatment initiation. Therefore, it is the preexistence of resistant cells that is important. The treatment phase
is unlikely to contribute significantly to resistance generation. Hence, the dynamics that are observed duringibrutinib treatment, including the lymphophagy phase, are not likely to contribute to the evolution of resistant mutants.

Second, drug-resistant mutants are unlikely to explain the lack of complete remission during ibrutinib therapy. The predicted number of resistant cells during this phase is too low to account for the observed plateau to which the CLL cell population converges during treatment.

Third, the time until resistant mutants grow to sufficient levels to contribute visibly to disease relapse is predicted to vary extensively across patients because of known variations in the basic parameters describing the rate of clonal expansion. This in turn defines the time until drug-resistant CLL cells divide and die in tissue during drug therapy. In general, we predict that significant interpatient variations in resistance dynamics will be caused by genetic differences in the tumors alone. Further variations can be caused by other factors, such as genetic differences among the patients and the specific types of resistance mutations acquired.

Fourth, although differences in cellular growth parameters across patients can lead to extensive variation in the time until visible relapse occurs, for a given set of parameters, stochastic variation in outcome is limited. Therefore, the expected time until disease relapse is a reliable indicator for how long ibrutinib monotherapy can control the disease for a particular patient.

Finally, depending on the relative rates of CLL cell division and cell death, it might be possible to increase the duration of progression-free survival during ibrutinib treatment by debulking the tumor with chemo-immunotherapy before ibrutinib therapy. These insights open up the possibility to use such computational models for personalized predictions about the outcome of treatment. For each patient, the division and death rate of cells tumor load before treatment initiation should be estimated with CT scans. Based on these measures, we can predict the average duration for which ibrutinib monotherapy can maintain control of the disease, when resistance is likely to emerge, and whether specific measures to prevent relapse are likely to be helpful.

Besides predictions about the dynamics of disease relapse, another important contribution of our calculations is that we were able to provide a first estimate of resistant-mutant fitness. This estimate will need to be refined when more clinical data become available that document the timing of disease relapse during ibrutinib treatment. The methodology presented here is indirect, and infers mutant fitness from epidemiological data; this is akin to similar approaches that have been used to infer in vivo processes from epidemiological data in cancer research (35–37). It is likely one of the more reliable ways to estimate mutant fitness. Although a direct measurement would obviously be preferable, this might be very difficult to achieve. The growth properties of CLL cells depend intrinsically on the tissue microenvironment in humans, which renders any experimentation and direct approaches challenging.

**Materials and Methods**

We use methods of evolutionary and computational biology to answer questions about the evolutionary dynamics of BTK inhibitor resistance in CLL. Deterministic and stochastic methods are used. Please see SI Appendix and refs. 38 and 39 for complete details.

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1 The data

Here we present the data that have been used in this work to parameterize our model.

1.1 Division/death rates and population sizes

In this section we describe the data informing us on the cellular kinetics of CLL. The first set of data comes from Ref. [1], where division and death rates of CLL cells were evaluated in untreated patients by labeling dividing CLL cells through deut erated (heavy) water administration to patients. Out of 16 patients studied, 9 exhibited a net growth of CLL cells, such that the measured division
rate was larger than the death rate. For these patients, we have converted the measured values (given in Ref. [1] as percentages) into the units of rates measured in days$^{-1}$, by using the following conversion formula:

$$\text{rate} = -\ln(1 - 0.01\text{percentage}).$$

The obtained values for division rates, $l_w$, and death rates, $d_w$, are presented in table 1.

<table>
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<tr>
<th>Div. rate, $10^{-3}$days$^{-1}$</th>
<th>2.30</th>
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<td>2.10</td>
<td>1.50</td>
<td>1.40</td>
<td>10.56</td>
<td>1.50</td>
</tr>
<tr>
<td>Death/Div ratio</td>
<td>0.96</td>
<td>0.19</td>
<td>0.59</td>
<td>0.90</td>
<td>0.88</td>
<td>0.33</td>
<td>0.78</td>
<td>0.60</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Table 1: Division rates, $l_w$, and death rates, $d_w$, and the ration $d_w/l_w$, from Ref. [1].

Further data used in this paper concerns the population sizes of CLL cells (in tissues) for patients at the start of treatment, $N$, as well as the stabilized number of CLL cells in the “plateau” stage after continuous treatment, $C$. These parameters were measured for 10 patients in Ref. [2], and are presented in table 2.

To run statistics, we used data in tables 1 and the second row of table 2 to create a population of artificial “patients” in the following way. For each “patient”, we randomly picked the value $l_w$ (the division rate of CLL cells) from the uniform distribution between the minimum and the maximum value of the data in the top row of table 1. Then, we picked the death-to-birth ratio, $r$, for the patient from the uniform distribution between the minimum and the maximum such values from the bottom row of table 1. The CLL cell death rate for this patient was then taken $d_w = rl_w$. Finally, we randomly chose he population size at start of treatment between the minimum and the maximum values of $N$ given in table 2. Usually, a population size of 1000 was used in the analysis.

