Chapter 14. The Philadelphia Chromosome Story and a new era of targeted cancer therapy 220826as3

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

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CHAPTER 14

The Philadelphia Chromosome Story and a new era of targeted 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 have learned 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 was needed to tell physicians what molecular defects are driving the malignant process in each particular patient and what drugs may provide a remedy. This chapter is about an unexpected observation more than 60 years ago that became a harbinger of this approach long before the idea of targeted cancer therapy was even conceived. It came from noticing under the microscope something strange 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 would 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 (CML), 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 cancer.

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 many types of 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. And why was it specifically associated with CML?

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* (or recently denoted *ABL1*) 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. (Italics are commonly used when the name refers to a gene, as opposed to its protein product that may go by the same name.)

On the positive side, however, it gave oncologists a target, namely the abnormal BCR-ABL protein, which only CML cells need to stay alive and actively dividing. 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 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, join so as to connect the *ABL* gene from chromosome 9 directly to the *BCR* gene from chromosome 22 (Figure 14.2). That rearrangement caused an abnormal protein to be made that included 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* stimulated the action of the slightly truncated *ABL* gene, thereby producing an abnormal ABL protein that induced the cells to divide without end: the attached *BCR* piece prevented the *ABL* part from being turned off. Consequently, the malignant cells continued to proliferate without control (Wang and Pendergast, 2015). The defective control however provided an opportunity for therapy.

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

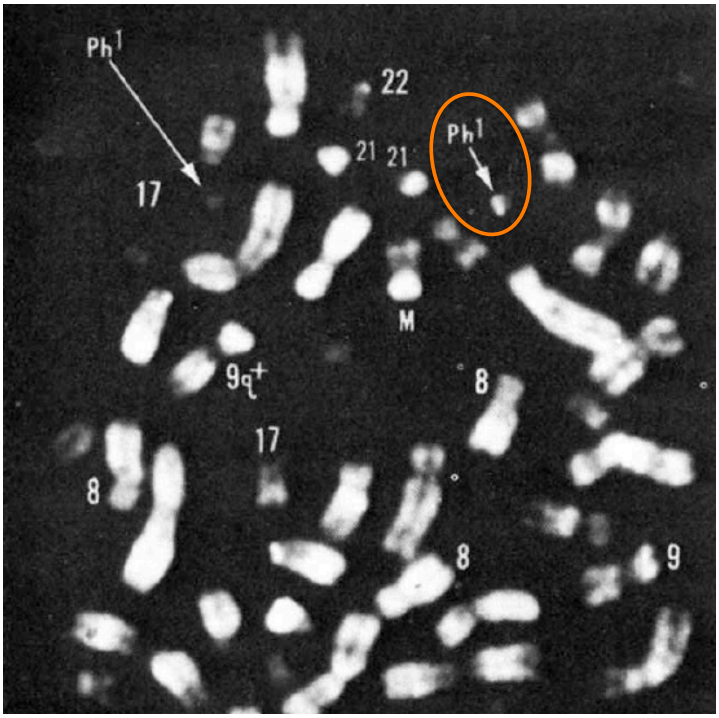


Figure 14.1. The Philadelphia chromosome (Ph^1) in a chronic myelogenous leukemia (CML) cell. The patient's CML cells were cultured, blocked in metaphase with vinblastine (see Chapter 10) and then stained to allow chromosomes to be identified. The identifiable chromosomes in this image were numbered.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

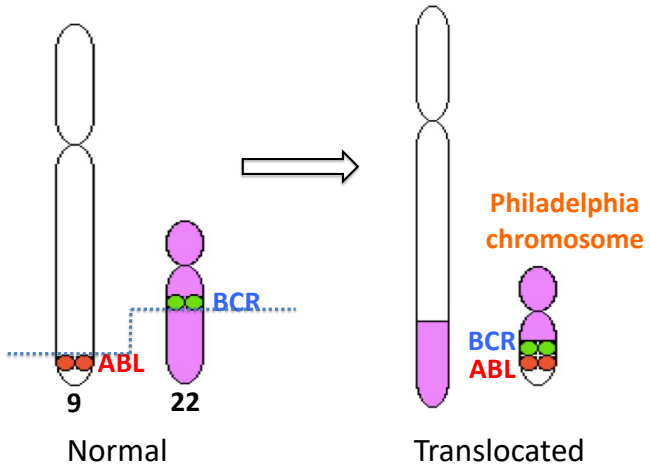


Figure 14.2. How a Philadelphia chromosome forms by translocation of parts from chromosomes 9 and 22. The translocation puts the *ABL* gene from chromosome 9 next to the *BCR* gene from chromosome 22 in a new chromosome, called the Philadelphia chromosome. The dotted line show where chromosomes 9 and 22 break; the parts of the two chromosomes then join (translocate) as shown on the right.

How the ABL gene was discovered.

In the 1960's several cancer-causing viruses had been discovered. Each of them had somehow incorporated a gene that drove the cancer, which was usually a leukemia, lymphoma or sarcoma. The viruses induced cancers in various strains of mice. In 1970, Herbert Abelson and Louise Rabstein reported a new cancer-causing virus in mice that was unusual in that it did not involve the thymus (Abelson and Rabstein, 1970). The new virus acquired the name *Abelson leukemia virus* and the cancer-causing gene (oncogene) harbored by the virus was duly dubbed *ABL* (or v-*ABL* to distinguish from c-*ABL* for the gene in normal cells).

That was during an era when much effort was directed to the idea that viruses were the major cause of cancer in humans, an erroneous idea that only slowly died; a large story could be told about that era. Nevertheless, the oncogenes in those viruses *were* major causes of human cancers (Chapter 15). The oncogenes were in fact mutated versions of normal genes that the viruses had picked up during their transfer in mice. The normal versions of those genes were capable of driving cell division, but that action was normally under control, so as to limit how often a cell would divide. Control was lost when the gene became mutated or otherwise altered; without the control, the excessive cell division led to cancer.

Taking advantage of the control defect caused by the translocation.

It was long 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. Those kinases each act on particular proteins that convey signals to turn particular cell functions on or off. The proteins phosphorylated by the ABL tyrosine kinase sends signals -- mostly via chains of phosphorylation events -- to the system that initiates processes leading to cell division. That's how the overactive *ABL* gene in the Philadelphia chromosome caused the malignant disease.

(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 phosphorylations on tyrosines that are most important.)

It turned out that, not only did inhibitors of the BCR-ABL protein stop the uncontrolled cell division, but they caused the malignant cells to die. It was as if the malignant cells had become addicted to the abnormally high levels of ABL tyrosine kinase activity; when that activity was cut off, the cells died.

