LETTERS

Dynamics of chronic myeloid leukaemia

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The clinical success of the ABL tyrosine kinase inhibitor imatinib in chronic myeloid leukaemia (CML) serves as a model for molecularly targeted therapy of cancer[1–4], but at least two critical questions remain. Can imatinib eradicate leukaemic stem cells? What are the dynamics of relapse due to imatinib resistance, which is caused by mutations in the ABL kinase domain? The precise understanding of how imatinib exerts its therapeutic effect in CML and the ability to measure disease burden by quantitative polymerase chain reaction provide an opportunity to develop a mathematical approach. We find that a four-compartment model, based on the known biology of haematopoietic differentiation[5], can explain the kinetics of the molecular response to imatinib in a 169-patient data set. Successful therapy leads to a biphasic exponential decline of leukaemic cells. The first slope of 0.05 per day represents the turnover rate of differentiated leukaemic cells, while the second slope of 0.008 per day represents the turnover rate of leukaemic progenitors. The model suggests that imatinib is a potent inhibitor of the production of differentiated leukaemic cells, but does not deplete leukaemic stem cells. We calculate the probability of developing imatinib resistance mutations and estimate the time until detection of resistance. Our model provides the first quantitative insights into the *in vivo* kinetics of a human cancer.

Chronic myeloid leukaemia (CML) represents the first human cancer in which molecularly targeted therapy leads to a dramatic clinical response[1–4]. In most patients, however, the rapid decline of the leukaemic cell burden induced by the ABL tyrosine kinase inhibitor imatinib fails to eliminate residual disease. Bone marrow studies have shown that the residual cells are part of the leukaemic stem cell compartment[6,7]. This observation raises the question of whether imatinib is capable of impairing the proliferation of leukaemic stem cells. Moreover, a substantial fraction of patients develops acquired resistance to imatinib. Mutations in the ABL kinase domain are the main mechanism of resistance and account for 70–80% of cases with treatment failure[8–13]. Sometimes, resistance mutations are present in leukaemic cells prior to imatinib therapy[13–15].

We design a mathematical model which describes four layers of the differentiation hierarchy of the haematopoietic system (see Methods and Supplementary Information). Stem cells give rise to progenitors, which produce differentiated cells, which produce terminally differentiated cells. This hierarchy applies both to normal and leukaemic cells. Therefore, the leukaemic cell population consists of leukaemic stem cells and three types of leukaemic differentiated cells; only leukaemic stem cells have an indefinite potential for self-renewal. The *BCR-ABL* oncogene is present in all leukaemic cells. It leads to a slow clonal expansion of leukaemic stem cells and accelerates the rate at which these cells produce leukaemic progenitors and differentiated cells.

We analysed 169 CML patients. The levels of BCR-ABL transcripts in the blood of the patients is measured by a quantitative real-time PCR (RQ-PCR) assay[10,16]. *BCR* is used as the control gene and BCR-ABL values are expressed as a percentage of the BCR transcript levels to compensate for variations in the RNA quality and efficiency of reverse transcription. Because the blood predominantly contains terminally differentiated cells, the obtained values give an estimate of the fraction of terminally differentiated leukaemic cells.

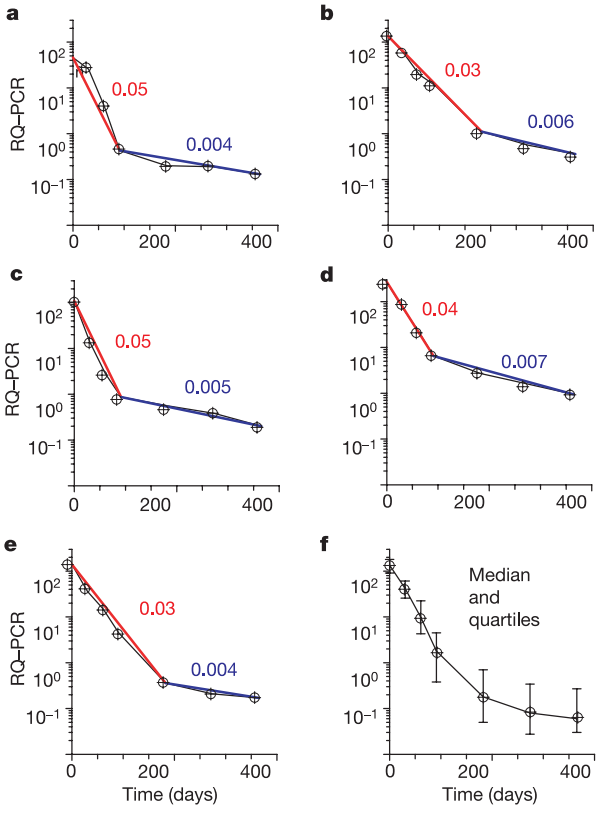

Figure 1 | Imatinib leads to a biphasic decline of leukaemic cells. a–e, The levels of BCR-ABL transcripts in the blood of five patients are shown during 12 months of therapy starting at day 0. In these patients, the first slope ranges from 0.03 to 0.05 per day and the second slope from 0.004 to 0.007 per day. The first slope represents the death rate of leukaemic differentiated cells and the second slope the death rate of leukaemic progenitors during imatinib therapy. Panel **f** shows the median with quartiles taken over all patients who do not have a rise in the leukaemic cell burden during the first 12 months of therapy. The circle represents the 50 percentile, and the bars the 25 and 75 percentiles.

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Successful therapy leads to a biphasic exponential decline of leukaemic cells (Fig. 1). The first slope is determined by calculating the exponential decline between 0 and 3 months; a mean value of 0.05 ± 0.02 per day is obtained, which corresponds to a decline of 5% per day. The second slope is determined by calculating the exponential decline between 6 and 12 months; a mean value of 0.008 ± 0.004 per day is obtained, which corresponds to a decline of 0.8% per day. This analysis considers only patients who do not have any rise in the leukaemic cell burden during the first 12 months of therapy, in order to exclude the effect of acquired resistance.

Fitting our model to the data, we conclude that the first slope represents the turnover rate of differentiated leukaemic cells. Therefore, these cells have an average lifespan of 1/0.05 = 20 days. Upon reaching a steady state with the leukaemic progenitors, the number of differentiated leukaemic cells decreases at the same rate as the number of leukaemic progenitors. The second slope represents the turnover rate of leukaemic progenitors. Hence, these cells have an average lifespan of 1/0.008 = 125 days. Both estimates denote average lifespans during imatinib therapy. Any process that removes cells from the corresponding subpopulation (including further differentiation) contributes to the average lifespan.

The first slope leads to an approximately 1,000-fold decline in the leukaemic cell burden. Therefore, imatinib reduces the rate at which leukaemic differentiated cells arise from leukaemic progenitors about 1,000-fold. This effect is as if imatinib prevented about ten rounds of cell division of leukaemic cells ($2^{10} = 1{,}024$), either by increasing their death rate or by reducing their division rate.

Some patients ceased imatinib therapy because of complications or side effects (Fig. 2). Even if imatinib had been administered for many months (up to three years), the numbers of BCR-ABL transcripts in those patients rose within three months after discontinuation of therapy to levels at pre-treatment baseline or above. Other studies of patients who ceased imatinib led to similar findings[17,18]. We conclude that long-term imatinib treatment does not deplete the cell population that drives this disease. This conclusion is consistent with the hypothesis that leukaemic stem cells are insensitive to chemotherapy[19–21]. The average rate of the exponential increase after stopping treatment is 0.09 ± 0.05 per day, corresponding to a doubling time of 8 days. This timescale indicates the rate at which terminally differentiated leukaemic cells arise from leukaemic stem cells in the absence of imatinib.