<table>
<thead>
<tr>
<th>$N \times 10^{-12}$</th>
<th>3.034</th>
<th>3.064</th>
<th>7.044</th>
<th>30.209</th>
<th>2.143</th>
<th>4.083</th>
<th>1.294</th>
<th>15.452</th>
<th>6.156</th>
<th>7.711</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C \times 10^{-9}$</td>
<td>0.4</td>
<td>47.5</td>
<td>94.7</td>
<td>392.1</td>
<td>86.3</td>
<td>35.7</td>
<td>1.0</td>
<td>376.4</td>
<td>507.8</td>
<td>155.9</td>
</tr>
</tbody>
</table>

Table 2: The number of CLL cells in tissues, $N$, at the start of treatment, and the plateau number of CLL cells, $C$, measured in [2].

Finally, important for the present study is the assumption that significant CLL cell division does not occur during ibrutinib therapy, as evidenced by preclinical data demonstrating that ibrutinib inhibits thymidine incorporation, CLL cell proliferation, and CLL cell migration and homing [3, 4, 5].

1.2 Bone marrow data

In Ref. [2], the total number of CLL cells was calculated based on the measured volume of the lymph nodes and spleen. The number of CLL cells in bone mar-
row was not quantified in that study, which led to a certain under-estimation of the total number of CLL cells in the tissues. Here we use additional data to quantify the additional amount of the CLL cells. In 7 out of the 10 patients, the percentage of CLL infiltration in the bone marrow was quantified, which averaged at 63% (with the range from 10% to 82%) at the start of Ibrutinib treatment. Further, for 6 patients, the infiltration percentage was quantified after several months of Ibrutinib treatment (between 13 and 30 months), resulting in the mean of 4.8% infiltration (with the range from 1% to 10%). These data can be used to calculate the correction to the values $N$ and $C$ that come from the bone marrow contribution to the total CLL load.

While we do not know the exact number of CLL cells in bone marrow of these patients, we can present a reliable upper bound estimate. In Ref [6] it is reported that the mass of hematopoietic bone marrow (BM) comprises 1.4% and 1.2% of total body mass for males and females. The mean body mass of US people between 60 and 74 years of age is 87.1 kg for males and 74.9 kg for females [7]. Given the nearly equal representation of males and females in the cohort and their age range, we obtain that the mass of the BM can be estimated to be 1.1 kg. The density of soft bone tissue was provided in [8] to be 1.14 kg/L, which gives the typical BM volume of 0.93 L. The volume of CLL cells is 166 fL [9]. Therefore, if we assume that all fat and connective tissue in the hematopoietic bone marrow of these patients has been replaced by CLL cells, we obtain that the maximum number of CLL cell in BM before treatment is $3.6 \times 10^{12}$. This gives a correction of about 40% to the total number of CLL cells at the start of treatment. For the plateau value of CLL cell numbers, the mean value for the 5 patients for whom we have the information changes from $2.4 \times 10^{11}$ cells to $5.0 \times 10^{11}$ cells.

These are the upper bounds for the corrections resulting from inclusion of the bone marrow compartment, and in reality these numbers are likely to be somewhat smaller. While in terms of the actual numbers, the correction is not negligible, for our calculations it does not make a significant difference. All the main calculations of this paper have been redone with the correction resulting from the inclusion of the BM CLL cell. They are presented in section 5 below. Because of a very large variability in the individual patient data, our calculations operate with ranges of numbers rather than individual numbers, and these span several orders of magnitude. All the conclusions that we draw are based on the “orders of magnitude” estimates rather than particular numbers, and therefore they remain the same when we include the corrections calculated here.

### 1.3 Disease progression data

We have analyzed several recent publications which reported patient response to ibrutinib treatment.

In [10], 56 patients with various B-cell malignancies were treated. 20 of them discontinued because of disease progression. Out of 56 patients, 16 had CLL/small lymphocytic lymphoma, and 11 had CLL. None of these patients have developed progressive disease (PD). Although the treatment duration was
not reported, the median progression-free survival in all patients was 13.6 months.

In abstract [11] results are reported for three cohorts of CLL patients. The treatment naive (age higher than 65 yrs) cohort (TN) contained 31 patients with the mean treatment duration of 16.6 months; no PD was observed. The Relapsed or Refractory cohort (R/R) contained 61 patients with the median treatment duration of 17.3 months; 2% of the patients had PD. Finally, the high risk cohort (HR) contained 24 patients with the median treatment duration of 10.3 months; 4% of the patients had PD. These data can be summarized as follows: with the total of 116 patients who received treatment for 15.67 months, 1.9% of patients developed PD.

The TN cohort results were also reported in more details in the paper [12]. 29 65+ treatment nave patients were studied. 2 of them withdrew consent early on (after less than a month and just over a month in treatment), two more dropped out because of adverse effects, and 1 developed Richters syndrome (RS). Out of the rest 24, there was not a single case of PD, and the median treatment time was 22.1 months.

Paper [13] reports results of ibrutinib treatment for 195 CLL patients. The number of PD is reported slightly different depending on whether it was assessed by the investigators (2 patients) or by independent researchers (5 patients). 2 patients were reported to have developed Richters syndrome and 1 Prolymphocytic leukemia; not clear if those were included in the PD count. Quoted is a 12 months follow-up, but the mean treatment duration was 8.6 months.

Paper [14] studied 85 patients with the median ibrutinib treatment time of 21 months. 13% (11 patients) showed PD (and their treatment discontinued). However, out of these, in 7 patients the progression was due to Richters transformation.

In abstract [15] it is reported that 3 out of 261 CLL patients developed resistance, which was defined as patients achieving partial response (PR) or better lasting 6 months, then developing PD without Richters transformation. For the three patients with resistance, the longest treatment time was 575 and the shortest was 388 days, averaging 17.2 months.

