Chapter 14. BcrAbl Ph CML Gleevec - version 200214d

Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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The remarkable story of the Philadelphia chromosome and the treatment of chronic myelogenous leukemia.
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The Molecular Basis of B Cell Malignancies (HHS Only)
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CCR Grand Rounds
The first in a new era of molecular targeted cancer therapy.

CHAPTER 14.

The extraordinary story of the Philadelphia chromosome and a new era of cancer therapy.

Normal cells have control systems that keeps cells well behaved; that is, they keep the cells from proliferating excessively. Malignant tumors, however, are often defective in those controls. We know a great deal about how those controls work, the molecules that carry them out, and the way they are defective in cancer. The challenge was how to take advantage of that knowledge for therapy.

In order to do that, a molecular diagnosis is needed to tell physicians what molecular defects are driving the malignant process in each particular patient and what drugs may provide a remedy. Modern cancer therapy is moving rapidly in that direction, but still has a long way to go. However, an unexpected observation more than 50 years ago became a harbinger of this kind of approach long before the idea was even conceived. It came from noticing something that came to be known as the "Philadelphia chromosome" in honor of the city where it was discovered.

In 1960, Peter C. Nowell and David A. Hungerford at the University of Pennsylvania noticed something strange about the chromosomes in the leukemia cells of 7 patients with chronic myelogenous leukemia (CML). One of the smallest of the 46 chromosomes that human cells normally have was even smaller than usual. They published their observation as a brief note in *Science* (Nowell and Hungerford, 1960). Even though their brief report was

immersed among other small reports, it did not go unnoticed. In modern parlance, one could say it went viral.

Cancer cells were long known to have scrambled and abnormal chromosomes, but the observation of a specific chromosome change in a particular type of malignancy was so novel and remarkable that it was soon confirmed in many laboratories, and the strange little one then came to be known as the Philadelphia chromosome (Figure 14.1). Nowell and Hungerford surmised correctly that the novel little chromosome was somehow connected with the cause of the disease, but they had no clue just how.

The Ph^+ chromosome, as it is now designated, is nearly diagnostic for chronic myelogenous leukemia (95% of patients with CML have it), although it also occurs occasionally in some other types of leukemia.

At last, in 1973, Janet D. Rowley at the University of Chicago figured out what the Ph^+ chromosome was (Rowley, 1973). Using new staining techniques, she saw that it consisted of a piece of chromosome 9 (a moderately long chromosome) stuck to a piece of chromosome 22 (one of the smallest chromosomes). A combination of parts from those two chromosomes is what the tiny Ph^+ chromosome was. Still, she had no idea how the Ph^+ chromosome caused the disease.

She did however know how such abnormal chromosomes form: by a phenomenon called "chromosome translocation" that tends to occur in cancer cells, as well as in cells exposed to radiation or mutagens that break chromosomes. Broken ends of chromosomes often stick to each other forming abnormal chromosomes from the joined-up pieces. Thus Ph^+ results from a t(9;22) translocation between chromosomes 9 and 22 (Figure 14.2). Chromosome translocations are common in cancer cells, but this particular one is closely associated with and by far the most frequent cause of CML. Ph^+ was noticed because of its unusually small size and frequent occurrence in CML. But it was still puzzling why this particular translocation tends to occur, considering the huge number of different translocation possibilities that might exist among the chromosomes of a cell.

It took another decade to work out what was going on in the Philadelphia chromosome (Ph^+). In chromosome 9, there is a gene called ABL that tends to push cells to divide and multiply. Normally, ABL is kept under control, so that it doesn't cause cells to keep on dividing like the brooms in The Sorcerer's Apprentice (from the film "Fantasia"). In chronic myelogenous leukemia (CML), an unregulated ABL keeps immature white blood cells dividing until they eventually overwhelm the body. ABL remains active and out of control in CML because of a gene region in a part of the Ph^+ chromosome (called BCR) that comes from chromosome 22 (Figure 14.2). The BCR-ABL combination is what causes the trouble: the BCR in the piece from chromosome 22 is right next to the greater part of the ABL gene that comes from chromosome 9. The BCR part stimulates the ABL part to produce a large amount of an abnormal ABL protein that continually pushes the cell into the cell division cycle.