A comparison between model and data suggests the following two concepts. (1) Imatinib treatment leads to a competitive disadvantage of leukaemic progenitors and leukaemic differentiated cells; their production rates are dramatically reduced. The consequence is a biphasic decline of the abundance of the BCR-ABL transcript in response to therapy. (2) Leukaemic stem cells, however, are not depleted during imatinib treatment. The total leukaemic cell burden rapidly returns to the baseline value (or beyond) when imatinib is discontinued.

Acquired imatinib resistance usually develops owing to mutations in the ABL kinase domain[8–13]. Resistant leukaemic cells emerge after an initially successful response to imatinib therapy and lead to a relapse of disease (Fig. 3). The average slope was determined by calculating the exponential increase after the first appearance of resistance mutations in 30 patients; a value of 0.02 ± 0.01 per day was obtained. Of those patients who start imatinib in the early chronic, late chronic, and accelerated phase of CML, respectively, 12%, 32%, and 62% develop detectable resistance mutations within two years of treatment[22].

Our basic model can be extended to include the stochastic evolution of resistance. Consider an exponentially growing leukaemia continually producing resistant cells at rate u per cell division. The probability of having resistance mutations once leukaemic stem cells have reached a certain abundance, y_0, is given by $P = 1 - \exp(-uy_0\sigma)$ where $\sigma = (1 + s)\log(1 + 1/s)$. The parameter s denotes the excess reproductive ratio of the exponentially growing leukaemia, which is the relative difference between birth and death rates. This calculation assumes that resistance mutations are neutral prior to therapy.

If the point mutation rate is about 10^{-8} per base per cell division[23], then resistance due to any one of about forty known mutations[7–14]

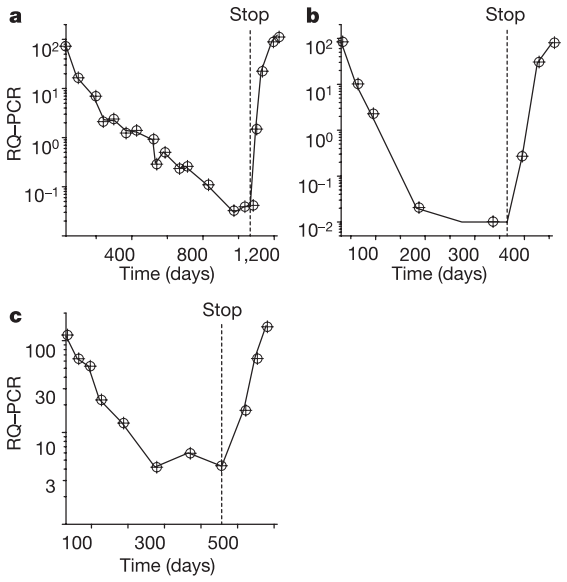

Figure 2 | Discontinuation of imatinib therapy in three patients after 1–3 years led to a rapid increase of leukaemic cells to levels at or beyond pre-treatment baseline. We conclude that leukaemic stem cells, which drive CML disease, are not depleted by imatinib therapy. The rapid upslope of 0.09 ± 0.05 per day corresponds to a doubling time of roughly 8 days, which characterizes the rate at which differentiated leukaemic cells are regenerated from leukemic stem cells. Each of panels **a**, **b** and **c** corresponds to one of the three patients.

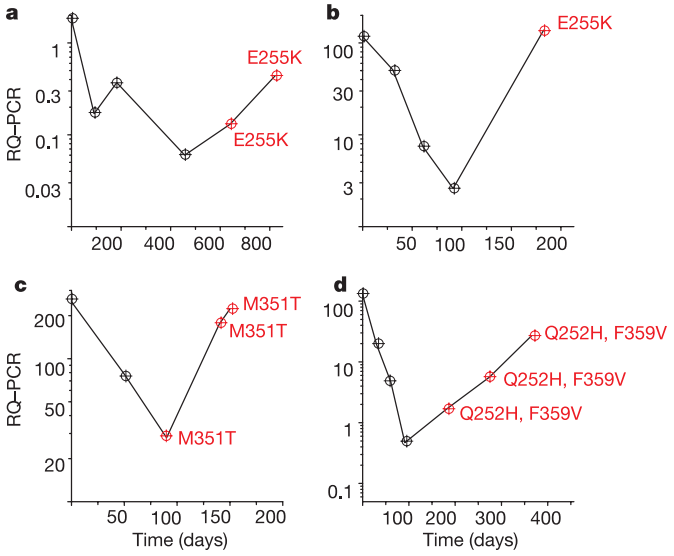

Figure 3 | About 40 different point mutations in BCR-ABL have been identified that confer various degrees of resistance to imatinib therapy. **a–d**, We show evolutionary dynamics of resistance in four patients. The labels denote the individual mutations that are detected at various time points. Resistance mutations lead to a relapse of leukaemic cells. The upslope ranges from 0.003 to 0.06 per day in 30 patients, with an average of 0.02 ± 0.01 per day. The characteristic timescale for the rise of resistance is given by the rate at which resistant leukaemic stem cells expand during therapy.

arises at rate $u = 4 \times 10^{-7}$ per cell division. The abundance of leukaemic stem cells at diagnosis in early chronic phase[24,25] is estimated to be about $y_0 = 2.5 \times 10^5$. For these values and $s = 1$, we calculate that about 13% of patients harbour resistance mutations at the time of diagnosis. If imatinib therapy commences at a later stage of the disease, when the leukaemic stem cell population has expanded to 10^7 cells, for example, then 100% of those patients have some resistant leukaemic stem cells. Therefore, the higher incidence of resistance in patients who start imatinib therapy in a later phase of disease can be explained by an increased leukaemic stem cell burden.

We expect that the frequency of resistance mutations at the start of imatinib therapy is below detection limit in most patients. We can use the deterministic model to calculate the time until detection of resistance mutations and treatment failure (see Supplementary Information). In our model, the characteristic timescale is given by the rate at which the resistant leukaemic stem cells are expanding during therapy. Therefore, a faster-growing leukaemia leads to an earlier emergence of resistance, while a slower-growing leukaemia might allow many years of successful therapy, even if resistant cells are present at low frequencies. The reason for this unusual behaviour is that leukaemic stem cells, with or without resistance mutations, continue to expand during treatment. Imatinib acts by reducing the abundance of leukaemic progenitors, differentiated and terminally differentiated cells without resistance mutations.

There is evidence that some resistance mutations confer a growth advantage even in the absence of imatinib[8,13]. These 'advantageous mutations' have a higher probability of being present in patients prior to treatment and can lead to a more rapid expansion during therapy.

Figure 4 summarizes the dynamical features of our mathematical model. Normal haematopoietic cells are in a steady state. Leukaemic stem cells expand exponentially at a slow rate. Imatinib reduces the rate at which leukaemic stem cells produce progenitors. Hence, the abundance of leukaemic progenitors declines once treatment is started. Similarly imatinib reduces the rate at which leukaemic progenitors produce differentiated cells. The abundance of differentiated leukaemic cells shows a biphasic decline. The first slope is determined by the average lifespan of leukaemic differentiated cells (20 days), while the second slope reflects the longer lifespan of leukaemic progenitors (125 days). We assume that imatinib does not affect the rate at which leukaemic differentiated cells produce terminally differentiated cells. This cell population has a fast turnover rate—on a timescale of one day—and simply tracks the biphasic decline of leukaemic differentiated cells. If imatinib is discontinued, there is a rapid resurgence of the leukaemic load because the cell population which drives the disease, the leukaemic stem cells, was not depleted during therapy. Resistant leukaemic stem cells might expand faster than leukaemic stem cells during therapy for two reasons: (1) either they have an inherent selective advantage; or (2) imatinib somewhat reduces the growth rate of leukaemic stem cells without depleting them. In any case, resistant leukaemic stem cells continue to produce large amounts of progenitors and differentiated cells during therapy. The total leukaemic cell burden declines initially, but rises again once resistant differentiated cells become abundant. In this case, the time to treatment failure is determined by the rate of expansion of resistant leukaemic stem cells.