In abstract [16], 267 patients from The Ohio State University Comprehensive Cancer Center were treated with either ibrutinib or with ibrutinib and ofatumumab, with the median treatment duration of 16 months. 22 patients developed PD, with 16 of them diagnosed with Richter’s syndrome.

Some of the above results are also presented in [17]. This information is summarized in table 3.

2 Stochastic modeling of CLL

To describe the stochastic dynamics of CLL, and in particular, to calculate the chances of generating resistant mutants by the time of disease detection, we formulate a fully stochastic model of cellular expansion, see also [18].

A stochastic continuous time birth-death process is considered, which represents the growth of a colony of CLL cells. The kinetics of susceptible cells
<table>
<thead>
<tr>
<th>Dataset Number</th>
<th>Reference</th>
<th># patients</th>
<th>Median treatment duration (m0)</th>
<th>Number of PD (excl. RS)</th>
<th>% PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[10]</td>
<td>16</td>
<td>13.6</td>
<td>0</td>
<td>&lt; 6.3</td>
</tr>
<tr>
<td>2</td>
<td>[11], TN</td>
<td>31</td>
<td>16.6</td>
<td>0</td>
<td>&lt; 3.2</td>
</tr>
<tr>
<td>3</td>
<td>[11], R/R</td>
<td>61</td>
<td>17.3</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>4</td>
<td>[11], HR</td>
<td>24</td>
<td>10.3</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>5</td>
<td>[12]</td>
<td>29</td>
<td>22.1</td>
<td>0</td>
<td>&lt; 3.4</td>
</tr>
<tr>
<td>6</td>
<td>[13]</td>
<td>195</td>
<td>8.6</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>7</td>
<td>[14]</td>
<td>85</td>
<td>21</td>
<td>4</td>
<td>4.7</td>
</tr>
<tr>
<td>8</td>
<td>[15]</td>
<td>261</td>
<td>17.2</td>
<td>3</td>
<td>1.1</td>
</tr>
<tr>
<td>9</td>
<td>[16]</td>
<td>267</td>
<td>16</td>
<td>8</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Table 3: Treatment response data from several studies. Progressive disease (PD) cases do not include Richter’s syndrome (RS).

before the start of treatment are described by the division rate, \( l_w \), and death rate, \( d_w < l_w \). The susceptible cells can mutate with probability \( u \) per cell division, and give rise to resistant cells, which are characterized by the division rate \( l_r \) and the death rate, \( d_r \). After the start of treatment, while susceptible cells effectively stop dividing, the resistant cells continue to grow with the same parameters \( l_r \) and \( d_r \). Below we assume that the growth starts with \( N_0 \) wild-type cells and 0 mutant cells. The size of the colony at the time when treatment starts is denoted by \( N \).

### 2.1 The probability of mutant generation

To describe the birth-death process with mutations, we can use the standard method of probability generating function, see e.g. [18]. Denoting by \( \varphi_{i_w, i_r}(t) \) the probability to have \( i_w \) wild type CLL cells and \( i_r \) resistant CLL cells at time \( t \), we can write down the Kolmogorov forward equation for this quantity:

\[
\varphi_{i_w, i_r} = l_w (1-u)\varphi_{i_w-1, i_r}(i_w - 1) + l_r \varphi_{i_w, i_r-1}(i_r - 1) + d_w \varphi_{i_w+1, i_r}(i_w + 1) + d_r \varphi_{i_w, i_r+1}(i_r + 1) - \varphi_{i_w, i_r}(l_w + l_r + d_w + d_r),
\]

(1)

where the terms on the first line represent divisions of both types of cells, the second line corresponds to mutations creating resistant cells, the third line is death of both types of cells, and the last line corresponds to the possibility of no change (the negative term). The probability function, \( \varphi_{i_w, i_r}(t) \) contains all the “microscopic” information about the stochastic process of interest. In fact, it contains “too much” information, and often one could make shortcuts to extract only the “useful” information needed to answer a specific question. One such shortcut is demonstrated by the method of the probability generating function.
We define the probability generating function as
\[ \Psi(\xi_w, \xi_r; t) = \sum_{i_w, i_r} \varphi_{i_w, i_r}(t) \xi_w^{i_w} \xi_r^{i_r}. \]  
(2)

In order to derive an equation satisfied by this function, we multiply equation (1) by \( \xi_w^{i_w} \xi_r^{i_r} \) and sum over \( i_w \) and \( i_r \). Then the left hand side becomes \( \frac{\partial \Psi}{\partial t} \). On the right hand side we have several types of terms. For example, the first term can be written as
\[ \sum_{i_w, i_r} \varphi_{i_w-1, i_r}(i_w - 1) \xi_w^{i_w} \xi_r^{i_r} = \sum_{k, i_r} \varphi_{k, i_r} k \xi_w^{k+1} \xi_r^{i_r}, \]
where \( k = i_w - 1 \). Next we notice this can be rewritten as
\[ \xi_w^2 \sum_{k, i_r} \varphi_{k, i_r} k \xi_r^{i_r} = \xi_w^2 \frac{\partial}{\partial \xi_w} \sum_{k, i_r} \varphi_{k, i_r} \xi_w \xi_r^{i_r} = \xi_w^2 \frac{\partial}{\partial \xi_w} \Psi. \]