To recapitulate the important point: when ABL's tyrosine kinase activity was continually on, as in the BCR-ABL fusion protein in CML, signals were continually sent to activate the proteins that initiated cell division. An inhibitor of the tyrosine kinase function of the BCR-ABL protein would therefore halt the malignant cell division process (Druker, 2002). (When we say "BCR-ABL", we have to specify whether we mean the fusion protein or the gene that codes for it. I use the convention of gene names in *italics*.) It was a long way to go from the discovery of the Philadelphia chromosome to effective treatment of CML that stretched to nearly 4 decades.

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 a great many of them of them. A normal cell can tolerate inhibition of the tyrosine kinase activity of its ABL protein, but inhibiting many of 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, 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 fusion protein. 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).

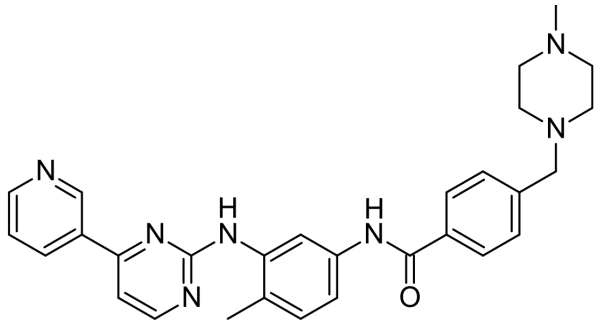
Researchers at Oregon Health Sciences University and Ciba-Geigy of Basel, Switzerland found a specific ABL inhibitor in 1996 that they called CGP57148 and which became known as imatinib or Gleevec. Importantly, the new drug inhibited the tyrosine kinase activity equally well of BRC-ABL and of normal ABL (c-ABL) and was inactive against a panel of other tyrosine kinase, as well as serine/threonine kinases (activity was also noted against the receptor of platelet-derived growth factor receptor (PDGF-R)) (Table 1).

Table 1 Profile of inhibition of protein kinases by CGP 57148

Protein kinase	Substrate phosphorylation IC_{50} value (μM)	Cellular tyrosine phosphorylation IC_{50} value (μM)
v-Abl	0.038	0.25
Bcr-Abl	0.025	0.25
c-Abl	0.025	
EGFR-R–ICD	>100	>100
Her-2/neu		>100
Insulin receptor		>100
IGF-1R		>100
PDGF-R		0.3
c-Src	>100	
v-Src		>100
c-Fgr	>100	
c-Lyn	>100	
v-Fms		>100
TPK-IIB	>100	
PKA	>500	
PPK	>100	
PKC α, β1, β2, γ, ε, σ, η, ζ	>100	
Casein kinases - 1 and 2	>100	
cdc2/cyclin	>100	

Abbreviations: EGF-R–ICD, epidermal growth factor receptor–intracellular domain; IGF-1R, insulin like growth factor-1 receptor; TPK, tyrosine protein kinase; PKA, protein kinase A; PPK, phosphorylase kinase; PKC, protein kinase C.

Table 1. Specificity of Ciba-Geigy CGP57148 for inhibition of the protein kinase activity of ABL. The drug became known as imatinib or Gleevec (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 in the treatment of chronic myelogenous leukemia (CML). 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. Compared to an equal number of patients who received the previous standard treatment with interferon plus cytosine arabinoside, treatment with imatinib was much superior (Druker, 2002). The success of the treatment of CML with imatinib opened the door to the era of targeted cancer therapy: it was the first time that a successful drug was designed to act on a specific protein target.

The clinical researchers were impressed by the low toxicity of effective imatinib treatments, which was very different from the experience with other cytotoxic chemotherapy. Less that 1% of patients had side effects severe enough to limit treatment with imatinib (Druker, 2002).

But why did imatinib give long-term survival of most chronic myelogenous leukemia (CML) patients, while it was not nearly as effective in treatment of other malignancies? One possibility that was considered was that the *ABL* gene is mainly needed only during development of the embryo and is dispensable in adults (Wang, 2014). Therefore, a drug that specifically targeted ABL could be given at high enough dosage to completely block the ABL component of the abnormally active BCR-ABL fusion protein that drives the disease.

Many tyrosine kinases are located in the cell surface membrane and convey signals from receptors in the outside to actions in the inside of the cell (Chapter 17). 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 is self-inactivated. 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, blocked ABL's tyrosine kinase activity, thereby blocking its ability to signal genes in the nucleus to initiate cell division.

How imatinib inhibits the ABL tyrosine kinase activity.

The molecular details of how imatinib inhibits ABL was revealed by crystallographic analysis that showed the structure of the protein and how imatinib binds to it (Figure 14.4). ABL was shown to work by first binding ATP within a pocket in the protein and then transferring the high energy phosphate bond from ATP to phosphorylate the substrate proteins. Imatinib binds to ABL in the pocket where ATP ought to bind but cannot because the drug is already bound there.

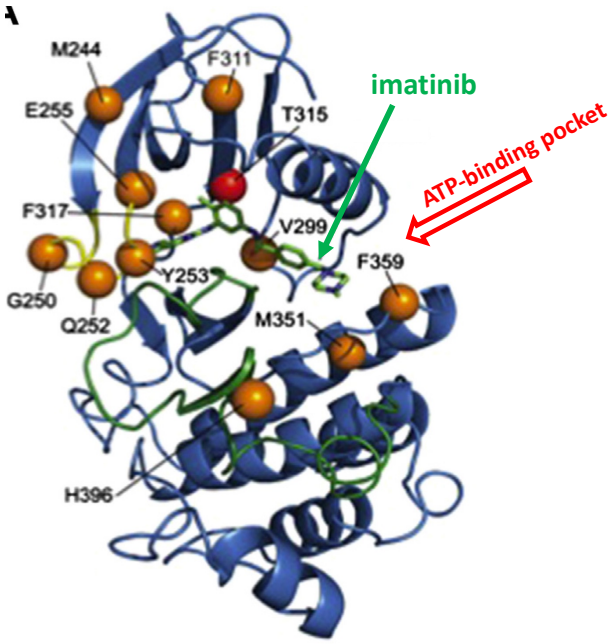


Figure 14.4. The crystallographic structure of the ABL protein, showing the pocket where ATP would bind. The structure shows imatinib binding within the pocket, thereby preventing ATP from binding there, and thereby inhibiting ABL's tyrosine kinase activity. (From (Patel et al., 2017) with labels added.)

Tyrosine kinase inhibitors FDA-approved for treatment of CML.

The large majority of chromic myelogenous leukemia (CML) patients treated with imatinib had long-lasting remissions. Nearly 90% of the patients survived more than 5 years without signs of any return of the disease (Eide and O'Hare, 2015). However, patients

eventually relapsed and became resistant to imatinib. Therefore, new drugs were sought for patients who had become resistant to imatinib. By 2015, four new tyrosine kinase inhibitors were approved by the U.S. Food and Drug Administration (FDA) for treatment of malignancies driven by chromosome translocations yielding a BCR-ABL fusion (Eide and O'Hare, 2015) (Figure 14.5).

