

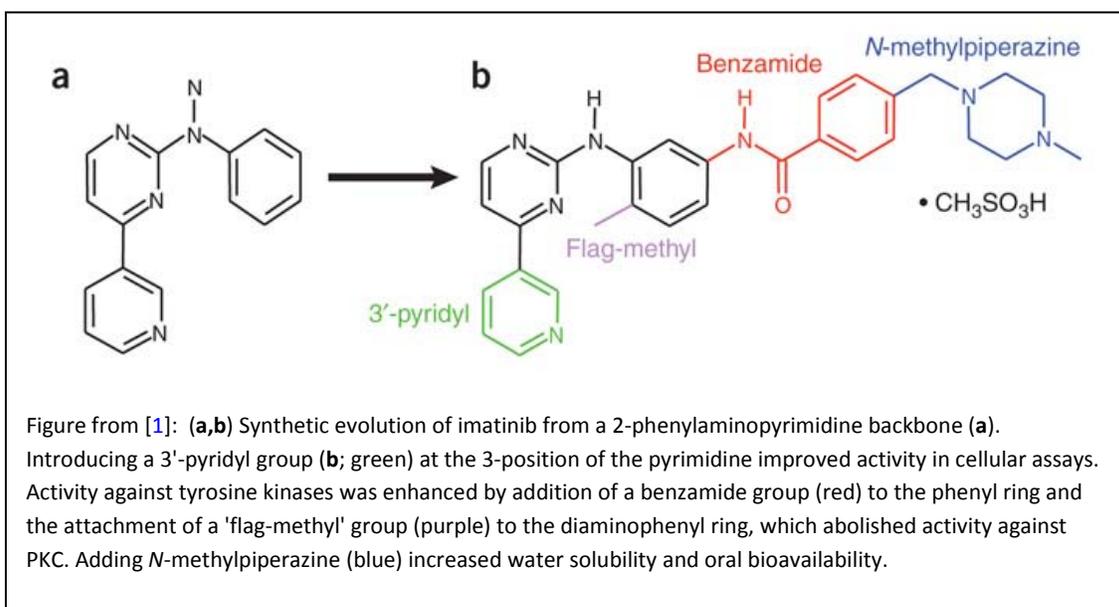
Modeling Disease

Today we will examine the process of drug discovery by modeling at many levels of abstraction, from the bimolecular interaction of a drug and its target all the way to the level of the organism.

CML, chronic myeloid leukemia is a relatively simple cancer caused by a single acquired genetic change. Ninety-five percent of people with CML have a particular chromosomal translocation that fuses the BCR protein to the ABL kinase creating a constitutively active kinase. It seems pretty clear that if you could inhibit this kinase you could cure the disease.

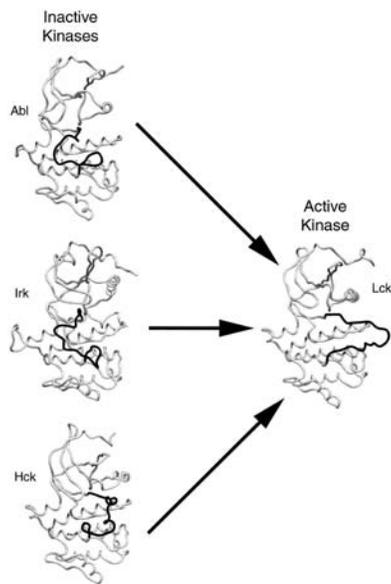
The drug discovery process for CML has been summarized in recent reviews [1, 2]. Additional information can be found in the following references: "A CONVERSATION WITH BRIAN J. DRUKER, M.D." <http://www.nytimes.com/2009/11/03/science/03conv.html> and the Lasker award citation: http://www.laskerfoundation.org/awards/2009_c_description.htm

Initial high-throughput screens, performed in vitro, produced a lead compound that inactivated the kinase. Analog-based design was used to improve the activity and ADME properties. This lead to the FDA-approved inhibitor of BCR-ABL (and wild-type ABL) called Imatinib (Gleevec), which was the first time that a drug had been developed to target the molecular basis of a cancer.