Similarly, the other terms can be rewritten in terms of partial derivatives of the function \( \Psi \) with respect to \( \xi_w \) or \( \xi_r \). We obtain the following first order partial differential equation:
\[ \frac{\partial \Psi}{\partial t} = \frac{\partial \Psi}{\partial \xi_w} (l_w (1 - u) \xi_w^2 + d_w - \xi_w (l_w + d_w - l_w u \xi_r)) + \frac{\partial \Psi}{\partial \xi_r} (l_r \xi_r^2 + d_r - \xi_r (l_r + d_r)). \]  
(3)

The calculations leading to equation (3) demonstrate the convenience of the probability generating function description: instead on an infinite system of coupled equations (1) for the original probability function, \( \varphi_{i_w, i_r}(t) \), we obtained a single first order PDE for the probability generating function. Solutions of this PDE can be related to important characteristics of the process such as the existence of resistant mutants.

PDE (3) can be solved by the method of characteristics. The initial value problem satisfied by the functions \( \xi_w(t) \) and \( \xi_r(t) \) is given by:
\[ \begin{align*}
\dot{\xi}_w &= l_w (1 - u) \xi_w^2 + d_w - \xi_w (l_w + d_w - l_w u \xi_r), \\
\dot{\xi}_r &= l_r \xi_r^2 + d_r - \xi_r (l_r + d_r), \\
\xi_w(0) &= 1, \quad \xi_r(0) = 0.
\end{align*} \]  
(4) (5) (6)

The quantity \( \xi_w(t)N_0 \) defines the probability of not having any resistant mutants at time \( t \). The time of interest is given by \( T: N_0 e^{(l_w - d_w)T} = N \). The quantity of interest is the probability \( P_{\text{res}}(T) \) to develop resistance by time \( T \) given that the colony has survived. To obtain this quantity, we can rewrite \( \xi_w(t)N_0 \) as
\[ \xi_w(t)^{N_0} = \text{Prob(Extinct)} + (1 - \text{Prob(Extinct)}) P_{\text{no res}}, \]
where the probability of colony extinction is well approximated by \( \text{Prob(Extinct)} = (d_w/l_w)^{N_0} \), and \( P_{\text{no res}} \) is the probability of no resistance conditional on the
colony non-extinction. Therefore, solving for $P_{\text{no res}}$, and using $P_{\text{res}} = 1 - P_{\text{no res}}$, we obtain

$$P_{\text{res}}(t) = \frac{1 - \xi_w(t)N_0}{1 - (d_w/l_w)N_0}.$$ 

Below we are using $N_0 = 1$. Therefore, we have

$$P_{\text{res}}(T) = \frac{1 - \xi_w(T)}{1 - d_w/l_w}. \quad (7)$$

In the parameter regime of interest, $N$ is of the order of $10^{12}$ cells, and the mutation rate $u$ is at least $u^{-3}$. Therefore, we have $Nu \gg 1$, and the following approximation holds:

$$P_{\text{res}} \approx 1.$$ 

In other words, in the regime of interest, resistant mutants exist with certainty by the time treatment starts. Equation (7) was used to plot figures 1 and 6 of the main text.

### 2.2 The mean number of mutants

The mean number of mutants (conditioned on the existence of resistance) at colony of size $N$ was found by [19]. In our notations, and assuming that mutants are neutral ($l_w = l_r$, $d_w = d_r$), we obtain

$$M_0 = \frac{Nu \ln N}{(1 - d_w/l_w)P}, \quad P = 1 - \left(1 - \frac{d_w}{l_w}\frac{N_0}{N}\right)^{\frac{1}{l_w/l_w}}.$$ 

In our regime of large $Nu > 1$, we have $P \approx 1$, and because having resistance is certainty, the unconditional expected number of mutants is simply given by

$$M_0 = \frac{Nu \ln N}{1 - d_w/l_w}. \quad (8)$$

This formula is easy to interpret. The numerator is exactly the expected number of mutants in the absence of cell death, calculated first by [20]. The denominator corrects for the actual expected number of divisions taken to each size $N$ in the presence of cell death. The same expression as (8) can be obtained by solving the initial value problem describing the expected number of cells,

\begin{align*}
\dot{x}_w &= (l_w(1 - u) - d_w)x_w, \quad (9) \\
\dot{x}_r &= l_wux_w + (l_r - d_r)x_r, \quad (10) \\
x_w(0) &= 1, \quad x_r(0) = 0. \quad (11)
\end{align*}

Setting $l_r = l_w$ and $d_r = d_w$ and evaluating the solution for $x_r$ at $t_* = \ln N/(l_w - d_w)$, we obtain exactly the expression in (8).
In the case of advantageous mutants a concise formula for the number of mutants is not available, and we will use a numerical solution of system (9-11). Solving numerically for \( t_* \) such that \( x_w(t_*) + x_w(t_*) = N \), we obtain the expected number of mutants at size \( N \) as

\[
M_0 = x_w(t_*).
\]

(12)

Note that this formula is valid for advantageous, neutral, or disadvantageous mutants.