Finding a cellular mechanism for drug resistance of leukaemic stem cells is a very important goal for future experimental research. Imatinib is a substrate for the multidrug resistance protein MDR p-glycoprotein and will therefore be excluded from cells that express significant MDR levels[26]. Stem cells naturally express higher levels of MDR[27]. It is unknown whether this is the actual mechanism for sparing leukaemic stem cells, because it has not been possible neither to measure accurately the imatinib concentration in leukaemic stem cells nor to measure BCR-ABL inhibition selectively in this compartment. Another possibility is that leukaemic stem cells are less dependent on BCR-ABL for growth and survival than are committed progenitors, and therefore BCR-ABL inhibition does not eliminate leukaemic stem cells. Indeed, there is evidence that BCR-ABL messenger RNA, but not protein, can be detected in a progenitor cell population[28].

In cell-culture systems using CML cell lines or murine haematopoietic cells transformed by BCR-ABL, imatinib leads to rapid inhibition of ABL kinase activity and subsequent induction of apoptotic cell death (on a timescale of hours). This very fast response to imatinib is in stark contrast to the 20 and 125 day half-lives we find in our *in vivo* data. This discrepancy might be explained by the fact that *in vitro* model systems are derived from (or resemble) blast crisis cells, wherease our data refer to chronic-phase CML. Since there are no *in vitro* models of chronic-phase CML, we cannot make a direct comparison. Curiously, the rate of clearance of leukaemic blasts from the blood of CML blast crisis patients is rapid (3–7 days) and may reflect increased dependence of blasts on the action of the *BCR-ABL* oncogene. In contrast, leukaemic cells from chronic-phase patients are not as dependent on the BCR-ABL signal and consequently do not undergo rapid apoptosis. Our analysis suggests that in chronic-phase

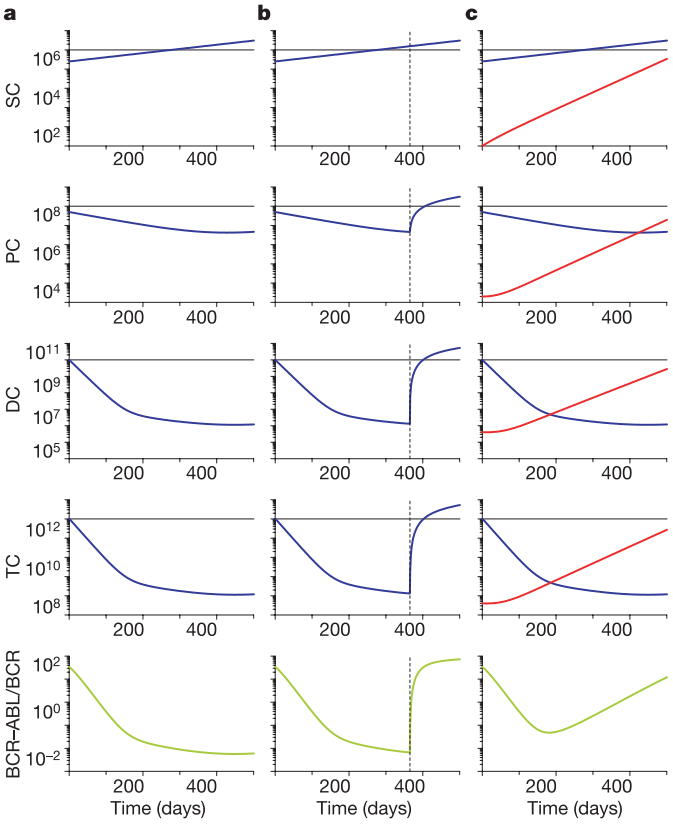

Figure 4 | Model dynamics of different treatment responses to imatinib. **a**, Without resistance mutations; **b**, when therapy is stopped after one year; and **c**, with resistance mutations. The rows show stem cells (SC), progenitor cells (PC), differentiated cells (DC), and terminally differentiated cells (TC); wild type cells are black, leukaemic cells blue, and resistant leukaemic cells red. The bottom row shows the ratio of BCR-ABL over BCR in % (green). **a**, Imatinib therapy is started at day 0. Treatment leads to a biphasic decline of the BCR-ABL/BCR ratio. Leukaemic stem cells continue to expand at a slow rate. **b**, Discontinuation of treatment (broken line) leads to a rapid rise of leukaemic cells to levels above the pre-treatment baseline, because leukaemic stem cells have not been depleted during therapy. **c**, Emergence of resistance mutations leads to an increase of the BCR-ABL/BCR ratio at a rate which is determined by the rise of resistant leukaemic stem cells. Parameter values are $d_0 = 0.003$, $d_1 = 0.008$, $d_2 = 0.05$, $d_3 = 1$, $a_x = 0.8$, $b_x = 5$, $c_x = 100$, $r_y = 0.008$, $a_y = 2a_x$, $b_y = 2b_x$, $c_y = c_x$, $r_z = 0.023$. During therapy we have $a_y' = a_y/100$, $b_y' = b_y/750$, $c_y' = c_y$, $a_z = a_z' = a_y$, $b_z = b_z' = b_y$, and $c_z = c_z' = c_y$. In **c**, we have $z_0(0) = 10$ and $u = 4 \times 10^{-8}$.

CML the mode of action of imatinib is to reduce the rate at which differentiated leukaemic cells are produced from progenitors and at which progenitors are produced from leukaemic stem cells. Hence, the *in vivo* action does not rely on massive and sudden apoptosis, but on a decline in the net proliferation potential of leukaemic cells.

We conclude that the comparison between model and data provides quantitative insights into the *in vivo* kinetics of CML. We obtain numerical estimates for the turnover rates of leukaemic progenitors and differentiated cells. Imatinib dramatically reduces the rate at which those cells are being produced from leukaemic stem cells, but it does not lead to an observable decline of leukaemic stem cells. The probability of harbouring resistance mutations increases with disease progression as a consequence of an increased leukaemic stem cell abundance. The characteristic time to treatment failure caused by acquired resistance is given by the growth rate of the leukaemic stem cells. Thus, multiple drug therapy[29] is especially important for patients who are diagnosed with advanced and rapidly growing disease.

METHODS

The basic mathematical model. The abundances of normal haematopoietic stem cells, progenitors, differentiated cells, and terminally differentiated cells are denoted x_0, x_1, x_2, and x_3. Their respective leukaemic abundances are given by y_0, y_1, y_2 and y_3 for cells without resistance mutations and by z_0, z_1, z_2 and z_3 for cells with resistance mutations. Stem cells produce progenitors, which produce differentiated cells, which produce terminally differentiated cells. The rate constants are given by a, b and c with appropriate indices distinguishing between healthy, leukaemic and resistant cell lineages. The death rates of stem cells, progenitors, differentiated and terminally differentiated cells are denoted by d_0, d_1, d_2 and d_3. Homeostasis of normal stem cells is achieved by an appropriate declining function, λ. Leukaemic stem cells divide at rate r_y, and resistant stem cells divide at rate r_z. The basic mathematical model is given by:

$$\dot{x}_0 = [\lambda(x_0) - d_0]x_0 \quad \dot{y}_0 = [r_y(1-u) - d_0]y_0 \quad \dot{z}_0 = (r_z - d_0)z_0 + r_y y_0 u$$

$$\dot{x}_1 = a_x x_0 - d_1 x_1 \qquad \dot{y}_1 = a_y y_0 - d_1 y_1 \qquad \dot{z}_1 = a_z z_0 - d_1 z_1$$

$$\dot{x}_2 = b_x x_1 - d_2 x_2 \qquad \dot{y}_2 = b_y y_1 - d_2 y_2 \qquad \dot{z}_2 = b_z z_1 - d_2 z_2$$

$$\dot{x}_3 = c_x x_2 - d_3 x_3 \qquad \dot{y}_3 = c_y y_2 - d_3 y_3 \qquad \dot{z}_3 = c_z z_2 - d_3 z_3$$