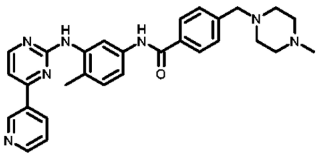
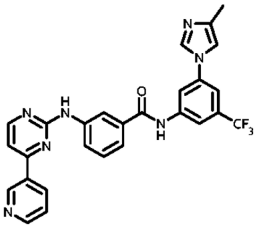
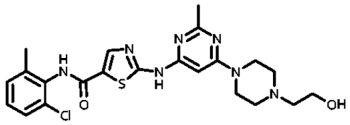
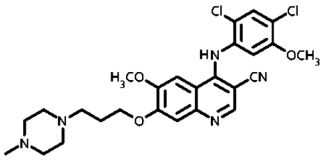
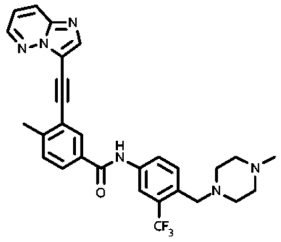
Inhibitor	Chemical structure	Binding site/ Inhibitor type	Regulatory status/ approval
Imatinib (Gleevec)		ATP-binding site/ ATP-competitive	FDA approved/ frontline therapy
Nilotinib (Tasigna)		ATP-binding site/ ATP-competitive	FDA approved/ frontline therapy
Dasatinib (Sprycel)		ATP-binding site/ ATP-competitive	FDA approved/ frontline therapy
Bosutinib (Bosulif)		ATP-binding site/ ATP-competitive	FDA approved/ 2nd-line therapy
Ponatinib (Iclusig)		ATP-binding site/ ATP-competitive	FDA approved/ 2nd-line therapy

Figure 14.5. The FDA had by 2015 approved these five tyrosine kinase inhibitors to treat malignancies driven by BCR-ABL (Eide and O'Hare, 2015).

How the function of the ABL protein is regulated.

I begin with the help of Figure 14.6 to relate what was learned about the function of the different parts of the ABL protein. It consists of a series of domains and motifs lined up from the amino-terminus to the carboxy-terminus of its amino sequence (Figure 14.6). This

kind of cobbling together of domains and motifs, as well as phosphorylation sites, is typical for many proteins that function in regulatory pathways. Starting at the amino end (*left*), we find an SH2 domain that is notable for its ability to bind phosphorylated tyrosines of proteins. It is followed by an SH3 domain that binds to some amino acid sequences that have two prolines separated by two other amino acids (PXXP). Then, there is a tyrosine (Y) that can be phosphorylated; it is within a short sequence of amino acids that links to the next domain where the tyrosine kinase enzyme function of the protein resides. We come next to an amino acid stretch that contains three PXXP motifs that can serve to bind to SH3 domains of various other proteins. Next is the DNA-binding domain that binds to certain gene promoters and turns on the transcription of those genes. Interestingly, ABL is both an enzyme and a gene regulator. The latter activity depends on the amount of ABL that is in the cell nucleus, which is regulated by two different motifs: one controls its entry into the nucleus (NLS, nuclear localization signal); the other controls its exit (NES). Finally, we come to two domains that bind to actin cytoskeleton; this binding tends to keep ABL in the cytoplasm and out of the cell nucleus.

Such an arrangement of SH2, SH3, PXXP, and phospho-tyrosine domains and motifs allowed ABL to link to other proteins to form multi-protein integrated networks of regulated functions. It turned out, however, that those domains and motifs formed bonds within the same protein molecule as well, forming an internal clamp that keeps the ABL protein inactive until the clamp was relieved by external interactions (Hantschel and Superti-Furga, 2004). This situation is the same in a closely related tyrosine kinase, SRC, for which I had some years ago prepared a molecular interaction map (Figure 14.7). The legend to the Figure describes the essential features of the regulation of this internal clamp.

ABL in cancers.

ABL was found to promote the development of several types of cancers other than leukemias. The activation of ABL in the solid-tumor-type cancers, however, was often not due to chromosome translocations. Sometimes the *ABL* gene in these cancers was amplified or mutated, thereby increasing its promoting of the cancers. An amplified gene has multiple copies of the gene that work together and increase the net expression of the gene. The possible roles of those actions on cancers however was not fully established (Wang and Pendergast, 2015). Some cases of *ABL* gene mutations were found but their role in cancer also was not entirely clear. Nonetheless, some patients benefitted from treatment with the ABL tyrosine kinase inhibitors dasatinib, bosutinib or nilotinib (Jones and Thompson, 2020).

Clearly, overactive ABL promoted the development of malignancies, particularly chronic myeloid leukemia (CML). As already explained above, the activity of ABL was normally kept in check by internal bindings: the SH3 domain with the PXXP motif and the SH2 domain with phospho-tyrosines Y245 and Y412 (Figure 14.6). When ABL was activated in the course of the normal functioning of the cell, this happened by controlled external interactions that competed with the internal inhibitory bindings (Figure 14 .7).

Uncontrolled activation of ABL was found to occur when a recombination deleted or disrupted its SH3 domain, which is at the N-terminal end of the protein (Figure 14.8).