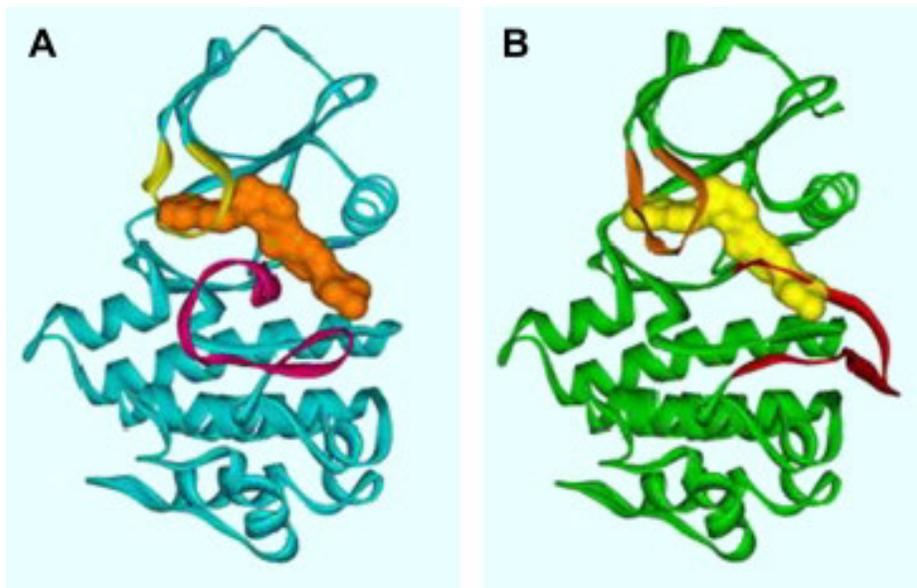
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Source: Lydon, Nicholas. "Attacking Cancer at its Foundation."
Nature Medicine 15, no. 10 (2009): 1153-7.

It is interesting to note that inventors failed in their efforts to use structural information to understand the changes in activity. It turns out that the compound binds to an inactive form of the kinase that differs significantly in structure from the active form that had been used in modeling efforts.



Top: The active forms of many kinases are structurally similar, but the inactive forms differ. Since imatinib binds to the inactive form of the kinase, it can be relatively specific. When imatinib was developed, only the active conformations were known. As a result, structure-based design failed. (Figure from Nagar B et al. *Cancer Res* 2002;62:4236-4243). To paraphrase Tolstoy, "Active kinases are all alike; every inactive kinase is inactive in its own way."

© American Association for Cancer Research. All rights reserved. This content is excluded from our Creative Commons license. For more information, see <http://ocw.mit.edu/help/faq-fair-use/>. Source: Nagar, Bhushan, William G. Bornmann, et al. "Crystal Structures of the Kinase Domain of c-Abl in Complex with the Small Molecule Inhibitors PD173955 and Imatinib (STI-571)." *Cancer Research* 62, no. 15 (2002): 4236-43.



Bottom: Figure 5 and legend from <http://dx.doi.org/10.1016/j.exphem.2007.01.023>. Ribbon representation of imatinib in complex with the kinase domain of ABL. (A) In the imatinib: ABL complex the activation loop (magenta) is in "closed" conformation, blocking the catalytic site for ATP and substrate binding. (B) Structure of ABL in an active conformation (green) with a surface representation of imatinib (yellow) superimposed. The activation loop is colored red and the glycine-rich, or P-loop, is colored orange. The N-methyl piperazine group of imatinib sits on the path of the activation loop in the active kinase, which is why imatinib cannot use this mode of binding for active ABL kinase

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Although Imatinib is highly effective at treating CML, it does not cure the disease. For many patients, that turns out not to be an issue. The median age of patients with Ph1-positive CML is 67 years. Since the median age at death in the US is in the mid-seventies, in many cases suppressing the disease for a decade or so is all that is needed. However, some young people do come down with CML and in these cases there is an urgent need for a cure rather than a treatment. The treatment regimen for these younger patients is controversial, and in some cases the recommendation can be for a bone marrow transplant, which carries with it a 10-25% risk of death!

For example, a recent review in the Lancet[3] said the following:

“The panel's recommendations corroborated the value of imatinib and allogeneic stem-cell transplantation. They recommended that the preferred initial treatment for most newly diagnosed patients in chronic phase should be imatinib 400 mg daily.”

“In a patient with high disease risk and low transplantation risk, first-line treatment with allograft can be appropriate. Such a patient **probably** should try imatinib since the early response to imatinib can provide prognostic information to reinforce or weaken the case for allograft. No data conclusively suggest a negative effect of a pretreatment with imatinib on allogeneic stem cell transplantation, provided imatinib is discontinued at least 2 weeks before transplantation.”