For further discussion and analysis of the model, see Supplementary Information.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Dynamics of Chronic Myeloid Leukemia
Supplementary Online Material

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The basic model. Our mathematical model is based on the architecture of the hematopoietic system as proposed by Irving Weissman and colleagues[1,2]. Denote by x_0, x_1, x_2, and x_3 the abundances of normal hematopoietic stem cells, progenitors, differentiated cells, and terminally differentiated cells. Their respective leukemic abundances are given by y_0, y_1, y_2, and y_3 for cells without resistance mutations and by z_0, z_1, z_2, and z_3 for cells with imatinib resistance mutations. We assume that normal hematopoietic stem cells are held at a constant level by a homeostatic mechanism. The growing leukemic cell population might eventually upset this homeostasis, but we do not consider this effect here. Leukemic stem cells escape from the homeostatic control and grow at a slow pace; it takes about 6-7 years from the first occurrence of the BCR-ABL oncogene to detection of disease[3]. If imatinib resistance mutations are neutral in the absence of treatment, then leukemic stem cells with these mutations grow at the same rate as the other leukemic stem cells. It is conceivable that some resistance mutations confer a selective advantage or disadvantage leading to different expansion rates of resistant clones. We assume, for simplicity, that the leukemia follows an exponential growth law; other dynamics are possible and need separate investigation.

Stem cells produce progenitors which produce differentiated cells which produce terminally differentiated cells. The rate constants are given by a, b, and c with appropriate indices distinguishing between healthy, leukemic, and resistant cell lineages. The current understanding is that the BCR-ABL oncogene increases the rate at which leukemic stem cells proliferate and differentiate into progenitors. Furthermore, the BCR-ABL oncogene is thought to increase the rate at which progenitors proliferate and turn into differentiated leukemic cells. Therefore, we expect that $a_y > a_x$ and $b_y > b_x$. Imatinib counteracts this effect and reduces the rates to $a'_y < a_y$ and $b'_y < b_y$. The death rates of stem cells, progenitors, differentiated, and terminally differentiated cells are denoted by d_0, d_1, d_2, and d_3. The 'death' rates can include further differentiation of some cell types. Progenitors, differentiated, and terminally differentiated cells have a limited potential for cell division. They divide a certain number of times before they differentiate further or undergo apop-

tosis. The actual dynamics of this process is more complicated than described in our simple model, but the essential features are captured: we assume that a stem cell leads to a progenitor lineage that divides i times thereby generating 2^i cells. The number 2^i is embedded in the rate constant a. Equally, a progenitor leads to a differentiated cell that generates 2^j cells, and this number is embedded in the rate constant b. Imatinib reduces the rate constants a and b, thereby preventing a certain number of cell divisions in each stage.

With these assumptions, we have the following system of differential equations:

$$\begin{aligned}
\dot{x}_0 &= [\lambda(x_0) - d_0]x_0 & \dot{y}_0 &= [r_y(1-u) - d_0]y_0 & \dot{z}_0 &= (r_z - d_0)z_0 + r_y y_0 u \\
\dot{x}_1 &= a_x x_0 - d_1 x_1 & \dot{y}_1 &= a_y y_0 - d_1 y_1 & \dot{z}_1 &= a_z z_0 - d_1 z_1 \\
\dot{x}_2 &= b_x x_1 - d_2 x_2 & \dot{y}_2 &= b_y y_1 - d_2 y_2 & \dot{z}_2 &= b_z z_1 - d_2 z_2 \\
\dot{x}_3 &= c_x x_2 - d_3 x_3 & \dot{y}_3 &= c_y y_2 - d_3 y_3 & \dot{z}_3 &= c_z z_2 - d_3 z_3
\end{aligned}$$

Homeostasis of normal stem cells is achieved by an appropriate declining function, λ. Normal cells remain at their equilibrium values, x_0, $x_1 = a_x x_0/d_1$, $x_2 = b_x x_1/d_2$, and $x_3 = c_x x_2/d_3$. At the start of therapy, leukemic cells are in steady state ratios with their precursors: $y_3 = c_y y_2/d_3$, $y_2 = b_y y_1/d_2$, and $y_1 = a_y y_0/d_1$. The leukemic stem cell population expands as $y_0(t) = \exp[(r_y - d_0)t]$ (ignoring resistance mutations). Imatinib dramatically reduces the rate constants, a_y to a'_y and b_y to b'_y. This leads to a bi-phasic decline. The first slope describes the exponential decline of differentiated leukemic cells to their new steady state, $y_2 = b'_y y_1/d_2$. The magnitude of this decline is around 1000-fold, suggesting that b'_y is 1000 times smaller than b_y. Thus, imatinib prevents about 10 rounds of cell division of differentiated leukemic cells. Note that $2^{10} = 1024$. The exponential decline is given by $\exp(-d_2 t)$. The observed slope establishes that the turnover rate of differentiated leukemic cells in the presence of imatinib is $d_2 = 0.05$ per day. Hence the average life-time of these cells is about $1/d_2 = 20$ days. After about 200 days of therapy, differentiated leukemic cells have reached their new steady state with leukemic progenitors and follow their decline that is given by $\exp(-d_1 t)$. The data suggest that the turnover rate of leukemic progenitors is $d_1 = 0.008$ per day corresponding to an average life-time of about $1/d_1 = 125$ days. A minimum estimate of the magnitude of this decline is about 7-fold, suggesting that a'_y is at the very least 7 times smaller than a_y. The overall 5000-fold decline is the combined effect of a'_y and b'_y.

We have assumed that imatinib primarily acts by reducing the proliferation rate of

leukemic cells, but it is in principle possible that imatinib also increases the death rates of those cells. In this case, we have to assign different death rates to the x, y and z populations, but all our conclusions remain the same; the numerical estimates for the decay slopes refer to the death rates of the corresponding cells during imatinib therapy. 'Death' includes any process that removes cells from the relevant subpopulation and does include cellular differentiation. It is also conceivable that the BCR-ABL oncogene changes the death rates of leukemic cells. In this case again, the observed slopes indicate the turnover rates of leukemic cells during imatinib therapy. Should BCR-ABL and imatinib, however, not affect the death rates of these cells, then our results apply to the turnover rates of healthy hematopoietic cells, too.

If therapy is interrupted, the model predicts an explosive recurrence of cancer cells. Since imatinib leads to an at least 5000-fold reduction of the production of differentiated leukemic cells, stopping therapy leads to a sudden 5000-fold increase of differentiated leukemic cells at the time scale of their cell division. If imatinib led to a decline in leukemic stem cells, then the rebound after therapy should be to a level below baseline (by whatever amount leukemic stem cells have declined during therapy). If imatinib does not lead to a decline of leukemic stem cells, the rebound should lead to baseline levels or beyond. The latter is observed in all three patients who stopped therapy (Fig. 2). Once terminally differentiated leukemic cells reach their new steady state, the further increase of the disease burden follows the characteristic time scale of leukemic stem cell expansion.

For comparison with the experimental PCR data, we calculate the BCR-ABL to BCR ratio as $(y_3 + z_3)/(2x_3 + y_3 + z_3)$ times 100%. A healthy cell has two copies of BCR. A leukemic cell normally has one copy of BCR and one copy of BCR-ABL. Most cells that are sampled by the PCR assay are terminally differentiated cells.

There are several other theoretical investigations of CML that analyze various aspects of the disease[4−7].