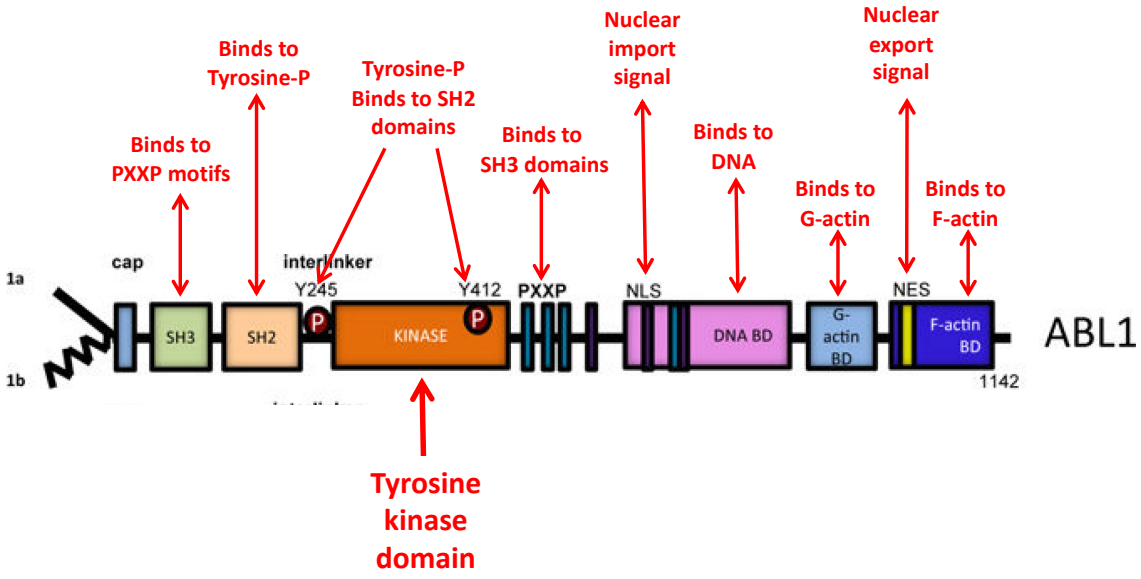


Figure 14.6. Domain structure of the ABL protein showing the motifs and domains and what they bind to. Like many signaling proteins, ABL is made up of a number of binding motifs and domains that are cobbled together into an integrated functional unit. Y = tyrosine; P = phosphate. ABL1 = ABL. (ABL2 = ARG, which is not included here.) (From (Wang and Pendergast, 2015) with labels in red added.)

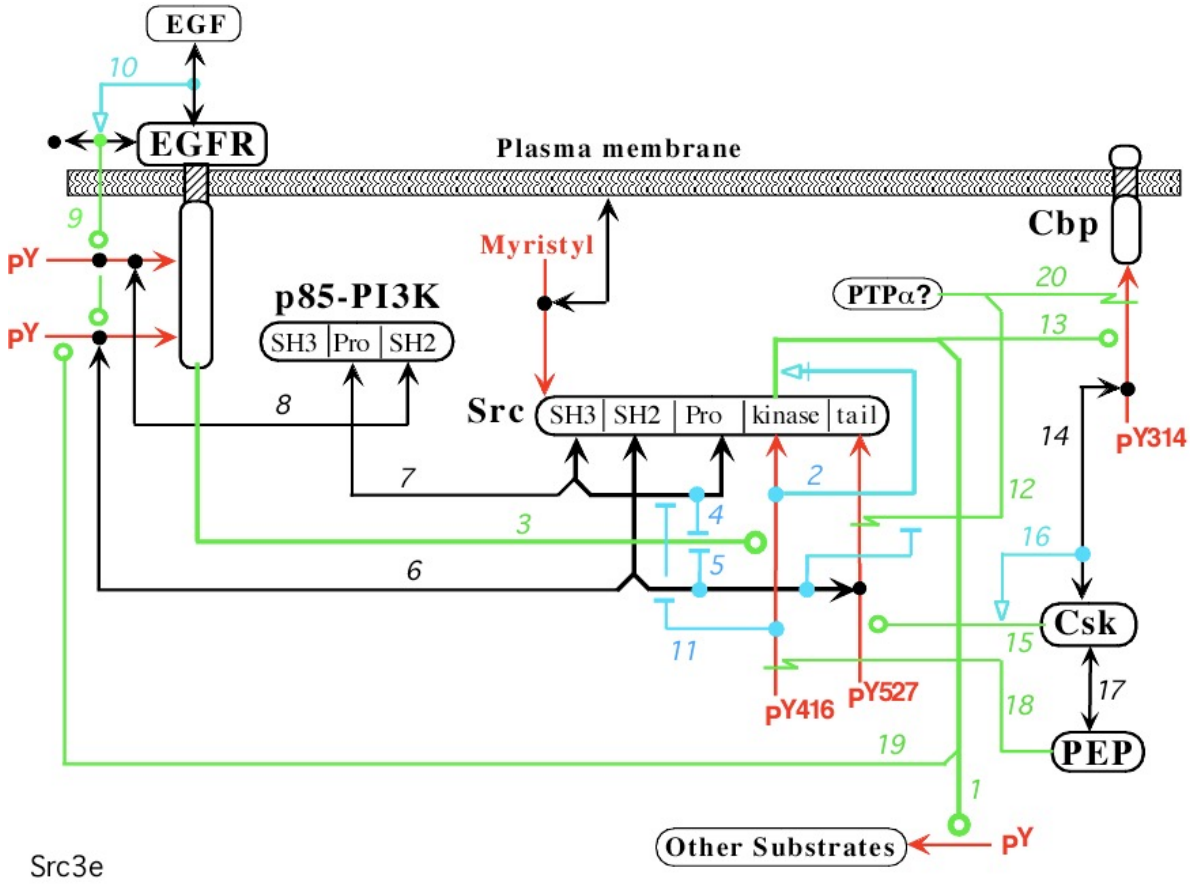


Figure 14.7. Molecular interaction map (Kohn, 1999) of the SRC tyrosine kinase, showing the internal clamp and its release, which are very similar in ABL. The double-arrowed black lines point between elements that bind to each other. Bindings between different parts of the protein form an internal clamp consisting of SH3 bound to Pro (PXXP) and SH2 bound to a phospho-tyrosine (P^Y); this is the same in SRC and ABL. The internal clamp can be released by the combined actions of (1) a Pro (PXXP) domain of p85-PI3K binding to SRC's SH3, displacing the internal bond from the SH3 to Pro; (2) a phospho-tyrosine of EGFR binding to SRC's SH2, displacing the internal bond from the SH2 to a phospho-tyrosine near the carboxy end of the protein. These interactions are similar for SRC and ABL, except that the clamp release proteins may differ. The two steps can happen in concerted fashion, because the p85-PI3K and EGFR are bound to each other. (The myristyl group that is linked to the amino-terminal region of ABL, rather than being bound to the cell membrane as shown in the diagram, actually binds to a hydrophobic pocket in the protein itself. This intramolecular binding further stabilizes the clamp that inhibits the kinase.)

Chromosome translocations drive leukemias.

It turned out that *BCR* on chromosome 22 (Figure 14.2) was not the only translocation that activated *ABL* in leukemias. Alternative translocations were found that occasionally drove

leukemias. In those cases, there was no Philadelphia chromosome, because the translocated chromosomes were not so tiny. Figure 14.8 shows some of those translocations. The break site of the translocation was often at a place that retained at least much of the SH3 domain at the amino end of the protein (upper part of Figure 14.8).

