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.

References

1. Spangrude, G. J., Heimfeld, S. & Weissman, I. L. Purification and characterization of mouse hematopoietic stem cells. *Science* **241**, 58-62 (1988).

2. Morrison, S. J., Uchida, N. & Weissman, I. L. The biology of hematopoietic stem cells. *Annu Rev Cell Dev Biol* **11**, 35-71 (1995).

3. Ichimaru, M., Ishimaru, T., Mikami, M., Yamada, Y. & Ohkita, T. Incidence of leukemia in a fixed cohort of atomic bomb survivors and controls, Hiroshima and Nagasaki October 1950-December 1978: Technical Report RERF TR 13-81. Radiation Effects Research Foundation: Hiroshima (1981).

4. Mackey, M. C. in *The Art of Mathematical Modeling: Case Studies in Ecology, Physiology and Biofluids* (eds. H. Othmer, F. Adler, M.Lewis, J. Dallon) 149-178 (Prentice Hall, New York, 1996).

5. Fortin, P., Mackey, M. C. Periodic chronic myelogenous leukemia: spectral analysis of blood cell counts and aetiological implications. *Br J Haematol* **104**, 336-345 (1999).

6. Moore, H., Li, N. K. A Mathematical model for chronic myelogenous leukemia (CML) and T cell interaction. *J Theor Biol* **227**, 513-523 (2004).

7. Fokas, A. S., Keller, J. B., Clarkson, B. D. Mathematical model of granulocytopoiesis and chronic myelogenous leukemia. *Cancer Res* **51**, 2084-2091 (1991).

8. Branford, S., Rudzki, Z., et al. Real-time quantitative PCR analysis can be used as a primary screen to identify imatinib-treated patients with CML who have BCR-ABL kinase

domain mutations. *Blood*. Prepublished July 15, 2004; DOI 10.1182/blood-2004-03-1134.
9. Branford, S., Hughes, T. P. & Rudzki, Z. Monitoring chronic myeloid leukaemia therapy by real-time quantitative PCR in blood is a reliable alternative to bone marrow cytogenetics. *Br J Haematol* **107**, 587-599 (1999).
10. Hochhaus, A., Lin, F., Reiter, A., Skladny, H., Mason, P. J., van Rhee, F., Shepherd, P. C., Allan, N. C., Hehlmann, R., Goldman, J. M. & Cross, N. C. Quantification of residual disease in chronic myelogenous leukemia patients on interferon-alpha therapy by competitive polymerase chain reaction. *Blood* **87**, 1549-1555 (1996).
11. Emig, M., Saussele, S., Wittor, H., Weisser, A., Reiter, A., Willer, A., Berger, U., Hehlmann, R., Cross, N. C. & Hochhaus, A. Accurate and rapid analysis of residual disease in patients with CML using specific fluorescent hybridization probes for real time quantitative RT-PCR. *Leukemia* **13**, 1825-1832 (1999).
12. Elmaagacli, A. H., Freist, A., Hahn, M., Opalka, B., Seeber, S., Schaefer, U. W. & Beelen, D. W. Estimating the relapse stage in chronic myeloid leukaemia patients after allogeneic stem cell transplantation by the amount of BCR-ABL fusion transcripts detected using a new real-time polymerase chain reaction method. *Br J Haematol* **113**, 1072-1075 (2001).
13. Branford, S., Rudzki, Z., Harper, A., Grigg, A., Taylor, K., Durrant, S., Arthur, C., Browett, P., Schwarer, A. P., Ma, D., Seymour, J. F., Bradstock, K., Joske, D., Lynch, K., Gathmann, I. & Hughes, T. P. Imatinib produces significantly superior molecular responses compared to interferon alfa plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. *Leukemia* **17**, 2401-2409 (2003).
14. Kantarjian, H. M., Talpaz, M., Cortes, J., O'Brien, S., Faderl, S., Thomas, D., Giles, F., Rios, M. B., Shan, J., Arlinghaus, R. Quantitative polymerase chain reaction monitoring of BCR-ABL during therapy with imatinib mesylate (STI571; gleevec) in chronic-phase chronic myelogenous leukemia. *Clin Cancer Res* **9**, 160-166 (2003).