The probability of resistance mutations. Table 1 shows the observed percentage of patients with acquired resistance mutations during the first and second year of treatment. In 5.9% of early chronic phase patients, resistant leukemic cells are detected within the first year of treatment; in 12%, resistant leukemic cells are detected within the first two years of therapy. These numbers increase to 14% and 32% in late chronic phase patients and to 38% and 62% in accelerated phase patients. 'Early chronic phase' refers to patients who commenced imatinib within one year of diagnosis.

We use a continuous-time branching process to calculate the probability that a patient has resistance mutations at the beginning of imatinib therapy. Assume that resistance mutations are neutral prior to therapy: this means mutated and unmutated stem cells expand at the same rate in the absence of imatinib. Leukemic stem cells, y_0, follow a branching process starting with a single cell at time $t = 0$. They go extinct with probability d_0/a_y and grow exponentially to give rise to leukemia with probability $1-d_0/a_y$. Resistant mutants are produced from normal leukemic cells with probability u per cell division. They reproduce and die at the same rate as normal leukemic cells. For the probability of having resistance mutations once the stem cell population has reached a certain size, y_0, we obtain $P = 1 - \exp(-uy_0\sigma)$. Here $\sigma = (1 + s)\log(1 + 1/s)$, where $s = (a_y - d_0)/d_0$ denotes the excess reproductive ratio of leukemic stem cells. For a wide range of plausible values of s, there is only little variation in σ: if s changes from 0.1 to infinity, then σ changes from 2.64 to 1.

We expect that patients who are diagnosed at later stages of CML disease tend to have a larger population size, y_0, of leukemic stem cells and consequently a higher chance of harboring resistance mutations at the time when therapy is started (Table 2). In addition, these patients might already have a more aggressive, faster growing leukemia and therefore the time to detection of resistance and treatment failure can be shortened (Table 3). Finally, especially in blast crisis it is conceivable that the mutation rate of leukemic cells is increased, which could also contribute to a higher incidence of resistance mutations.

RQ-PCR measurements. BCR-ABL transcript levels are determined by a quantitative real-time polymerase-chain-reaction (RQ-PCR) assay[8,9]. BCR is used as the control gene. BCR-ABL values are expressed as percentage of the BCR transcript levels to compensate for variations in the RNA quality and efficiency of reverse transcription. Every leukemic cell has one copy of the normal BCR gene and at least one copy of BCR-ABL. The values sometimes exceed 100%because BCR-ABL expression can be upregulated in leukemic cells.

The PCR measurement determines the fraction of leukemic cells among all terminally differentiated cells in the blood because peripheral blood predominantly contains such terminally differentiated cells. The standard method to estimate the level of leukemic cells in CML patients is conventional cytogenetic analysis of bone marrow metaphases. The quantitative PCR method for measuring BCR-ABL levels in blood correlates closely with the cytogenetic assessment of leukemic cells in the bone marrow and is an accurate and

reliable method for measuring the leukemic burden[9−14].

In total we have analyzed data for 169 patients, but 46 of those patients were studied for resistance and treatment failure. Of the remaining 123 patients, 68 patients had enough data points and never showed an increase in leukemic cell count during the first 12 months of therapy. We have chosen these 68 patients to calculate the bi-phasic decline slopes under successful therapy attempting to exclude the confounding effects of acquired resistance. Figure 5 shows the molecular response to imatinib in these 68 patients.

The first slope is determined by calculating the exponential decline between 0 and 3 months; a mean value of 0.05 ± 0.02 per day is obtained, which corresponds to a decline of 5% per day. The second slope is determined by calculating the exponential decline between 6 and 12 months; a mean value of 0.008 ± 0.004 per day is obtained, which corresponds to a decline of 0.8% per day. Figure 1f shows the median with quartiles as calculated from all patients shown here.

Oncogene addiction. There is a fundamental unanswered question at the heart of molecularly targeted cancer therapy: if drugs impair the action of an oncogene and thereby reduce the growth of tumor cells, then why do these tumor cells have a competitive disadvantage relative to normal cells? The term 'oncogene addiction' has been coined to describe the phenomenon of tumor cells becoming dependent on a single molecular driver, but its cellular basis is unknown[15]. Most hypotheses are focused on the notion that tumor cells lose the redundant signaling machinery that normal cells retain, but this remains to be proven.

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Figure legends.

Figure 5: Molecular response to imatinib. The figure shows the leukemic cell load in all patients used to calculate the bi-phasic decline. This analysis excludes patients who had a rise in their leukemic burden during the first twelve months of therapy. This approach attempts to exclude the effect of acquired resistance mutations.

Table 1: Observed percentage of patients with acquired resistance mutations during the first and second year of treatment. In 5.9% of early chronic phase patients, resistant leukemic cells are detected within the first year of treatment; in 12%, resistant leukemic cells are detected within the first two years of therapy. These numbers increase to 14% and 32% in late chronic phase patients and to 38% and 62% in accelerated phase patients. 'Early chronic phase' refers to patients who commenced imatinib within one year of diagnosis.

Table 2: Predicted percentage of patients which harbor resistance mutations depending on the abundance of leukemic stem cells, y_0, and the mutation rate conferring resistance, u. Resistant leukemic cells can be below detection limit in some patients. We use $s = 1$.

Table 3: Predicted time until detection of resistance mutations ($y_3 = z_3$) and until treatment failure ($z_3 = 10^{12}$) in dependence of the abundance of resistant stem cells at the start of therapy, $z_0(0)$. Parameter values are $d_0 = 0.003$, $d_1 = 0.008$, $d_2 = 0.05$, $d_3 = 1$, $a_x = 0.8$, $b_x = 5$, $c_x = 100$, $r_y = 0.008$, $a_y = 2a_x$, $b_y = 2b_x$, $c_y = c_x$, $r_z = 0.023$, $a'_y = a_y/100$, $b'_y = b_y/750$, $c'_y = c_y$, $a_z = a'_z = a_y$, $b_z = b'_z = b_y$, $c_z = c'_z = c_y$, and $u = 4 \cdot 10^{-8}$.