In figures of the main text, 2 - 4, for each parameter combination we calculated the expected number of mutants (as explained above), and then modeled their growth during treatment as exponential by multiplying the initial number by \( e^{(l_r - d_r)t} \), where \( t \) is time after start of treatment. The artificial “population was created by taking random parameter combinations in the ranges given by the data cited above. Using this formalism, we can evaluate the mutant population size at time \( t \), or calculate the time it takes for the mutants to reach a threshold value. We can also calculate the percentage of the population that develops resistance after a certain treatment time. The latter calculation was used to test different mutant fitness values and find the best fit when comparing with ibritinib follow-up data.

2.3 Multiple mutant generation

The probability of double-hit (or in general, \( m \)-hit) mutant generation in a colony of size \( N \) has been studied in [21, 18]. Each cell can acquire resistance to each of the \( m \) drugs, by means of a certain mutation. In general, there are \( m \) types of mutations, with rates \( u_1, \ldots, u_m \). Each mutation event corresponding to rate \( u_i \) leads to a phenotype resistant to drug \( i \). We assume that resistance to one drug does not imply resistance to another drug (in general, this is not always true and cross-resistance is often observed, see [22]). In order to develop resistance to all \( m \) drugs, a cell must accumulate \( m \) mutations. Simple combinatorics suggests that there can be up to

\[
n = 2^m - 1
\]

different resistant phenotypes. The total number of types is \( 2^m \), and the system’s state is characterized by an integer-valued vector of length \( 2^m \). In particular, there are \( \binom{m}{k} \) phenotypes resistant to \( k \leq m \) drugs. Figure 1 illustrates the mutation network for \( m = 3 \). We label each phenotype by a binary number of length \( m \), where “1” indicates resistance to the drug corresponding to its position and “0” indicates susceptibility. For example, if \( m = 3 \), type 101 is resistant to drugs number 1 and 3 and it is susceptible to drug number 2.

Each type \( i \) is characterized by the division rate \( l_i \) and the death rate \( d_i \). In particular, the wild type (of fully susceptible type) has the division rate \( l_0 = l_w \) and the death rate \( d_0 = d_w \). To describe stochastic evolution of a cellular colony with mutations, we introduce the function

\[
\varphi_{i_0, \ldots, i_n}(t),
\]
Figure 1: Mutation diagram corresponding to three drugs. The binary number, $s$, for each node describes the resistance properties of the corresponding phenotype: 0 stands for “susceptible” and 1 for “resistant”.

the probability to have $i_s$ cells of type $s$ at time $t$, where $0 \leq s \leq n = 2^m$ are binary numbers. We can write down the Kolmogorov forward equation,

$$\dot{\varphi}_{i_0, \ldots, i_n} = \sum_{s=0}^{n} Q^{(s)},$$

where $Q^{(s)}$ is the contribution obtained from considering probabilities of reproduction and death of cell-type $s$,

$$Q^{(s)} = \varphi_{...,i_s-1,...}(i_s-1)L_s \left(1 - \sum_j u_j^{s,\text{out}}\right) + i_sL_s \sum_j \varphi_{...,i_s,...,i_j-1,...}u_j^{s,\text{out}} + \varphi_{...,i_s+1,...}(i_s + 1)d_s - \varphi_{...,i_s}(L_s + d_s).$$

In this equation we used the following short-hand notation: $\varphi_{...}$ stands for $\varphi_{i_0, \ldots, i_n}$, and the only explicit subscripts indicate the indices which are different from $(i_0, \ldots, i_n)$. We further denoted by $u_j^{s,\text{out}}$ the mutation rates corresponding to the arrows originating at type $s$ in a mutation diagram, see e.g. figure 1. In equation (14), the first term is faithful reproduction, the second term represents all possible mutations, the third term is death, and the last term comes from the probability of no change.

In analogy with equation (2), we define the probability generating function, $\Psi(\xi_0, \ldots, \xi_n; t)$,

$$\Psi(\xi_0, \ldots, \xi_n; t) = \sum_{i_0, \ldots, i_n} \varphi_{i_0, \ldots, i_n} \prod_{s=0}^{n} \xi_s^{i_s}.$$

Let us multiply equation (13) by $\prod_{s=0}^{n} \xi_s^{i_s}$ and sum over all indices to obtain the equation for the generating function. The rule for rewriting various types of terms in terms of the generating function can be summarized as follows:
• Terms with $\varphi_{i_s}$ give $\xi_s \frac{\partial}{\partial \xi_s}$;
• Terms that multiply $\varphi_{i_s-1}(i_s-1)$ give $\xi_s \frac{\partial}{\partial \xi_s}$;
• Terms that multiply $\varphi_{i_s+1}(i_s+1)$ give $\frac{\partial}{\partial \xi_s}$;
• Terms like $\varphi_{i_s-i_{j-1}}$ give $\xi_s \xi_j \frac{\partial}{\partial \xi_s}$;
• Terms with $\varphi_{i_s+1-i_{j-1}}$ give $\xi_j \frac{\partial}{\partial \xi_s}$.