“Management recommendations might be dependent on economic conditions, such as cost of expensive drugs or modern diagnostic technologies. Allogeneic stem-cell transplantation offers an option that could be more cost effective than a life-long therapy with imatinib.”

Pharmacology at the organism level

To understand better why Imatinib does not cure CML, and therefore to understand the complexity of drug development, we need to examine the problem from the level of the organism. There are several mechanisms at work that limit the effectiveness of even a highly specific therapy. The following is based largely on references [4, 5]:

1. **Resistance mutations:** Imatinib binds to the ATP-binding pocket and stabilizes the inactive form of the BCR-ABL kinase. At least 50 mutants have been identified that prevent the kinase from adopting this conformation. Identification of these resistance mutations set off a rush for second generation inhibitors that would be active against the mutants. Dasatinib and nilotinib are new inhibitors that are active against some of these mutants. However, they are not active against the T315I mutant. The new compounds are associated with other problems as well. Dasatinib has broader specificity (inactivates more kinases) than nilotinib or imatinib, which could be the cause of some of the observed side effects.

All three of these compounds target the same binding pocket. Other inhibitors are currently being developed that bind to different sites, such as GNF-2 and its derivative GNF-5, which bind a pocket far away from the ATP binding site and functions through an allosteric mechanism [6]. There are also compounds that function as competitive inhibitors of the substrate. Some of these show promise as overcoming the T315I mutation.

Why do so many mutations occur? It appears that it is not just the normal level of random mutagenesis. Recent evidence [7] indicates that some BCR-ABL-carrying cells turn on a protein called activation-induced deaminase (AID), which normally is only turned on in mature B cells and which causes somatic hypermutation leading to an increase in antibody diversity.

2. **Amplification of BCR-ABL:** this turns out to be common in cell lines but relatively rare in patients. Is there some difference between in vivo and in vitro that is relevant here?
3. **ADME**
 - a. **Absorption:**
 - i. **Decreased import:**
 1. many drugs don't diffuse through the lipid membrane, but use transporters.
 2. polymorphisms in the organic cation transporter (OCT1) can reduce the effectiveness of the response to the diabetic medication, metformin.
 3. Imatinib also uses OCT1, among other transporters. Does variation in this transporter affect treatment? Do mutations induce resistance?
 - ii. **Increased export:** It has been known since the 1970s that overexpression of some transporters confers resistance to chemotherapy by pumping the drug out of cells.
 1. Resistance can occur due to export out of cells in the GI tract. This would lower bioavailability.
 2. There is some evidence that fruit juices can interfere with transport by the (OATP)1A2 transporter, which can influence uptake of drugs
 - b. **Distribution:**
 - i. **Lower levels of free drug:** About 90% of imatinib is bound to serum proteins including albumin. In principle, a mutation in a serum protein that

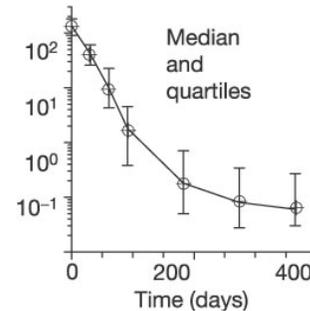
increased affinity for Imatinib could bring the levels so low that they would not be therapeutic.

c. **Metabolism:**

- i. **Liver metabolism:** Imatinib is mostly metabolized by one particular cytochrome (**CYP3A4**). Variations in this cytochrome among individuals might cause different levels of the drug and different levels of response. This is also the cytochrome that is inhibited by grapefruit juice

The difference between a treatment and a cure

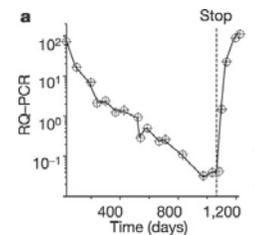
Many of the mechanisms listed above contribute to the difficulties in curing CML. However, there is another important component that is only beginning to be fully understood. An important observation was made by Michor et al.[8], who examined the time-course of the disease. They took patient blood and used quantitative real-time PCR to measure how many cells expressed the BCR-ABL fusion protein. To control for variations in the purification and PCR they normalized to BCR transcripts. Their data from 169 patients showed a very striking trend.