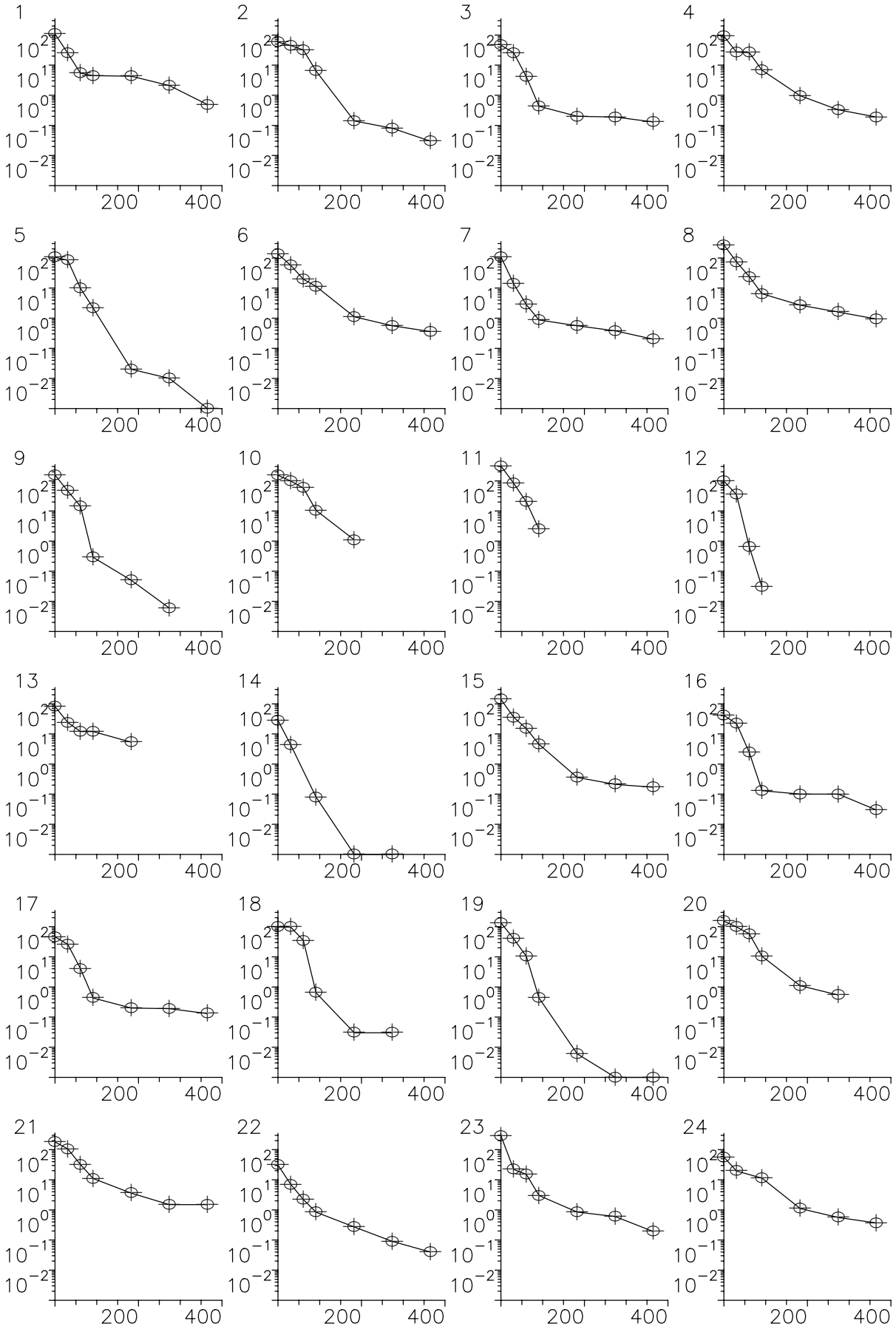
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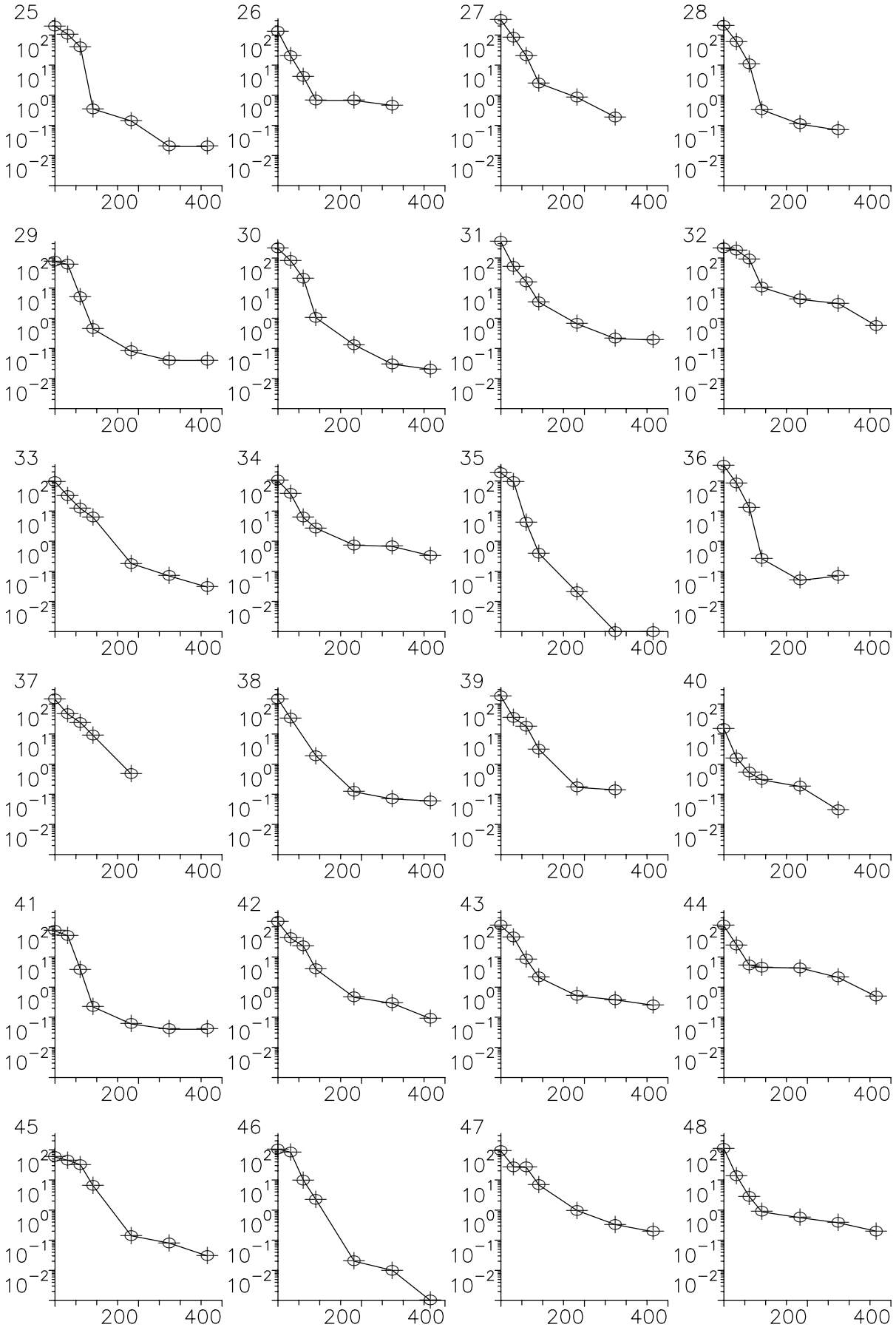
Figure 5 contd.