It is convenient to introduce the following shorthand notation,

$$u_{s,\text{out}} = \sum_j u_{s,\text{out}}^j.$$

Then the function $\Psi(\xi_0,\ldots,\xi_n; t)$ satisfies the following first order partial differential equation (a generalization of (3)):

$$\frac{\partial \Psi}{\partial t} = \sum_s \frac{\partial \Psi}{\partial \xi_s} \left[ \xi_s^2 L_s \left(1 - u_{s,\text{out}}\right) + \xi_s L_s \sum_j \xi_j u_{j,\text{out}} - (L_s + d_s) \xi_s \right].$$

(16)

As before, this equation can be solved by the standard method of characteristics. The equations for characteristics are given by:

$$\dot{\xi}_s = L_s \left(1 - u_{s,\text{out}}\right) \xi_s^2 + \left[ L_s \sum_j u_{j,\text{out}} \xi_j - (L_s + d_s) \right] \xi_s + d_s, \quad 0 \leq s \leq n.$$

(17)

Although the methodology outlined here is very general, in this paper we assume the existence of certain symmetries in the coefficients. First we suppose that all the mutation rates are equal, that is, $u_i = u$. Further, we assume that all the types resistant to 1, 2, etc drugs, have equal kinetic parameters. To implement these assumptions and simplify equation (17), we note that all the resistant types can be separated into classes such that in each class $k$, all the types are resistant to exactly $k$ drugs (and susceptible to $m-k$ drugs). For each $k$, the class consists of all variables $\xi_s$ such that the binary numbers $s$ contain exactly $k$ nonzero entries. Therefore, we can denote by $\xi_k$, with $0 \leq k \leq m$, the class of variables describing resistance to $k$ drugs out of $m$. Let us suppose that within each class, the birth and death rates are equal, and also that all mutation rates are equal to each other. Because within each class, the birth and death rates are equal, it does not matter which $k$ out of $m$ drugs the cell is resistant to. The total number of distinct equations in this case is not $n = 2^m + 1$, but $m + 1$. Then, simplifying equation (17), we obtain the following initial value problem:

$$\dot{x}_j = l_j (1 - (m - j)u)x_j^2 + d_j - x_j (l_j + d_j - (m - j)l_j u x_{j+1}), \quad 0 \leq j \leq m,$$

$$x_j(0) = 1 \text{ for } 0 \leq j \leq m - 1, \quad x_m(0) = 0.$$
This is a multi-drug generalization of system (4-6). The probability to have \( m \)-resistant mutants at time \( T \) is given by equation similar to equation (7):

\[
P_{\text{res}}(T) = \frac{1 - \xi_w(T)}{1 - d_w/l_w}.
\]

This equation is used to plot figure 6(c,d) of the main text.

3 Deterministic and stochastic calculations

Here we examine the applicability of simple deterministic calculations and compare them with stochastic results. The mutant dynamics after the start of treatment is described by

\[
y(t) = y_0 e^{(l_m - d_m)t},
\]

where \( y_0 \) is the number of mutants accumulated at start of treatment, and \( l_m - d_m \) is the net growth rate of mutants. To estimate the time until the mutants reach a level \( M_1 \), we assume that the initial number of mutants is given by its mean value, \( M_0 = \langle y_0 \rangle \), and calculate

\[
t_{\text{det}} = \frac{1}{l_m - d_m} \ln \frac{M_1}{M_0} = \frac{1}{l_m - d_m} (\ln M_1 - \ln \langle y_0 \rangle).
\]

(18)

In the stochastic calculation, we are interested in the mean time reaching level \( M_1 \). Then

\[
\langle t \rangle = \frac{1}{l_m - d_m} \left\langle \ln \frac{M_1}{y_0} \right\rangle = \frac{1}{l_m - d_m} (\ln M_1 - \langle \ln y_0 \rangle).
\]

(19)

Comparing the two expressions, we can see that in formula (18), we subtract the logarithm of the (arithmetic) mean of the values \( y_0 \). In formula (19) we subtract the mean logarithm, which is the logarithm of the geometric mean of \( y_0 \). Because the arithmetic mean is larger than the geometric mean, we conclude that the deterministic estimate is an underestimation of the expected time of mutant growth.

Next, we examine the role of stochastic fluctuations. It is known (see e.g. \cite{23}) that the variations of the number of mutants found in a colony of a given size are very large (with the standard deviation that can be orders of magnitude larger than the mean). This means that the knowledge of the mean values of mutant numbers is not very informative. An altogether different picture emerges when we talk about the time of reaching a given level by a mutant colony. It follows that

\[
\text{Var}(t) = \frac{1}{t^2} \left( \langle (\ln y_0)^2 \rangle - \langle y_0 \rangle^2 \right),
\]

that is, the (relative) standard deviation scales with the standard deviation of the logarithm of the mutant population size, which is logarithmically small compared with the standard deviation of the mutant population size itself.
Figure 5 of the main text contains results of stochastic simulations. A fully stochastic simulation becomes computationally very costly because treatment is initiated when the overall number of tumor cells is of the order to $10^{12}$. At the same time, once the cell populations have risen to relatively large values, the trajectory of the stochastic simulation is not significantly different from a deterministic simulation using ordinary differential equations. Hence, to reduce computational time, the stochastic simulation (by using the Gillespie algorithm [24]) was run until the number of drug-resistant mutant cells reached 1000. At this time, the simulation was switched to ordinary differential equations, see equations (9-10). Both neutral and advantageous (in the absence of treatment) resistant mutants were also investigated. Typically, simulations were repeated 5,000 times and the distribution of the outcomes was recorded.

4 Disease progression data analysis and the fitness of resistant mutants

4.1 Mutant fitness analysis

So far we assumed that mutants were neutral with respect to the wild type. Let us now assume that the mutants have an advantage or a disadvantage. Namely, they have a division rate which is increased with respect to the wild type division rate by a certain percentage,

$$t_m = (1 + s) t_w,$$

where the parameter $s$ is negative for disadvantageous mutants and positive for advantageous mutants. We would like to find out what value of $s$ is consistent with the data on resistant mutant generation presented in Section 1.3. Figure 2 shows the data of table 3 with large black circles connected by dashed line, where the percentage of PD is plotted as a function of median treatment time.