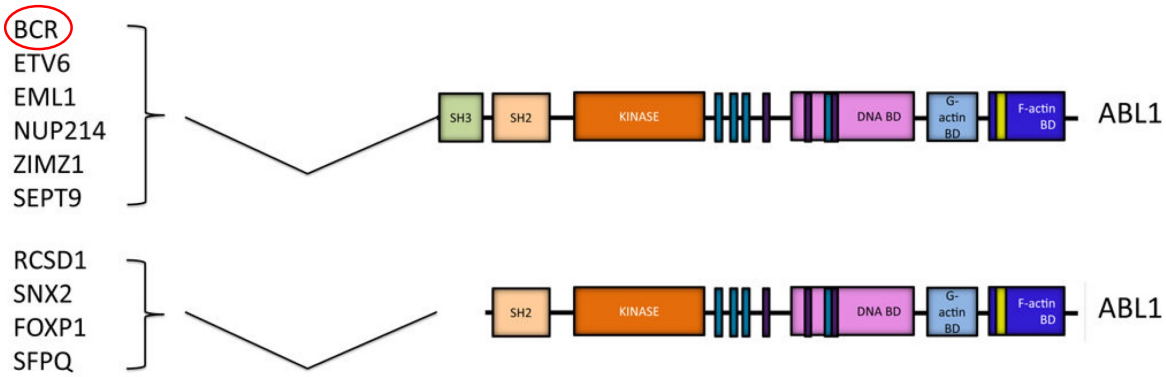


Figure 14.8. Alternative chromosome translocations in leukemias (Wang and Pendergast, 2015). The names on the left are of the genes that became placed next to the *ABL* gene. These translocations did not result in a Philadelphia chromosome. In the cases shown in the lower part of the Figure, the translocation cuts the *ABL* after the SH3 domain, which therefore is not included in the product of those translocations, and internal self-inactivation cannot occur. (From (Wang and Pendergast, 2015) with red oval added.)

Chronic myelogenous leukemia (CML) becomes resistant to imatinib.

Although the great majority of CML patients (as well as occasional BCR-ABL-positive acute lymphatic leukemia (ALL) patients) responded well to imatinib for several years, the disease eventually recurred and was then resistant to the drug. The resistance was usually due to a mutated BCR-ABL that did not bind the drug. Two new ABL inhibitors, nilotinib and dasatinib, were active against some of those mutants; the chemical structure of nilotinib closely resembles imatinib, while dasatinib is more different (Figure 14.5).

However, there was a particular mutant, BCR-ABL^{T315I} (threonine at position 315 of ABL replaced by isoleucine) that was resistant to all three drugs. To meet this problem, a new inhibitor, ponatinib (Figure 14.5; originally AP24534), was developed that worked against that mutant, as well as against other forms of BCR-ABL (O'Hare et al., 2009). Another drug, axitinib, found to work against CML cells harboring the BCR-ABL^{T315I} mutation is discussed in the next section.

The most common way that resistance developed was by a mutation in the cell's ABL protein that altered the drug-binding pocket in a manner that prevented the drug from

binding there (Greuber et al., 2013). Many mutations around the pocket region of the ABL protein were discovered that reduced the effectiveness of imatinib. Drugs were developed that could bind to ABL despite the most common mutations, and some of those single mutations could be circumvented by one or another of the new inhibitors listed in Figure 14.5. There were however cases of double mutations for which no drug therapy was available (Eide and O'Hare, 2015).

Other albeit less common mechanisms of resistance were discovered that had to do with the fact that ABL acts inside the cell nucleus. The membrane that encompasses the nucleus has channels that pump ABL into the nucleus or that pump it out. Resistance occasionally developed due to defective exit pumps or to overactive input pumps. Either way, there would be too much ABL in the nucleus for the drugs to inhibit it all (Yaghmaie and Yeung, 2019). These pathways to drug resistance remained a challenge for medicinal chemists.

New drug combinations for chronic myelogenous leukemia (CML).

Since 1986, the National Cancer Institute (NCI) and cooperating institutions have been accumulating vast amounts of data on gene expression and drug sensitivities of many human patient-derived cell lines. The data contain much information about correlations and patterns that could be used in clinical and experimental studies but accessing and making sense of the vast data was a big challenge. Software tools to access and analyze the data were therefore developed, led by a Genomics and Pharmacology group within NCI's Developmental Therapeutics Branch. As the final part of this chapter, I used one of the tool sets, CellMinerCDB (Luna et al., 2021), to look for relationships between the expression of the *ABL* gene and the drug-sensitivities of various human cancer cell lines, with special attention to lines from chronic myelogenous leukemia (CML) patients (Figures 14.9 - 14.11 and Table 14.2). CellMinerCDB is freely accessible at https://discover.nci.nih.gov/rsconnect/cellminercdb/.

CellMinerCDB has data for several datasets from different institutions. The most useful for the current analyses were the CTRP-Broad-MIT and the GDSC-MGH-Sanger datasets. Figure 14.9 shows that results from these two datasets agreed with each other quite well. They show that most of the CML cell lines (red dots) expressed the *ABL* gene (*ABL1*) more than did the great majority of other leukemia cell lines (Figure 14.9, *left*) or of all the other lines in the datasets (*right*). It is interesting, as well as reassuring, that these cell line data gave results consistent with the clinical and experiment findings: namely, that CML cells expressed ABL1 to an unusually high degree. However, they also showed that there were a few non-CML lines that also exhibited high ABL1 expression; this too was consistent with the clinical finding of occasional cancers other than CML that had high ABL1 expression.

In further exploration, I focused on the CTRP-Broad-MIT dataset, because it had the larger number of CML cell lines. Figure 14.10 shows that CML cell lines, which had relatively high ABL1 expression, were highly sensitive to imatinib (Gleevec), as expected since the drug

was developed as a specific ABL inhibitor (Table 1). The other two drugs at the top of Figure 14.5 and Table 14.2, nilotinib and dasatinib, gave results similar to imatinib.

The next step was to use CellMinerCDB to get a list of drugs whose action against leukemia cell lines was most highly correlated with ABL1 expression (Table 14.2). We see that the top three entries in the table, as well as one at the bottom, were among the five drugs approved for treatment of ABL-positive CML, and whose chemical structures were shown in Figure 14.5. However, Table 14.2 also included several drugs that were approved for treatment of other cancers and that were reported to act on molecular targets other than or in addition to ABL1. Combining these different targets by combing the drugs could perhaps improve treatment.

The first of these possible combinations was imatinib (or dasatinib) together with axitinib (Figure14.11A and Table 14.2). We see that the CML cells were substantially more sensitive to both drugs than nearly all the other lines in the database. A literature search then disclosed a report that axitinib could overcome resistance mediated by a mutation of BCR-ABL (Halbach et al., 2016), which followed up on a brief letter that this might be the case (Okabe et al., 2015). There was also a more recent report of a CML patient who had become resistant to imatinib and dasatinib who then responded to a combination of axitinib and dasatinib (Deng et al., 2020). Other than those reports, I found nothing in the literature to relate axitinib to ABL or to CML, findings that came independently from CellMinerCDB (Figure14.11A and Table 14.2). Axitinib had been extensively investigated as an inhibitor of vascular endothelial growth factor receptors (VEGFR) that nourish cancers by stimulating blood vessel production in the tumors, and it had been combined with other drugs for treatment of several cancers other than leukemias. Clinical trials of axitinib in the treatment of BCR-ABL-mutated CML were planned.