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 $u = 4 \cdot 10^{-8}$.

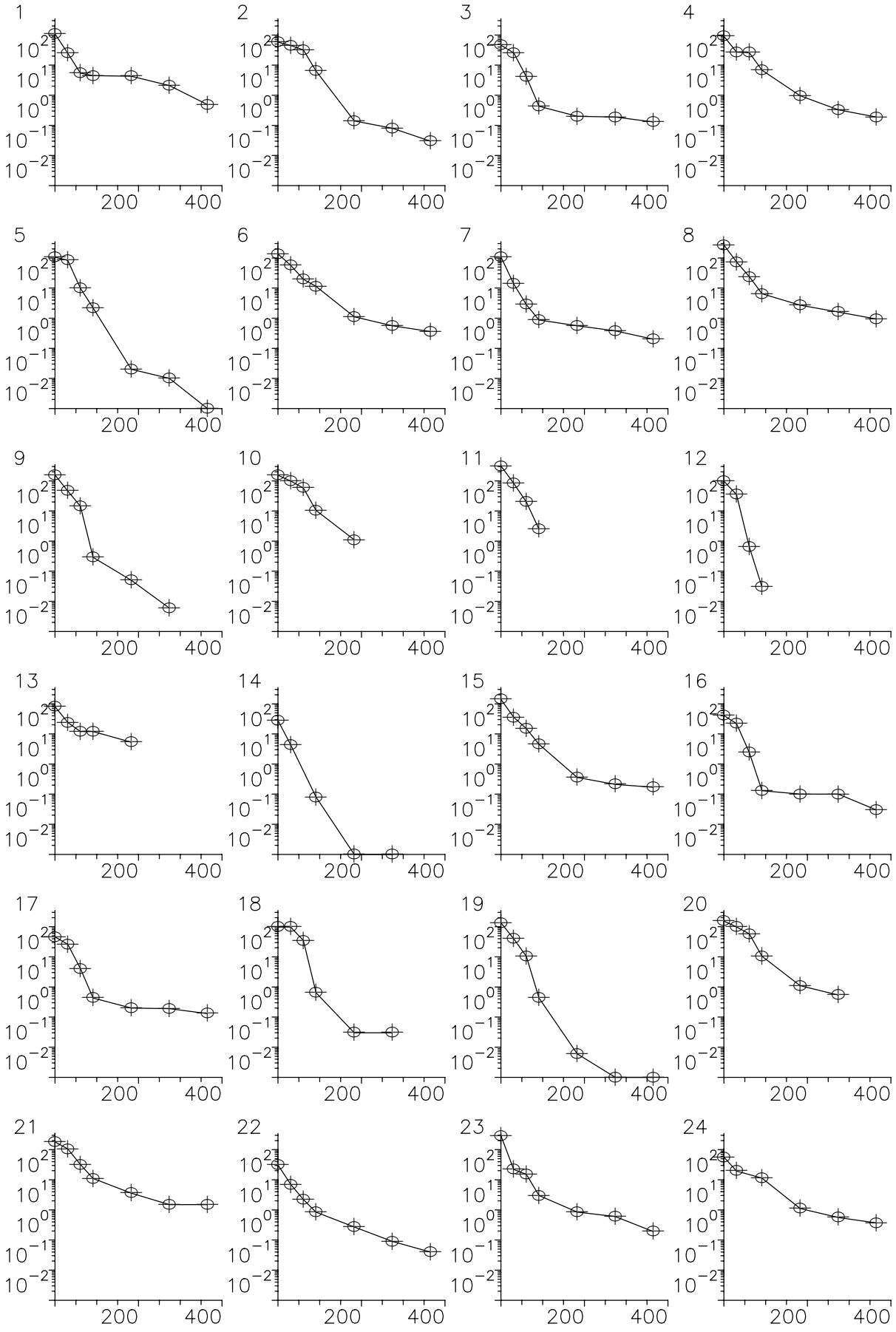