Next, we compare these data with simulation results. To do this, we create an artificial “patient” population as described in Section 1.1. For each parameter combination, we first calculate the expected mutant population size, $M_0$, at the beginning of treatment (that is, when the total population reaches size $N$). Then we calculate the time it takes the mutants to reach a certain detection size, $M_1$:

$$t_{det} = \frac{\ln M_1/M_0}{t_m - d_m}.$$

The expected mutant population size $M_0$ is calculated in Section 2.2, formula (8) for neural mutants and formula (12) for advantageous, neutral, or disadvantageous mutants.

The time $t_{det}$ is calculated for 1000 parameter combinations (or artificial “patients”). For each value of the treatment time (the fourth column in table 3), we calculate the percentage of “patients” whose mutant detection time is smaller than the treatment duration. This gives us an estimate of the percentage of
Figure 2: The data in table 3, and examples of calculations for different fitness of mutants in the model. The horizontal axis corresponds to treatment time, and the vertical - to the percentage of patients that show PD. The thick dashed line connects the percentages of PD in different datasets (the rightmost column of table 3). The other lines connect points that represent the model prediction for the percentages of PD under treatment duration of each dataset. The line connecting circles corresponds to neutral mutants in the model. The line connecting squares corresponds to the model with 1.6% advantage for the mutants. The line connecting diamonds corresponds to the model with 3.0% advantage for the mutants. The gray bars under three data points represent the fact that those three points only give an upper bound for the values. The mutant detection threshold is given by $M_1 = 10^{10}$, and $u = 10^{-8}$.

patients with PD for each treatment time. These points are shown in figure 2 for three values of mutant fitness: red squares represent the calculations for neutral mutants ($s = 0$), blue circles - the calculations for mutants with $s = 0.005$, and yellow diamonds - the calculations with $s = 0.01$.

We can see that the assumption of mutant neutrality gives an obvious underestimation of the percentage of PD. On the other hand, the fitness advantage of 3% gives a clear overestimation. To study this more systematically, we varied the fitness advantage from $-10\%$ to $+10\%$, and calculated the mean square error between the calculated values and the data of table 3.

There is one aspect of the data in table 3 that deserves a closer scrutiny. Three of the datasets did not contain any patients with PD; the corresponding data points are denoted by gray vertical bars in figure 2. These datasets are three out of four smallest datasets in table 3, and they can be said to be the least informative of all, because we assume that, given more patients, these studies would have shown some cases of PD. Therefore, it would be misleading to count these points as 0%. Instead, we can say that these three datasets give us an upper bound on the percentages of PD in the three studies, which is calculated as the reciprocal of the number of patients in the study. Because there is some uncertainty on how to account for this in the fitting procedure, we have used three different methods:

1. Include the upper bound value for the three uncertain points as the percentage of PD.
2. Use 1/2 of the upper bound as the percentage of PD.

3. Exclude these three points entirely from the calculations.

While all three methods gave very similar results. Method 1 gives a slight overestimation of fitness advantage.

Figure 3 shows the results of the fitting procedure. We can see on the left panel of the figure that the error is the smallest for disadvantageous and neutral mutants (with possibly a small advantage), and it is significantly larger with strongly disadvantageous mutants and mutants with an advantage more than about 1-2%. As we zoom in (the right panel of figure 3) we can see that the error reaches its minimum at around 1% advantage, that is, for \( s = 0.01 \). In other words, the data are consistent with the model where mutants have a fitness advantage of less than 2%.

Results obtained so far suggest that in order to be consistent with the experimental data, the mutants in the model have to have an advantage or around 1−2%. For the calculations in figure 3(b), the minimum least square error corresponds to \( s = 0.016 \). We have run similar simulations 10 times and obtained the mean estimate for the fitness advantage of mutants to be \( s \cdot 100\% = (1.54 \pm 0.4)\% \). With either value of \( M_1 \), we can see that mutant advantage, if any, is relatively small.

In the next section we will check the robustness of the calculations in figure 3(b).

4.2 Exploring several scenarios for mutant fitness calculations

In the calculations presented in Section 4.1 and in figures 2 and 3 we used artificial populations created as described in Section 1.1, and used this population
Figure 4: The mean square error for different values of mutants advantage in the model. In (a) and (b), the mutant detection threshold is $M_1 = 10^{10}$, and in (c,d) it is $M_1 = 10^9$. In panels (a) and (c), 50 artificial “patients” are created for each value of the mean treatment time. Two typical stochastic runs are presented. In (b) and (d), 1000 artificial patients are created, which stay the same for each treatment duration. In panel (b), the 2nd method of accounting for the least informative data points is employed. The mutation rate is $u = 10^{-8}$. 

\[ M_1 \]
to calculate the percentage of PD for all treatment durations. We also used the detection threshold for mutants of $M_1 = 10^{10}$. Here we explore some alternative methodologies and parameter settings.