Another potentially effective combination in the findings with CellMinerCDB was imatinib together with crizotinib (Figure 14.11B and Table 14.2). A literature search then disclosed a recent report that crizotinib inhibited resistant mutants including BCR-ABL^{T315I} (Mian et al., 2021). The drug was known to block several protein kinases, most notably hepatocyte growth factor receptor (HGFR, also known as MET) and anaplastic lymphoma kinase (ALK) and was approved for treatment of advanced lung cancers that had an ALK fusion protein that was continually active. Here again, CellMinerCDB independently predicted this effective drug combination.

Tivozanib, which appears below axitinib in Table 14.2, received FDA approval in March 2021 for treatment of advanced renal cell carcinoma (Chang et al., 2022). The drug is a tyrosine kinase inhibitor that targets vascular endothelial growth factor receptors (VEGFR), a platelet-derived growth factor (PDGFR), and KIT. However, there were no papers relating tivozanib to leukemia, CML, ABL1, or BCR-ABL in the PubMed literature. Figure 14.11C shows the selective responses of CML cells to tivozanib and imatinib. This is very much like the results with axitinib and crizotinib and suggests that the combination of tivozanib with imatinib or one of its approved relatives (nilotinib or dasatinib) would be another useful treatment of CML with a different range of kinase targets upon which the combination would act and counter drug resistance.

Below tivozanib in Table 14.2, we come to a drug called pluripotin. Although it has received little or no attention in the recent literature and we still do not know its targets of action, a report suggested that pluripotin may affect cancer stem cells in culture (Mertins et al., 2013). In view of its selective action on CML cells (Figure 14.11D), it would be interesting to explore its targets of action on the possibility that it may offer a novel therapy for drug-resistant CML. Notably, CML cells were the only type of cells that showed a selective response to pluripotin.

Next in Table 14.2, we come to masitinib, a tyrosine kinase inhibitor that targets PDGFR, FGFR, and KIT and that was in clinical trial against pancreatic cancer but showed little benefit (Aljoundi et al., 2019; Waheed et al., 2018). Masitinib was another drug that inhibited CML cells specifically and that could be considered for testing in combination with imatinib, nilotinib or dasatinib for treatment of drug-resistant CML (Figure 14.11E).

Finally worth noting is that Nilotinib showed a remarkably strong selectivity for action on CML cells (Figure 14.2F) and might be particularly effective in drug combinations.

Thus, CellMinerCDB can help reveal possible cancer targets, such as CML, including drugs already approved for treatment of other cancers.

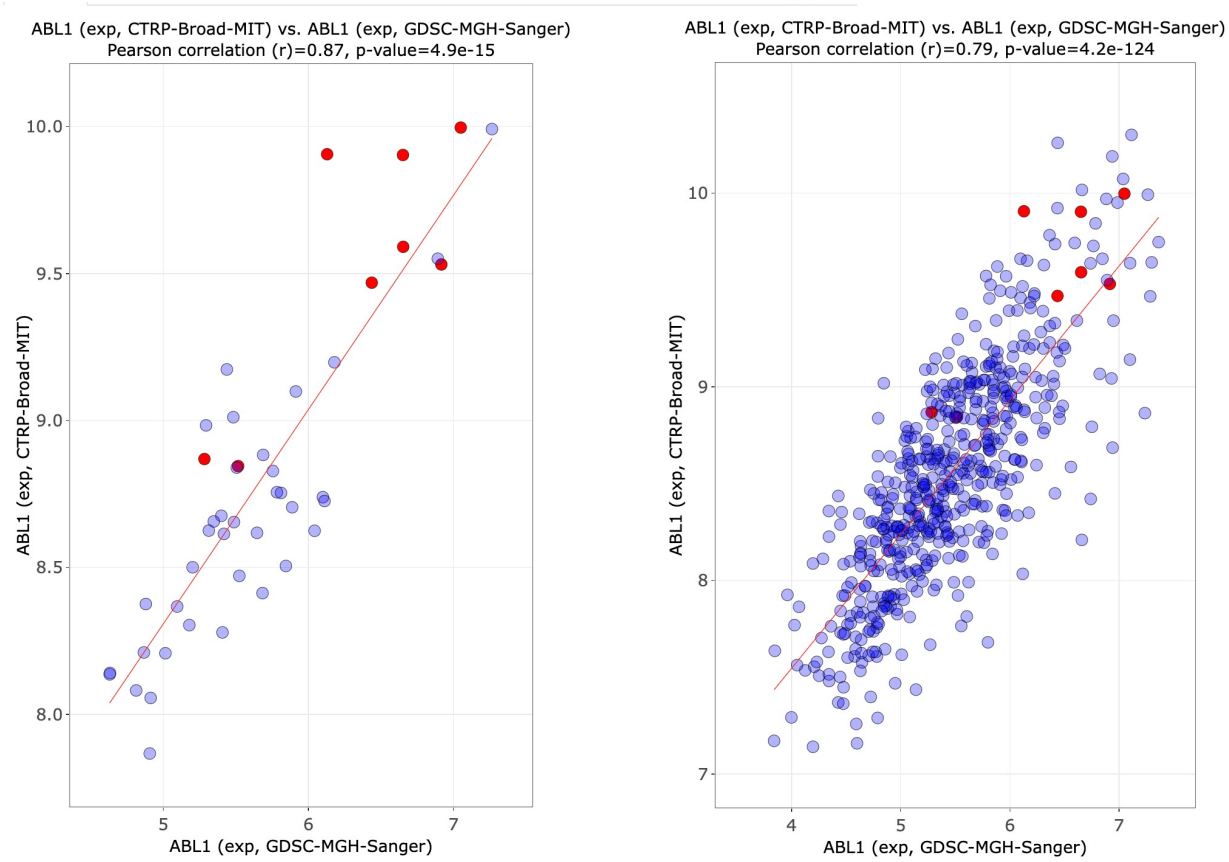


Figure 14.9. Six of eight chronic myelogenous leukemia (CML) cell lines (*red*) had high ABL1 expression relative to other cell lines. The cell lines shown were those for which there was data in both the CTRP-Broad-MIT (vertical axis) and the GDSC-MGH-Sanger (horizontal axis) data sets, showing the consistency between the two data sets. *Left*, data for CML relative to other leukemia cell lines. *Right*, data for CML relative to all other cell lines in both data sets. (I created the graphs using CellMinerCDB version 1.4 (Luna et al., 2021). Scales are in log2 units.) https://discover.nci.nih.gov/rsconnect/cellminercdb/