Courtesy of Macmillan Publishers Limited. Used with permission. Source: Michor, Franziska, Timothy P. Hughes, et al. "Dynamics of Chronic Myeloid Leukaemia." *Nature* 435, no. 7046 (2005): 1267-70.

The data show a very consistent decay of BCR-ABL transcripts to levels that are almost undetectable. However, in a few cases, patients had to stop treatment due to side effects. In each case, the levels of BCR-ABL transcripts rebound, even though one patient had been treated for three years!

What we see here is reminiscent of what happens to HIV patients on anti-retroviral therapies. As long as the drugs are being taken and resistance is overcome using new inhibitors, if necessary, the disease is kept in check. However, once the treatment stops, the disease returns very rapidly. The important questions are why this happens and what to do about it.



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Cancer Stem Cells

(This section is based, in part on Reya et al (2001)[9])

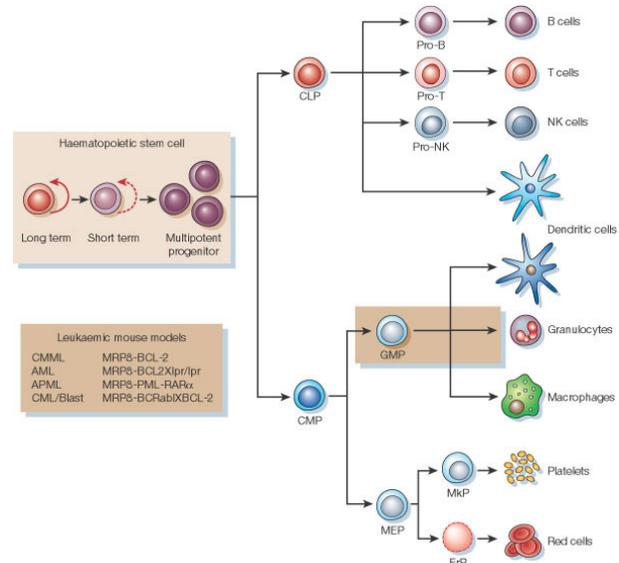
It has been known since the 1960s that the fraction of cells in a tumor that can form a new tumor when transplanted into a new animal is low.

What could explain this?

1. Each cell might have a low probability of proliferating, or
2. most cells may not proliferate at all and only a small sub-population is tumorigenic.

In some cases, it has been shown very clearly that there is a special population of cells that are the only ones to proliferate. Bonnet and Dick, back in 1997, took peripheral blood from patients with AML and used FACS to identify subpopulations. They then injected these cells into an immuno-compromised mouse and measured the number of human cells in the bone marrow at various times. There is a CD34+ CD38- subpopulation of acute myeloid leukemia cells that is uniquely capable of dividing.

When this was first discovered, it remained possible that it was unique to hematologic cancers. That's because normal blood cells consist of a large population of cells that have limited or no ability to divide and a small population of cells that are actively dividing. The most primitive of these dividing cells form a very small, self-renewing population that is called "hematopoietic stem cells." It was possible that the actively dividing subpopulation of the tumor was derived from these more primitive cells.



Courtesy of Macmillan Publishers Limited. Used with permission. Source: Reya, Tannishtha, Sean J. Morrison, et al. "Stem Cells, Cancer, and Cancer Stem Cells." *Nature* 414, no. 6859 (2001): 105-11.

However, over the last few years, it has been shown that several solid tumors also contain at least two very distinct types of cells: a small subset of cells that divide and are tumorigenic and a large population that has limited ability to divide. This has been shown now for cancer of the colon[10], breast[11] and brain[12].

These tumor initiating cells are often referred to as "cancer stem cells." However, the term remains somewhat controversial [13]. The term stem cell is usually reserved for cells that are multipotent – that can give rise to many different types of terminal cells. This may not be the case for the tumor initiating cells. In addition, cancer stem cells may not originate from normal stem cells and may even be generated from more terminal stem cells through a process called the epithelial mesenchymal transition (EMT). Nevertheless, we will use the term "cancer stem cell" convenience.