In panels 4(a,c) we used a slightly different way to randomize “patient” populations. Instead of creating one population for each fitness value and using it to calculate the percentage of PD for different treatment duration, we created a different artificial population for each treatment duration. This is a little closer to the way data in table 3 were created. Panel 4(a) shows the result of two typical stochastic runs, where for each mean treatment duration, we created 50 patients. We can see that while the exact position of the minimum varies, it is clear that models with strongly advantageous mutants are not compatible with the data, and slightly advantageous mutants minimize the error.

Panel 4(b) presents a different modification with respect to figure 3(b). Here, instead of using method 1 for dealing with the least informative points, we used method 2 (including the upper bound with a factor $1/2$). As we can see, the results are very similar.

Next, we changed the threshold value for mutant detection from $M_1 = 10^{10}$ to $M_1 = 10^9$. The results corresponding to $M_1 = 10^9$ are presented in panels 4(c-d). Panel 4(c) should be directly compared with panel 4(a), and panel 4(d) with panel 3(b). We can see that lowering the mutant threshold size by an order of magnitude is not compatible with resistant mutants having a fitness advantage. In other words, in order to develop a mutant population of size $10^9$ in the timeframe of experiments of table 3, the mutants do not have to possess any fitness advantage.

We can argue however that the value $M_1 = 10^{10}$ is more realistic. In the datasets presented in table 3, there were no specific essays used to find mutant/resistant CLL cells, and only the percentage of patients with PD was measured. Having a PD correlates with the rise of mutant population, which causes a visible increase in the overall CLL population. This means that the population should rise above the plateau. The mean level for the “plateau” level of CLL cells in tissue, $C$, has the order of magnitude of $10^{11}$ cells, see table 2. We can reasonably expect to see an increase in the CLL level if the mutant population reaches about 10% of that amount, which corresponds to $M_1 = 10^{10}$.

Finally, we explore the possibility that not all ibrutinib-resistant mutants are advantageous. Suppose that only fraction $\nu$ of all mutants possesses fitness advantage $s$, and the rest of the mutants are neutral. Figure 5 explores different values of the fraction $\nu$. For each fixed percentage of advantageous mutants, we repeated the simulation of figure 4(d). The four curves of figure 5 correspond to assuming the existence of 100%, 50%, 20%, and 10% mutants of fitness advantage $s$ (the horizontal axis). The result is very intuitive. If we assume that 100% mutants are advantageous, then the fitness advantage that is most consistent with the data is about 1.5% (as reported above). If only 10% of the mutants possess a fitness advantage, it must be proportionally larger, about 15%, as shown by the blue curve of figure 5. Similarly, for intermediate percentages of advantageous mutants, the parameter $s$ seems to vary accordingly, such that the mean advantage of the mutants is about 1.5%.
Figure 5: Same as in figure 3(b), except only a certain fraction, $\nu$, or ibrutinib-resistant mutants is assumed to have fitness advantage. The four curves correspond to $\nu = 1$, $\nu = 0.5$, $\nu = 0.2$, and $\nu = 0.1$.

Figure 6: The influence of the bone marrow correction on the mutant population size, to be compared with Figure 2 of the main text. A histogram of mutant population sizes: (a) at start of treatment, (b) after 300 days of treatment. Plotted are results for a population of artificial “patients of size 1000 (with parameters from table 1 and 2 with a factor of 1.4 correction for the $N$ values and a factor of 2 for the $C$ values). Blue histograms correspond to the assumption that resistant mutants are neutral and dar gray that they have a 1.5% fitness advantage in the absence of treatment. The vertical bar in (b) marks the mean value of the plateau of CLL cells achieved upon treatment. The mutation rate is $10^{-8}$.
Figure 7: The influence of the bone marrow correction on the time-to-resistance calculations, to be compared with Figure 3 of the main text. Numerically obtained probability distribution function for the expected time when a resistant colony reaches detection level, for different combinations of kinetic parameters. We combined colony sizes in the range of table 2 with a 1.4 factor correction, with the parameter pairs $(l_w, d_w)$ in the ranges of table 1; the mutation rate is $10^{-8}$. (a) The probability density function. (b) The cumulative distribution function.

Figure 8: The influence of the bone marrow correction on the time-to-resistance calculations, to be compared with Figure 3 of SI. The mean square error between the % of PD predicted by the model and the data from table 3, as a function of the resistant mutant fitness advantage in the model. Panel (a) shows the overall picture with a plateau for neutral and slightly advantageous mutants, and panel (b) zooms in on the region around zero fitness advantage. The artificial “patient” population size was 1000 in (a) and 1500 in (b), and the second method for accounting the least informative points was used. The rest of the parameters are as in figure 2.
5 The correction resulting from the inclusion of the bone marrow CLL cells

In the previous sections, as well as in the main text, for our statistic we used data from table 2 without the correction resulting from the inclusion of the bone marrow CLL cell numbers. The maximum correction is calculated in section 1.2, and here we present the main calculations of this paper generated with the inclusion of this correction.

In figure 6 we present the histograms of numerically obtained mutant cell numbers at the start of treatment (a), as well as after 300 days of treatment (b), which have to be compared with figure 2 of the main text. In figure 7 we present the numerically calculated PDF (a) and CDF (b) of the time it takes for mutant clones to reach significant numbers. This figure should be compared with figure 3 of the main text. Apart from stochastic variations, there is no noticeable difference between the figures that include the correction and the figures that do not. Finally, figure 8 should be compared with figure 3 of the previous section of SI. This figure demonstrates the calculations of the best-fitting mutant fitness advantage.

References


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