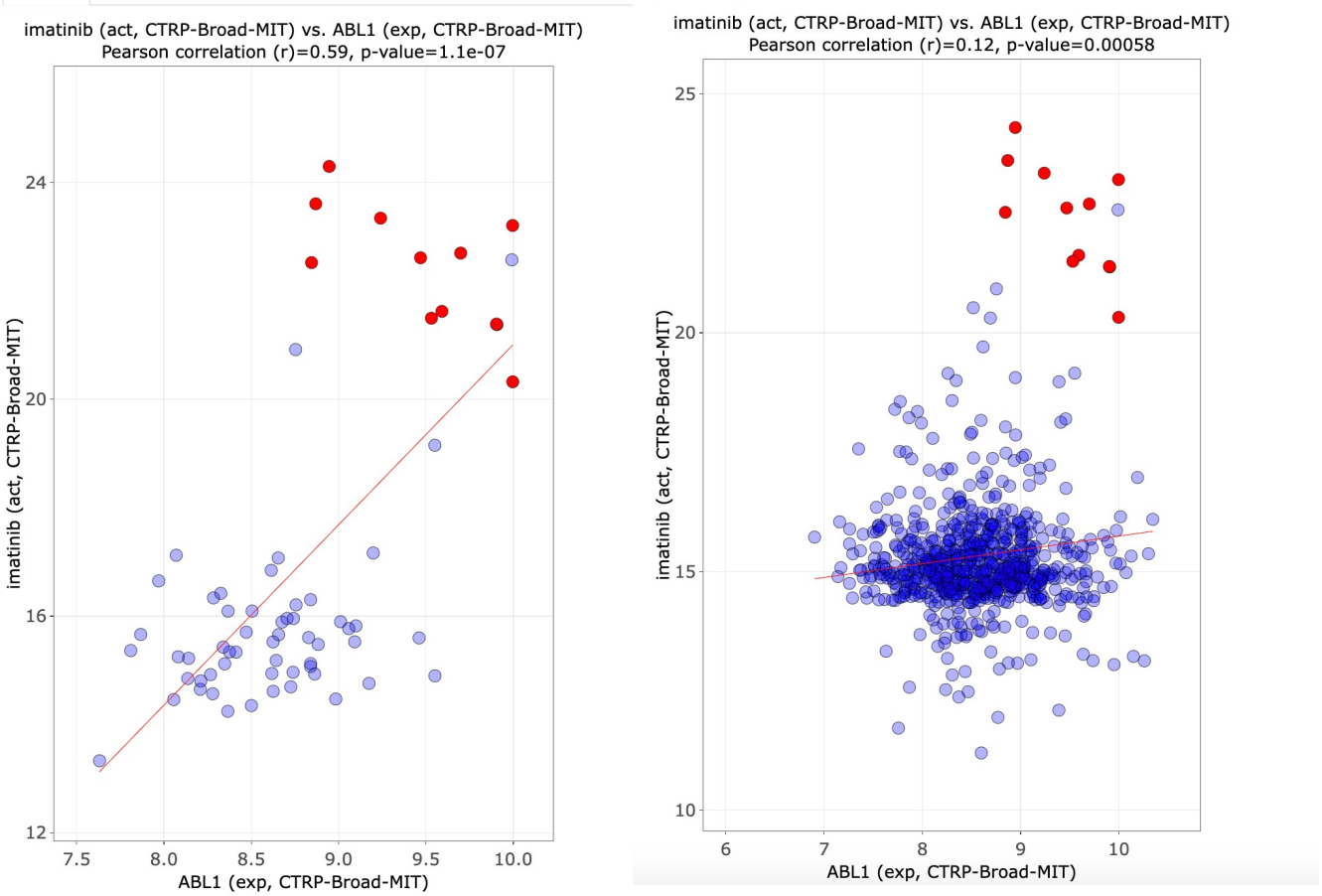
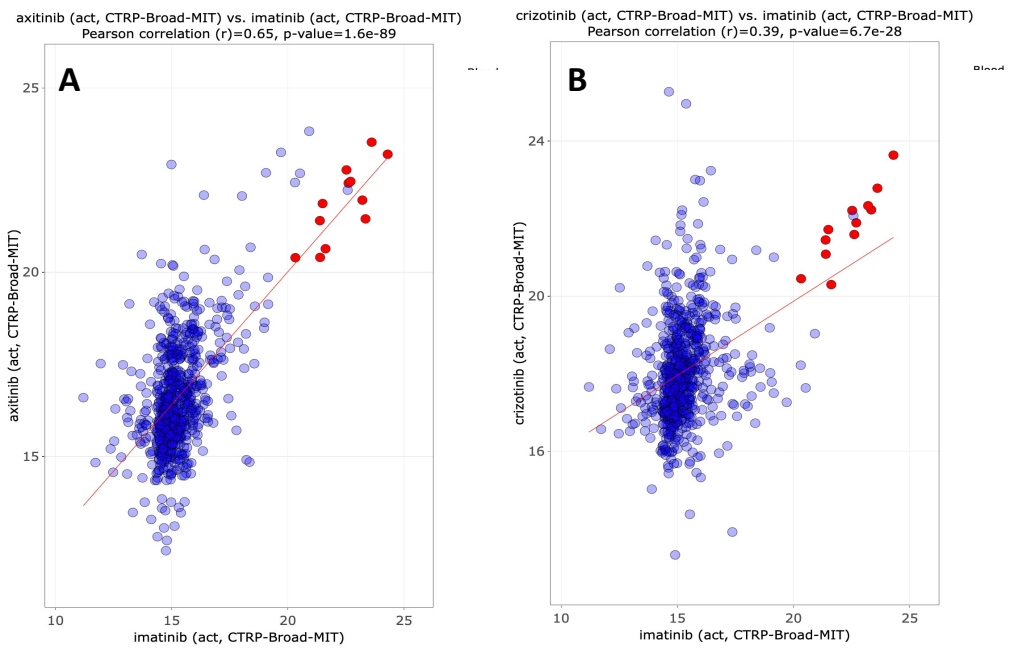


Figure 14.10. Response to imatinib (vertical axis) versus expression of ABL1 (horizontal axis) of cell lines in the CTRP-Broad-MIT data set. *Left*, CML cell lines (red) relative to other leukemia cell lines. *Right*, CML cell lines (red) relative all other cell lines. (Graphs created using CellMinerCDB version 1.4)

Table 14.2. Correlation of drug activities with ABL1 gene expression. (Table created using CellMinerCDB version 1.4, data set CTRP-Broad-MIT for all leukemia cell lines.)