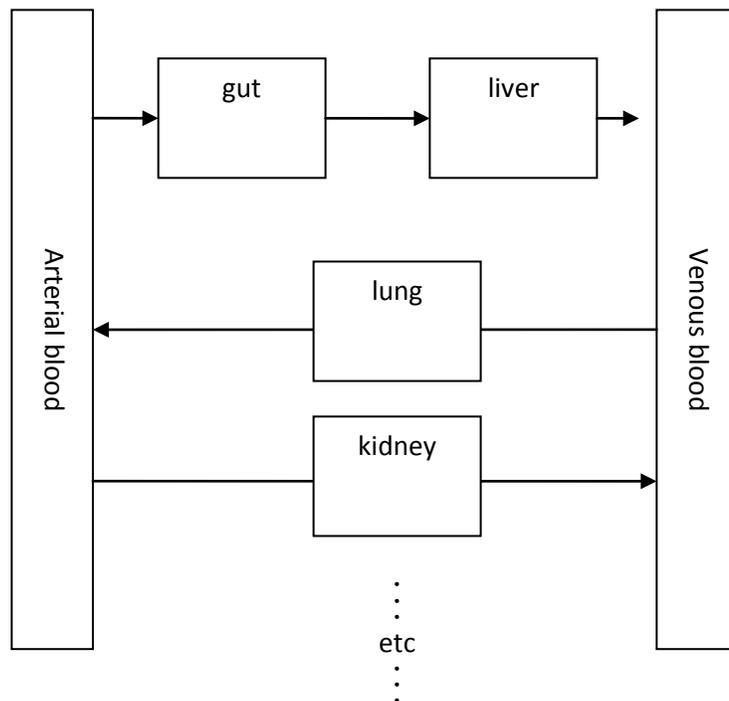
Modeling CML

The existence of this population of special cells requires consideration when designing a therapy, as we will see by examining why CML rebounds when Imatinib treatment stops. It has been proposed that the rapid rebound is due to the existence of Imatinib-resistant cancer stem cells. There are several plausible mechanisms to explain why the stem cells are not affected by Imatinib, including the fact that stem cells express higher levels of the MDR protein P-glycoprotein, an efflux pump, and evidence that stem cells are less dependent on BCR-ABL for growth and survival.

Modeling the number of cancer cells of various types in a patient during treatment can aid in designing better therapeutic measurements. In the section below, we follow the analysis of Michor[8, 14], but you may be interested to consult Komarova and Wodarz[15] for a very different approach. The figure above shows the normal hematopoietic lineage, which is often thought of as consisting of four major stages: A normal stem cell gives rise to progenitors (CLP, CMP); these produce differentiated cells (pre T, pre B, etc.), which in turn generate terminally differentiated cells.

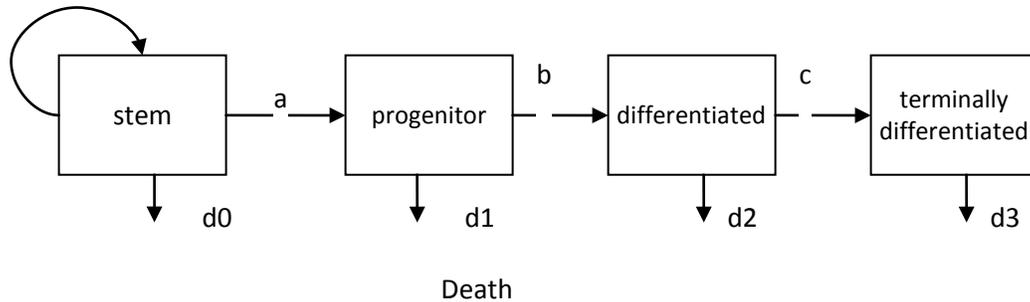
In our model, we want to track the different types of cells with different variables, since they will show different rates of growth. A common approach is to use a “**Compartmental Model.**” Compartmental models are widely used to treat complex problems in a modular way. In these models, we assume that there exist several homogeneous “compartments,” and we focus on the flow between them. For example, pharmacokinetic models might treat the concentration of drug in each organ with a single parameter and examine the flow of drug between organs (see figure).

I



The same type of analysis can be applied to model the types of cells in CML. We create one imaginary compartment for each type of cell. So we have a stem cell compartment, a progenitor compartment, etc. If a cell differentiates, it is treated as if it moved from one compartment to the next. These movements can be modeled by mass action kinetics.