On the positive side, however, it gave oncologists a target, namely the abnormal BCR-ABL protein, which only CML cells need. It looked like a perfect chance to kill those malignant cells without harming normal cells. To understand how that therapy works and why it is not by itself the whole solution, we must delve a little deeper into how BCR-ABL causes its effects: how it induces cells to keep on dividing.

To summarize to this point: what is important about the Ph^+ chromosome is not its small size, but the way the two chromosomes, 9 and 22, are joined so as to connect the ABL gene from chromosome 9 directly to the BCR gene from chromosome 22 (Figure 14.2). That arrangement causes an abnormal protein to be made that includes almost all of the normal ABL protein plus a piece that is coded by the BCR gene (Ben-Neriah et al., 1986) (Heisterkamp et al., 1983). The attached piece of BCR stimulates the translation and transcription of the truncated ABL gene, thereby producing an abnormal ABL protein that induces cells to divide without end: the attached BCR piece prevents the ABL part from being turned off. Consequently, the malignant cells continue to proliferate without control (Wang and Pendergast, 2015). The defective control however provides an opportunity for therapy, as we shall now see.

The breakage and rejoining points on chromosomes 9 and 22 (a bit of minor detail here) are not always in exactly the same place, which means that the resultant BCR-ABL protein can vary somewhat. The clinical picture of the disease therefore may vary somewhat (Lugo et al., 1990). In fact, the reason that the break is in approximately the same place in chromosome 22 is that BCR, which stands for "breakpoint cluster region" is a region of the chromosome that, as its name implies, is particularly prone to break.

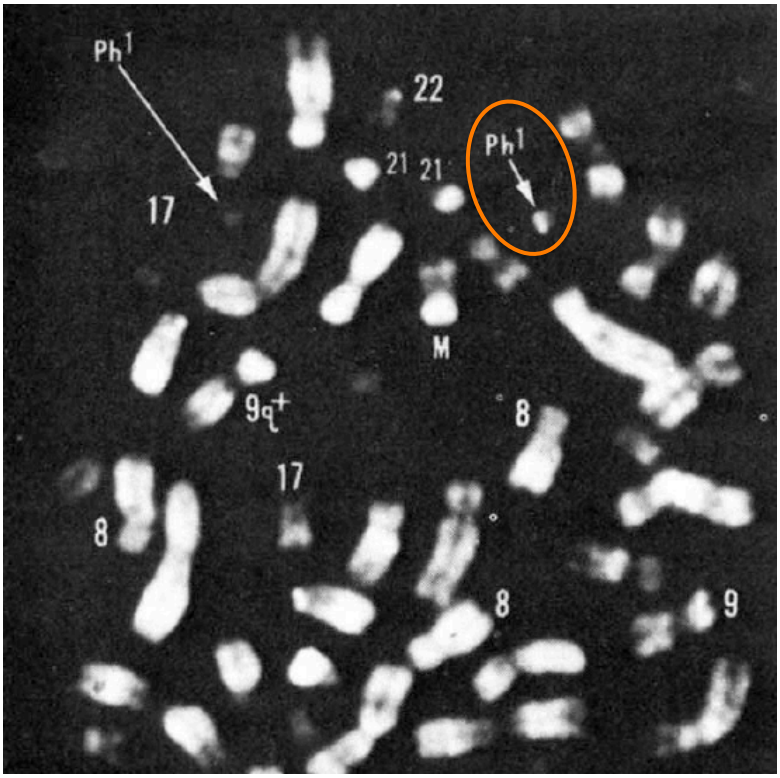


Figure 14.1. The Philadelphia chromosome (Ph^1) in a chronic myelogenous leukemia (CML) cell. The patient's CML cells were cultured, blocked in metaphase with vinblastine (see Chapter 10) and then stained in a way that allows chromosomes to be identified. The identifiable chromosomes in this image are numbered. From Rowley (1973) *Nature* (Rowley, 1973).