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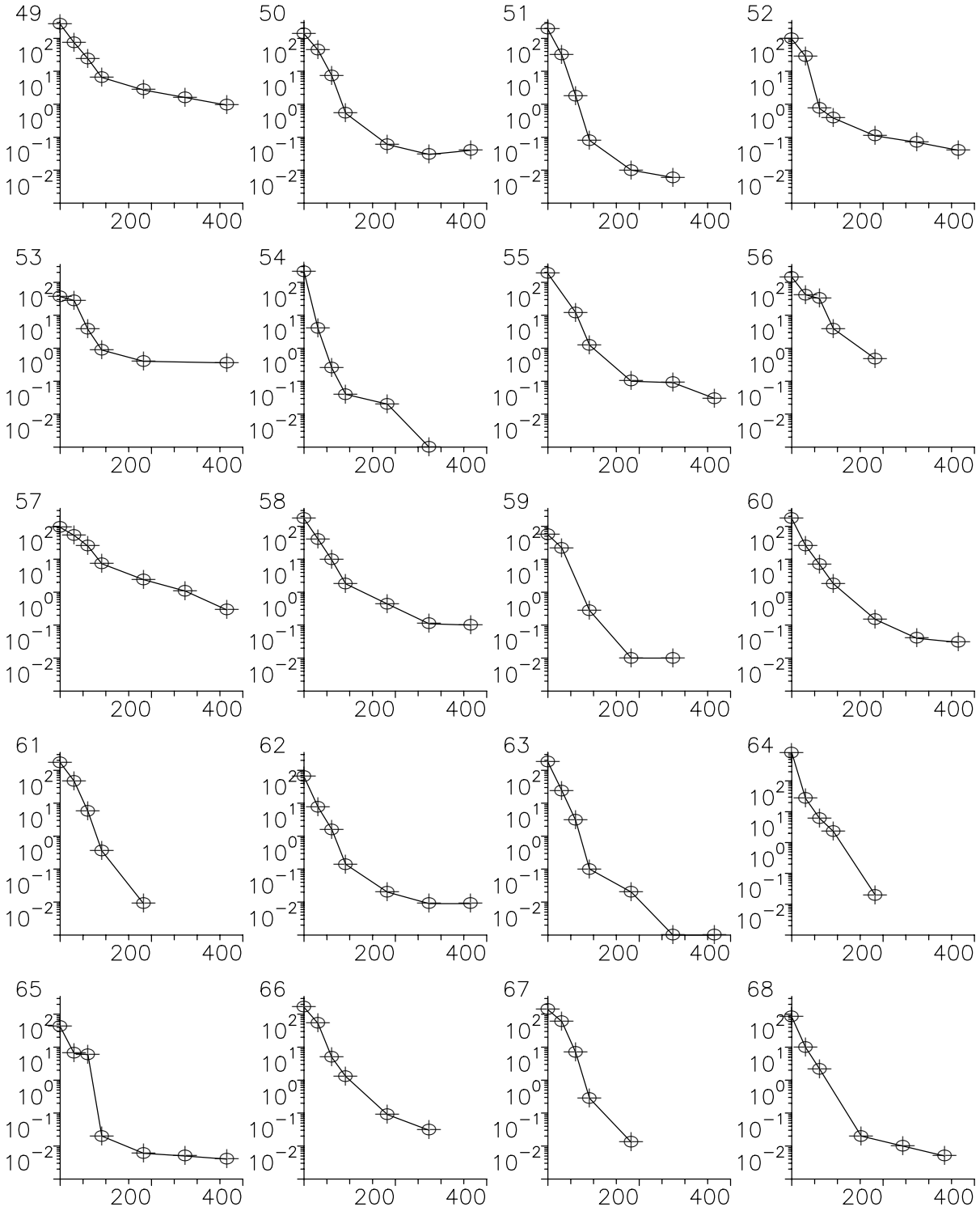

Table 1

Observed incidence of imatinib resistance

	early chronic phase	late chronic phase	accelerated phase
1 year	5.9%	14%	38%
2 years	12%	32%	62%

Table 2

Predicted fraction of patients with resistance mutations

u \ y_0	10^5	10^6	10^7	10^8
$4 \cdot 10^{-7}$	5.4%	43%	100%	100%
$4 \cdot 10^{-8}$	0.6%	5.4%	43%	100%

Predicted time to detection of resistance and treatment failure

$z_0(0)$	detection	failure
10	186 d	566 d
100	141 d	470 d
1000	101 d	357 d
10 000	61 d	241 d
100 000	9.2 d	119 d

— on the glass, except the spreading is much quicker owing to the energy of the falling drop.

This is a spectacular finding because it unambiguously demonstrates the effect of air in the splashing story. And it might also have practical consequences. The pressure reduction required to suppress the splash is modest — the air is only as rarefied as that on top of Everest. Such a partial vacuum is easily generated, providing a simple and efficient way of suppressing the emission of droplets that limits the resolution in ink-jet printing, for example. Moreover, an understanding of the role of air might also help to enhance the generation of drops in situations where it is desired, as in combustion or the production of sprays.

Xu and colleagues[1] have a surprising proposition to explain their results. As a liquid drop approaches a solid surface, the liquid can either displace or compress the air. Based on the rapid initial spreading of the liquid on the surface (the radius of the spreading disk increases as the square root of time), the authors suggest that the liquid front compresses the air. At a standard pressure, the gas resists compression, forcing the liquid edge to lift, after which this free liquid sheet cannot avoid breaking. The corresponding force (per unit area) is proportional to the gas density, speed of sound (resulting from air compression) and liquid velocity. Surface tension, which maintains the liquid cohesion, opposes this destabilizing force. Splashing occurs when the two forces are equal, and so is reduced as the gas density vanishes.

This subtle argument needs to be confirmed, because it assumes a speed on the order of the speed of sound, which is only true in the first fraction of a microsecond after impact (a much shorter timescale than observed experimentally). But it also allows us to understand why different gases behave differently; Xu *et al.* observed that splashing occurs more easily with a heavier gas (such as krypton or sulphur hexafluoride), showing an unexpected way to tune a splash.

The idea that air can be used to control the behaviour of a drop can be exploited for other purposes. A drop deposited on a pool of the same liquid will coalesce with that pool, but only after the film of air between the drop and the surface has disappeared. This typically takes a tenth of a second — a time hardly appreciable to the human eye. But if one finds a trick to renew this film, coalescence can be inhibited. Couder and colleagues[4] have applied such a trick. Vibrating the pool at a sufficiently high frequency prevents coalescence 'for ever' (more than three days). The drop simply bounces on the surface of the pool, provided that the acceleration of the vibrating bath is at least the same as the acceleration due to gravity (9.8 m s^{-2}). The impact of the liquid on the surface is much softer than in Xu and colleagues' experiment. This maintains the integrity of the floating drop, whose mobility is enhanced through the lubricating effect of the air, making it easy to manipulate. By 'feeding' the drop, Couder *et al.* can maintain floating globules of liquid several centimetres across, which persist long after the vibration has stopped, because of the slow 'drainage' of air at these large scales.

The two experiments[1,4] demonstrate that air is not as passive as we thought, and that it can be used for tuning the behaviour of drops. Our understanding of such problems as air entrapment or jet impact[5,6] should benefit from this amended vision. ■

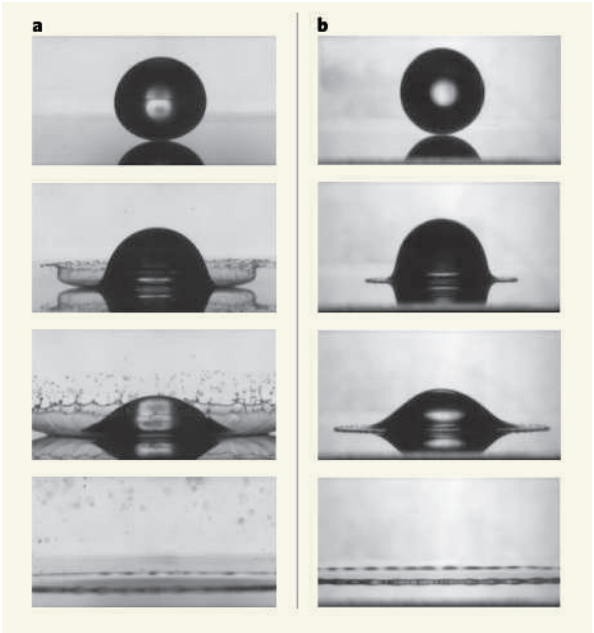

Figure 1 | Impact zone. As shown in these high-speed photographs taken by Xu *et al.*[1], air pressure determines the outcome of the impact of an ethanol drop on dry glass. **a**, At ambient pressure there's a splash. **b**, In a rarefied atmosphere — 0.3 atm, equivalent to that found at the summit of Everest — a corona fails to form and there is no subsequent splash.

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CANCER BIOLOGY

Summing up cancer stem cells

Brian J. P. Huntly and D. Gary Gilliland

Are current cancer drugs targeted at the wrong kinds of cells? A pioneering approach to the development of treatments uses a mathematical model to follow how different types of tumour cells respond to therapy.

In this issue, Michor and colleagues (page 1267)[1] address the vexing problem that although many cancer drugs dramatically reduce the size of tumours, most cancers will eventually recur, often fatally. The authors model the dynamic changes in populations of cancer cells during treatment, and their data fit with the theory that there is a small population of cells — 'cancer stem cells' — that are ultimately responsible for the growth of tumours and are resistant to current therapies. Furthermore, the results imply that these cells may provide a reservoir for the generation and propagation of mutant cells that are also resistant to therapy. This innovative modelling strategy should allow prospective evaluation of drugs aimed at eradicating cancer stem cells.