The normal developmental process can be modeled as follows:



Let x_0 , x_1 , x_2 and x_3 be the populations of the normal cells of each of these types (stem, progen, differ, term). The normal cells can be modeled by the equations at right. Note that the growth of the stem cells is determined by a parameter $\lambda(x_0)$ that varies in order to maintain a relatively constant population of normal stem cells.

$$\frac{dx_0}{dt} = [\lambda(x_0) - d_0 - a_x]x_0$$

$$\frac{dx_1}{dt} = a_x x_0 - d_1 x_1 - b_x x_1$$

$$\frac{dx_2}{dt} = b_x x_1 - d_2 x_2 - c_x x_2$$

$$\frac{dx_3}{dt} = c_x x_2 - d_3 x_3$$

Michor et al. assume that the differentiation rates are small with respect to the death rates and thus ignore the loss of cells to differentiation to produce the following simplified equations:

$$\frac{dx_0}{dt} = [\lambda(x_0) - d_0]x_0$$

$$\frac{dx_1}{dt} = a_x x_0 - d_1 x_1$$

$$\frac{dx_2}{dt} = b_x x_1 - d_2 x_2$$

$$\frac{dx_3}{dt} = c_x x_2 - d_3 x_3$$

Now, let's imagine we have a population of stem cells in which the BCR-ABL fusion protein is created by a random event. We can assume that this mutation is sufficient for the stem cell to

become a cancer stem cell, although it is possible that additional uncharacterized mutations are also needed. Let y_0, y_1, y_2, y_3 represent the populations of the leukemic cells.

We assume that the leukemic stem cells expand exponentially, with kinetics $y_0(t) = \exp[(r_y - d_0)t]$

Then we have the following system of rate equations:

$$\begin{aligned} \frac{dy_0}{dt} &= [r_y - d_0]y_0 \\ \frac{dy_1}{dt} &= a_y y_0 - d_1 y_1 \\ \frac{dy_2}{dt} &= b_y y_1 - d_2 y_2 \\ \frac{dy_3}{dt} &= c_y y_2 - d_3 y_3 \end{aligned}$$

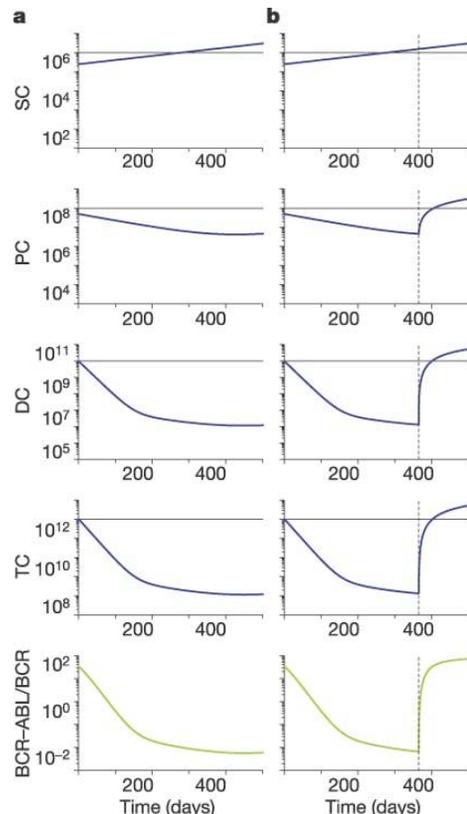
This model is based on a number of assumptions:

- normal stem cell population is constant
- leukemic stem cells grow exponentially
- BCR-ABL increases rate of stem cell proliferation $r_y > \lambda(x_0)$ AND differentiation: $a_y > a_x$
- BCR-ABL increases rate of differentiation of progenitors ($y_1 \rightarrow y_2$): $b_y > b_x$.
- imatinib reverses these changes so $a'_y < a_y$ and $b'_y < b_y$
- death rates are the same in wt and mutant cells.

Our goal is to use these equations to understand the changes in populations that occur during treatment. Recall that the experiment measures BCR-ABL transcripts in circulating blood, not cells. Since the stem cells do not circulate, the observed measurements will be proportional to the sum of y_1, y_2, y_3 . Fitting these equations to the data, we can accurately reproduce the biphasic response.

The model predicts the populations shown in the figure, where Black=wt and blue=leukemic. The main features of the model are as follows:

- The leukemic stem cells are expanding exponentially with a slow rate constant, regardless of treatment. Imatinib reduces the rate at which leukemic progenitors are produced.



- The differentiated cells show a biphasic decline. The first phase is due to the natural death of the short-lived differentiated cells (20 days), which, due to imatinib, are being replaced very slowly. Once these are exhausted, the number of differentiated cells follows the progenitors, which are also declining in number.
- Terminally differentiated cells track the differentiated cells. (The model assumes that their rate of production is unchanged).
- Once treatment is terminated (the dashed line in “b”), all the populations take off because there has been no loss of stem cells.