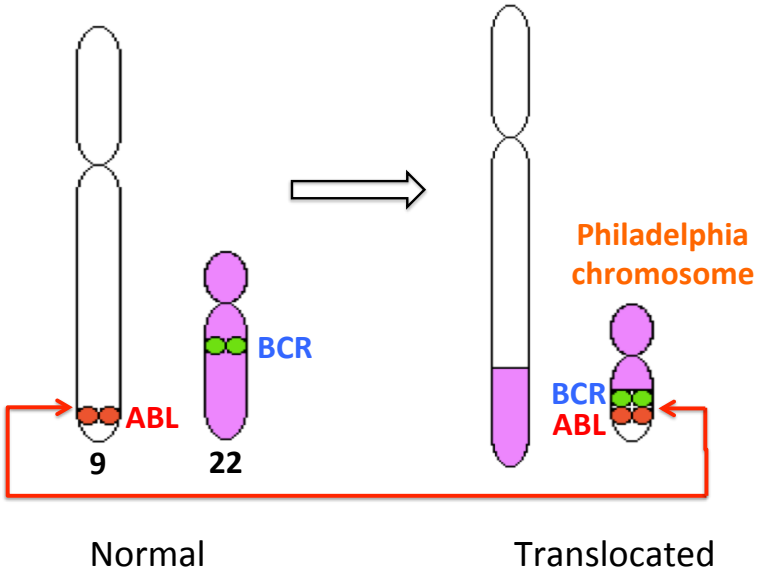


Figure 14.2. How a Philadelphia chromosome forms by translocation of pieces from chromosomes 9 and 22. This puts the ABL gene from chromosome 9 next to part of the BCR gene.

Taking advantage of the control defect

It was already known that the ABL gene codes for a protein tyrosine kinase. Tyrosine kinases are proteins (enzymes) that can stick phosphate groups onto tyrosine amino acid units of other protein molecules. Many of those proteins are then turned on or off, depending on which of several possible tyrosine units become phosphorylated. The proteins phosphorylate by ABL then send signals (mostly via chains of phosphorylation events) to the system that initiates processes leading to cell division.

(The important thing about a phosphate group, by the way, is that it has a negative charge. An electric charge on a protein can have a big effect on its structure and function. A cell's regulatory network is in that respect somewhat like an electronic computer: presence or absence of a phosphate on a particular protein is like on/off in an electronic unit. Phosphorylations on serine or threonine units, too, can regulate protein functions, but, for many initiators of cell division, it is usually the tyrosines that are most important.)

To recapitulate the important point: when ABL's tyrosine kinase activity is continually on, as in the BCR-ABL fusion protein in CML, signals are continually sent to activate the proteins that initiate cell division. An inhibitor of the tyrosine kinase function of the BCR-ABL protein should therefore halt the malignant cell division process (Druker, 2002). (Note that, when we say "BCR-ABL", we have to specify whether we mean the fusion protein or the gene that codes for it.)

It turns out that, not only do inhibitors of the BCR-ABL protein stop the uncontrolled cell division, but they cause the malignant cells to die. It is as if the malignant cells have become addicted to the abnormally high levels of ABL tyrosine kinase activity; when that activity is cut off, the cells die.

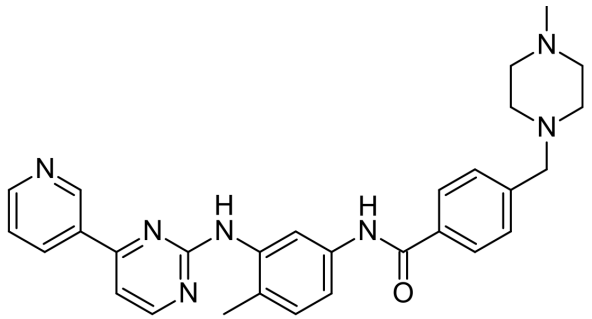
Before continuing the BCR-ABL story, however, we should note that ABL is only one of a large family of tyrosine kinases that have important signaling functions. Many of them also stimulate the cell division process, and some are protagonists in the stories of other tumors and opportunities for their treatment. Those stories, as well as the story of the early search for tyrosine inhibitors, are the subject of the next chapter.