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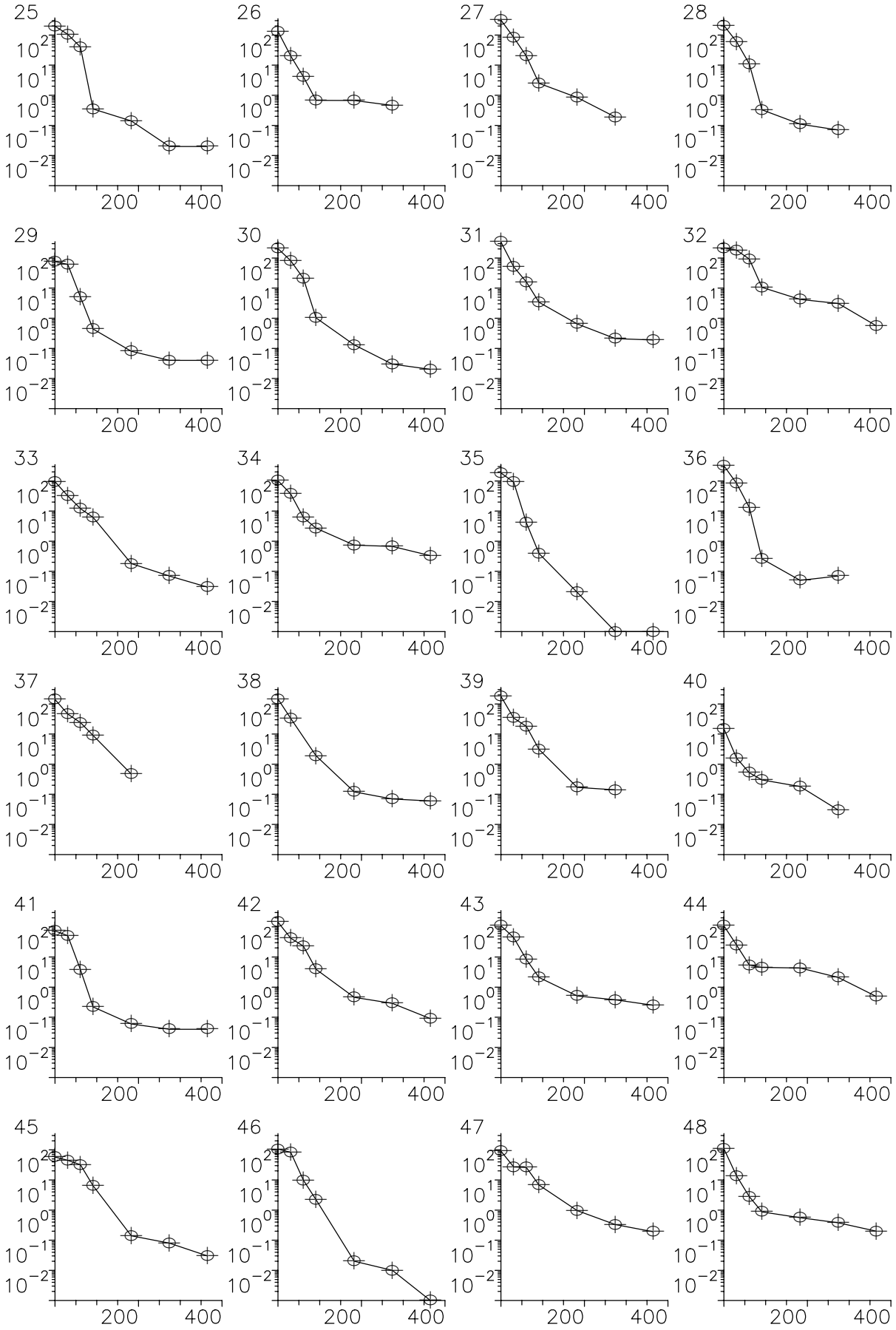
Figure 5 contd.