The conclusion from this model is that you could get the level of detectable BCR-ABL transcripts down to zero, but the cancer would still come back because of the small number of cancer stem cells.

It is important to realize that there are an infinite number of models consistent with the data. This problem is especially severe here, where we do not have direct measurements of any of the key species. The RT-PCR values give us the aggregate number of differentiated cells, but do not detect the stem cells which can only be measured by FACS on a bone marrow biopsy sample.

It is useful to consider which of the model’s assumptions have the most impact on the conclusions. Why does the stem cell population y_0 not decline? One reason is that we assumed Imatinib has no effect on d_0 . Is this true? We can’t be sure, because it is so hard to detect the stem cells in vivo. However, in vitro, there is evidence to support this assumption: imatinib does not appear to induce apoptosis[16]. If so, is there anything that can be done to eliminate y_0 ? Perhaps. The ideal way to treat the disease would be to design compounds that target the stem cell. However, this may not be possible. Recall that it is currently believed that stem cells are resistant because they express higher levels of the MDR protein P-glycoprotein, an efflux pump and are less dependent on BCR-ABL for growth and survival. It is possible that new compounds can be found that cause apoptosis in stem cells, but this does not seem very likely.

More recent studies have come to contradictory conclusions about the ability to target stem cells. In a recent paper, Michor and colleagues analyze very long-term data from patients to suggest that there are some who do see a decline in the stem cell population, with others either showing no effect or an increase [17]. Other studies have taken different approaches and reached different conclusions. See for example [18, 19].

A very different type of therapy would be to increase the rate of stem cell differentiation a_y in order to reduce the stem cell population below the level at which it is self-sustaining. Of course, this will also make the disease worse, since it would increase the population of differentiated cells. So any such treatment would have to be done in conjunction with treatment to kill off more differentiated cells using compounds like imatinib. Since imatinib reduces a_y , we would need modeling to help design an effective protocol, just as was described for the use of MEK-inhibition together with adenovirus treatment for p53-deficient cancers.

Implications for clinical trials

The fact that cancer stem cell population is invisible to RT-PCR, one of the chief diagnostic tools, is an example of a broad problem. Clinical measurements are not just used to monitor patient health after the drugs are licensed. They are also one of the main techniques we have for clinical trials to test drugs before they are approved. Unless a disease has a truly horrible prognosis, most patients in the control arm of the study won't die during the course of testing. To figure out if a drug is beneficial, we need to track something other than death. If we are testing drugs for CML, it makes sense to measure BCR-ABL transcripts. But as we have seen, a treatment that eliminates these transcripts might not be curative. Conversely, a treatment that is slowly reducing the stem cell population might not have any measurable effect on the bulk tumor during the time of the study.

A similar problem can be seen in other diseases where prognosis is not well-correlated with clinical measurements[20]. For example, indolent non-Hodgkin's lymphoma (NHL) is currently considered to be an incurable disease, with a median survival of 6-8 years. For many years, there was no consensus on a standard treatment. This has changed somewhat with a new therapy that is based on an antibody-based therapy called rituximab. However, before rituximab, some studies indicated that chemotherapy could eliminate all the symptoms of the disease for several years. Some doctors prescribed chemotherapy while other prescribed 'watchful waiting'. It turned out that the two groups had roughly the same life expectancy.

In multiple myeloma, the cancer cells derive from a B-cell and thus produce a monoclonal antibody. The disease also has no known cure. Because every cancer cell is producing the same antibody, it is easy to track. Surprisingly, there are several studies in the literature indicating that survival is not correlated with either the size or the kinetics of the changes in antibody levels. There are also papers that assert that a correlation exists, but the presence of the controversy indicates that the effect must be a small one.

Conclusions

Many of the topics of the course can be seen in the context of treating CML. This disease at first appears simple, since it arises from a spontaneous mutation in a kinase. In the first part of the course we examined how to design small molecules to inhibit a kinase, and in the second part of the course we looked at how we could model the effects of inhibition on signaling pathways. In fact, it has been possible to develop an inhibitor of this kinase that is a highly effective treatment, but it is not a cure. To understand why, we must examine the kinetics of various cell populations. Such models can provide insights into new therapeutic approaches that may require careful timing of treatments that have opposing effects on particular cell populations.

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