Drug name	Clinical status	Correlation	P-Value
Nilotinib*	FDA approval	0.636	4.19E-09
Dasatinib*	FDA approval	0.590	9.33E-08
Imatinib*	FDA approval	0.591	1.14E-07
saracatinib	Clinical trial	0.567	3.86E-07
crizotinib	FDA approval	0.521	6.14E-06
axitinib	FDA approval	0.512	7.98E-06
tivozanib	FDA approval	0.501	2.09E-05
pluripotin		0.470	4.13E-05
masitinib	Clinical trial	0.445	2.07E-04
vandetanib	FDA approval	0.356	3.87E-03
GW-843682X		0.381	8.26E-03
Bosutinib*	FDA approval	0.329	9.02E-03

* ABL1-inhibitor drugs whose structure is shown in Figure 14.5.



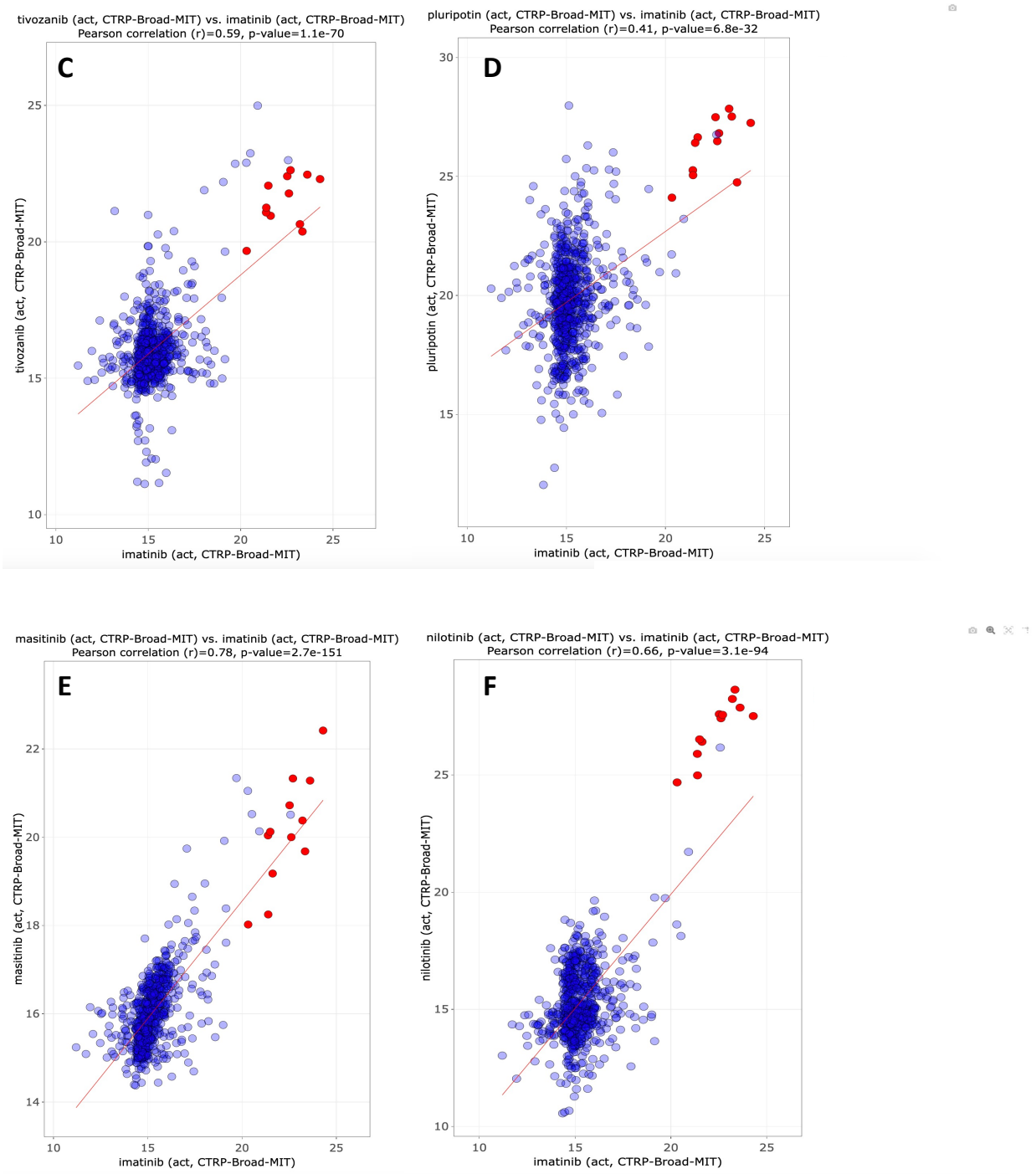


Figure 14.11. Chronic myelogenous leukemia (CML) cell lines (red) responded strongly to drugs from Table 14.2. The data were from the CTRP-Broad-MIT data set; CML cell lines in red, other cell lines in blue. The response levels shown numerically on the axes are in log2 units. Horizontal axes: response to imatinib. Vertical axis: response to **A**, axitinib; **B**, crizotinib; **C**, tivozanib; **D**, pluripotin; **E**, masitinib; **F**, nilotinib. These represent possible

drug combinations with imatinib (or with nilotinib or dasatinib). (Graphs created using CellMinerCDB version 1.4.)

Summary

It was a long path from the time that Peter Nowell and David Hungerford first noticed the tiny Philadelphia chromosome in patients with chronic myelogenous leukemia (CML) in 1960. The next landmark did not occur until 1973, when Janet Rowley figured out that the Philadelphia chromosome resulted from a translocation between chromosomes 9 and 22. Further elucidation came indirectly from an unusual mouse leukemia virus discovered by Herbert Abelson and Louise Rabstein. The virus was found to harbor a cancer-causing gene – a mutated normal gene – that came to be known as the *ABL* oncogene. ABL was found to be a tyrosine kinase: an enzyme that puts phosphate groups onto particular tyrosines in proteins. Hard work by medicinal chemists then came up with the selective ABL tyrosine kinase inhibitor, imatinib, popularly known as Gleevec. Imatinib changed the world of patients suffering from chronic myelogenous leukemia (CML): 90% of them had long-term survival and appeared to be cured without having to endure severe toxicity. Eventually however, mutant CML cells appeared that were resistant to the drug. Medicinal chemists then went to work and developed several drugs that were effective against some of the resistant cases and that received FDA approval. But certain of the CML mutations resisted all of the approved drugs. Finally, a study using a software tool, called CellMinerCDB, of the selective responses of CML cell lines to the drugs found several drug combinations for possible testing against drug-resistant CML. The development of a drug, imatinib (Gleevec), that was specific for cancers caused by a specific oncogene (ABL1) issued in the new era of targeted cancer therapy.

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