Selective inhibitors of ABL tyrosine kinase

At this point in the story, the basic science information indicated that, if oncologists could inhibit the tyrosine kinase activity of the ABL part of the BCR-ABL fusion protein, the uncontrolled proliferation of CML cells would be stopped, and the malignant cells might even die. Several tyrosine kinase inhibitors were already known in the 1980's (Druker, 2002), but the problem was that the cell has many different tyrosine kinase proteins that it needs in order to regulate many essential processes. The previously known inhibitors of tyrosine kinases were non-selective: they inhibited almost all of them. A normal cell can tolerate inhibition of the tyrosine kinase activity of its normal ABL protein, but inhibiting the cell's other tyrosine kinases would not be good. It was necessary to find inhibitors that mostly inhibited the tyrosine kinase activity of only the ABL protein.

An enormous effort was made to find the right kind of selective tyrosine kinase inhibitors. Many compounds were synthesized by chemists or obtained from natural sources and tested for their abilities to inhibit different tyrosine kinases needed by the cell. In addition, researchers tested the ability of their potential drugs to selectively kill or inhibit the proliferation of cells that had the BCR-ABL fusion protein.

To guide the search for the best chemical structure, the researchers studied the relationship between the chemical structures of the compounds and their abilities to selectively inhibit the ABL tyrosine kinase or to kill only cells that have the BCR-ABL abnormality. The first drug to come out of that endeavor and promising enough to put into clinical trial against CML was imatinib, also called Gleevec (Figure 14.3) (Druker, 2002; Druker et al., 1996).



Imatinib (Gleevec)

Figure 14.3. Chemical structure of imatinib (Gleevec), the first clinically effective inhibitor of the BCR-ABL tyrosine kinase. The drug inhibited BCR-ABL, as well as the normal ABL tyrosine kinase, but had no effect on a panel of other protein tyrosine kinases (Buchdunger et al., 1996; Druker et al., 1996).

Results in the early clinical trials were spectacular: in a phase III trial of 553 newly diagnosed CML patients, 96% of those treated with imatinib had a complete disappearance of visible CML cells from the blood and bone marrow, and in 68% there was no longer any trace of the Philadelphia chromosome. These remissions of the disease lasted more than 14 months, which was the time limit of that trial. In an equal number of patients in this randomized phase III comparison with the previous standard treatment with interferon plus cytosine arabinoside, treatment with imatinib was much superior (Druker, 2002).

The clinical researchers were impressed by the low toxicity of effective imatinib treatments, which was very different from the experience with cytotoxic chemotherapy (less that 1% of patients had side effects that limited treatment with imatinib) (Druker, 2002). The reason for the low toxicity of imatinib might be that ABL tyrosine kinase has a critical role in embryonic development but not in adults (Wang, 2014). The ABL tyrosine kinase protein is turned on in adult CML because of its fusion with the BCR fragment. That is why CML cells that have the ABL-BCR fusion protein respond to specific therapy with imatinib/Gleevec. To go from the discovery of the Philadelphia chromosome to effective treatment of CML however took nearly 4 decades.

Many patients who initially respond well to imatinib, eventually encounter drug resistance, where the malignant cells no longer respond. In order to inhibit BCR-ABL, the drug must bind to a site on the ABL part of the protein. Resistance develops when the malignant cells develop a mutation in the critical site of the protein, which prevents the drug from binding there (Greuber et al., 2013). Drugs are therefore being developed that will bind to ABL at a different site.

The next chapter will be about receptor tyrosine kinases, which are located in the cell surface membrane and convey signals from the outside to the inside of the cell. ABL

however is a non-receptor tyrosine kinase, not localized to the cell surface. Instead, it shuttles information between cytoplasm and nucleus. It can be activated by certain receptor tyrosine kinases, from which it then transmits signals to the nucleus to activate genes for cell division. When not engaged in this signal transmission task, ABL normally self-inactivates. The BCR-ABL combination gets around this self-inactivation and causes the ABL signaling to continue non-stop, thereby inducing non-stop cell division and cancer. ABL-inhibitor drugs, such as imatinib/Gleevec, block ABL's tyrosine kinase activity, thereby blocking its ability to signal genes in the nucleus to initiate cell division.

{Kaelin, 2005 #657}
(Wang and Pendergast, 2015)
Search for actives for resistant CML
Sausville NIH lecture March 10, 2016

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