The cancer-stem-cell hypothesis posits a very rare population of cells within tumours that are the only tumour cells with the capacity for limitless self-renewal (reviewed in ref. 2). This concept has important therapeutic implications, and may explain why it is possible to treat many cancers until the tumour cannot be detected, and yet the cancer returns. It is plausible that current treatments do not eliminate cancer stem cells, which can then regenerate the tumour once the treatment stops. This theory is not new, but it is only recently that advances in technology have allowed the prospective identification and purification of cancer stem cells, a goal first accomplished in leukaemias[3], and now extended to breast cancer[4] and tumours of the central nervous system[5].

Michor *et al.*[1] took advantage of such advances in the understanding of chronic myelogenous leukaemia (CML) to model how leukaemic stem cells respond to therapy. CML is a blood cancer caused by a mutation that splices together the genes encoding the BCR and ABL proteins; the resulting BCR–ABL fusion protein is expressed in the bone marrow of affected individuals. ABL is an enzyme that regulates normal cell growth, but when fused with BCR it becomes permanently active, causing dysregulated overproduction of white blood cells — leukaemia.

A striking advance in the treatment of CML has been the development of imatinib, a drug that inhibits BCR–ABL activity and induces complete remission in the majority of patients with CML[6,7]. The response to imatinib can be monitored in the clinic by measuring the level of BCR–ABL gene transcript during treatment[8]. However, several problems remain. First, as with many cancer therapies, imatinib does not cure CML. Second, some tumours develop resistance to imatinib, in most cases because new mutations result in an ABL enzyme to which imatinib cannot bind (reviewed in ref. 9).

To understand these problems better, Michor *et al.*[1] developed a mathematical model of CML based on the normal biology of blood-cell development (Fig. 1a). The model includes a hierarchy of cells at four developmental levels: stem cells, the most immature cells that can continuously renew themselves; progenitor cells, which have begun to develop but can no longer self-renew; differentiated cells, which have begun to form a particular cell type; and terminally differentiated cells, the fully mature, specialized blood cells.

The authors then tested the model to see how well it reflected the response of CML patients to imatinib, as monitored by serial measurements of BCR–ABL transcript levels. At first, there was a rapid decline in BCR–ABL transcript number (around 5% per day) that corresponded to the model's prediction of a reduction in the differentiated leukaemic cells (Fig. 1b). Thereafter, there was a slower rate of decline of transcript number (around 0.8% per day), consistent with the modelled decrease in the leukaemia progenitor cells (Fig. 1c).

These fascinating observations are the first to characterize the kinetics of reduction of distinct subpopulations of tumour cells in response to therapy, and provide important insights into disease responsiveness and resistance. For example, the rate of increase of BCR–ABL transcript copy number after stopping therapy (Fig. 1c–e), or development of resistance to imatinib through acquired point mutations in the ABL gene, is consistent with the presence of leukaemic stem cells that have not been eradicated with treatment and are fully capable of reconstituting fulminant leukaemia. Although more work is needed to validate the model, it should be possible to test this hypothesis directly by prospective purification and measurement of distinct BCR–ABL-positive blood-cell populations during treatment.

Although there have been remarkable advances in the development of molecularly targeted drugs against cancer, such as imatinib, it is clear that additional agents will be needed to eradicate cancer stem cells — literally the root of the problem. The kinetic snapshots of how different tumour-cell subpopulations respond to treatment, derived from Michor and colleagues' model, may prove an invaluable tool as our attention shifts to the cancer stem cell as a therapeutic target. Using this model, prospective *in vivo* monitoring of response to new drugs for CML should help to identify those that strike at the leukaemic stem cell itself. ■

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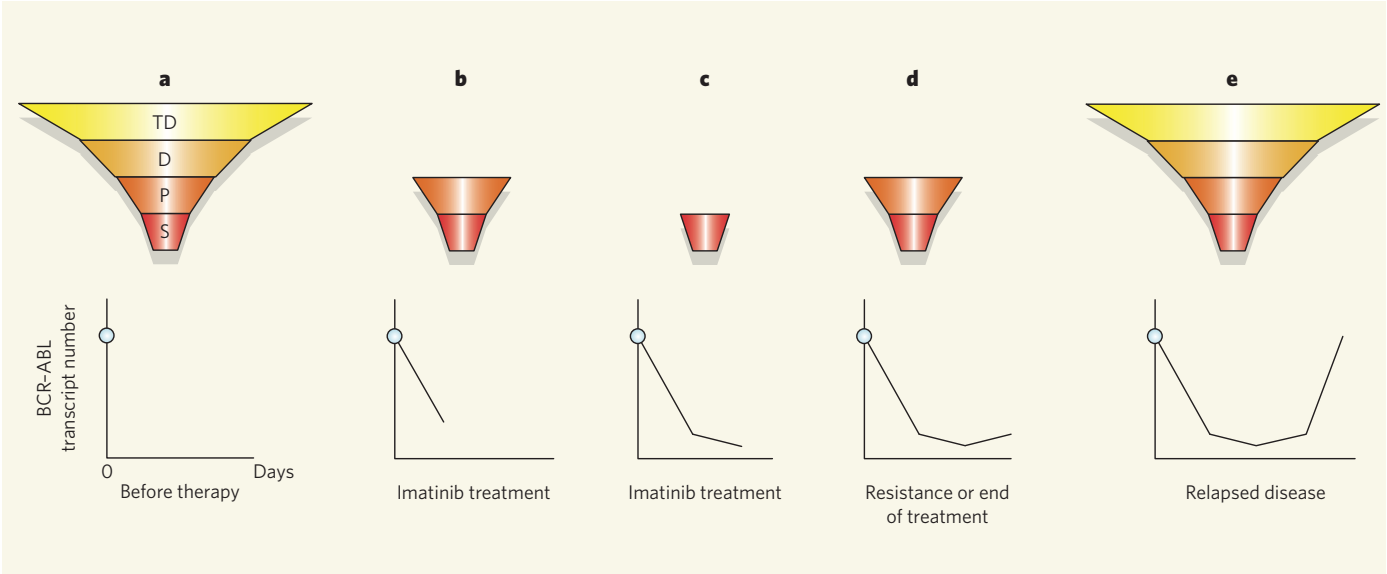

Figure 1 | Modelling leukaemia. **a**, Michor *et al.*[1] have developed a mathematical model of chronic myeloid leukaemia (CML) as four 'compartments' based on stages in the normal development of blood cells. These compartments are: leukaemic stem cells (S), which are the most immature cells and can continuously renew themselves; leukaemic progenitor cells (P), which have developed to the extent that they can no longer self-renew; differentiated leukaemic cells (D), which have begun to develop into specialized cell types; and terminally differentiated cells (TD), fully mature, specialized cells. **b**, Treatment of CML with imatinib, which inhibits the BCR–ABL enzyme that causes CML, results in an initial rapid reduction of BCR–ABL gene-transcript copy number, corresponding in the model to the depletion of the more-differentiated cells (TD and D). **c**, With continued therapy, there is a slower decline in BCR–ABL transcripts that corresponds to a reduction in the leukaemic progenitor-cell population, but the leukaemic stem cell compartment is preserved. **d**, Stopping therapy, or the development of resistance in the leukaemic stem-cell compartment, allows an initial slow rate of rise of BCR–ABL transcripts corresponding to an increase in the leukaemic progenitors, fuelled by the leukaemic stem cells. **e**, There is a rapid rate of rise in BCR–ABL transcripts that corresponds to relapse of disease. Monitoring of the rate of decline of progenitor-cell pools may aid the development of agents to target the progenitor and leukaemic stem-cell compartments in CML and other cancers.