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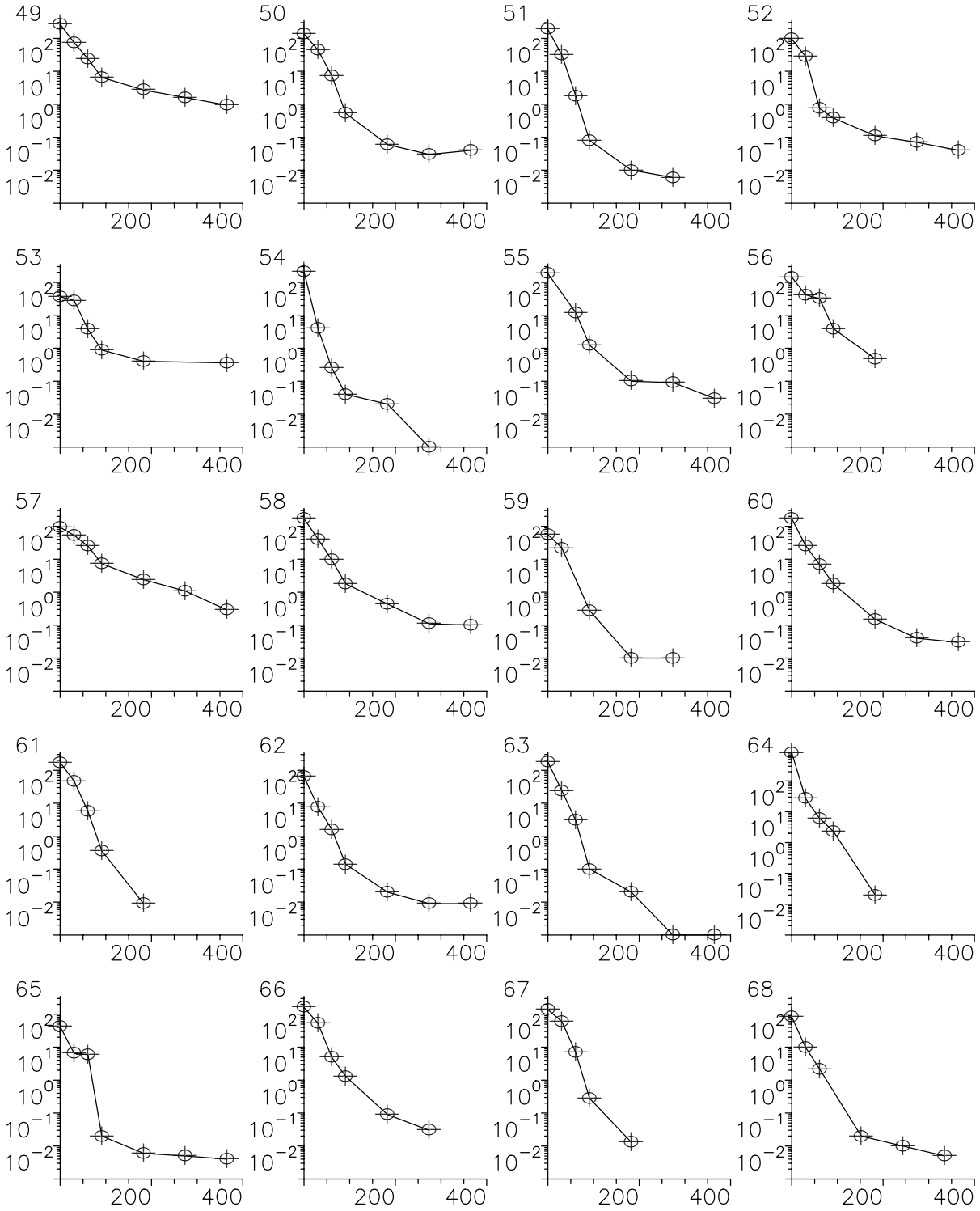

Table 1

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