Shifting paradigms: the seeds of oncogene addiction

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Thomas Kuhn's *The Structure of Scientific Revolutions* argues that new insights often come from scientific renegades who champion new paradigms to account for observations that cannot be adequately explained by existing theory[1]. His views intrigued me while I was a Princeton undergraduate interested in the history of science. Imagine the scientific upheaval in 1543 when Copernicus proposed a heliocentric model to explain various astronomical observations, challenging the geocentric view of Ptolemy that had been in place for centuries. Cancer biologists and physicians completing their training today are likely to assume that the imatinib story—the discovery that imatinib (Gleevec) is an effective treatment for chronic myeloid leukemia (CML)—is a logical extension of earlier landmark discoveries that CML is caused by BCR-ABL, the tyrosine kinase inhibited by imatinib. Although true in a broad sense, the backstory is not quite so simple.

In 1995, the year that imatinib was first described by Nick Lydon and his colleagues, the general consensus was that cancers such as CML could be initiated by single oncogenic events or driver mutations. But there was skepticism about whether such tumors would remain dependent on the initial lesion, owing to the innumerable additional oncogenic events that accumulate in most cancers. This widely held view had important implications, because it predicted that inhibitors targeting the initiating lesion would fail unless a cocktail of drugs could be developed to target multiple lesions. Just 14 years later, this conclusion has been turned on its head. Most cancer drug discovery efforts are now focused on targeting individual oncogenic lesions, in the belief that many cancers remain dependent on driver mutations. Although the success of imatinib was not a revolution in the Copernican sense, it spawned a transformation in cancer research that has fueled an urgency to characterize cancer genomes comprehensively and discover the driver mutations in all cancers.

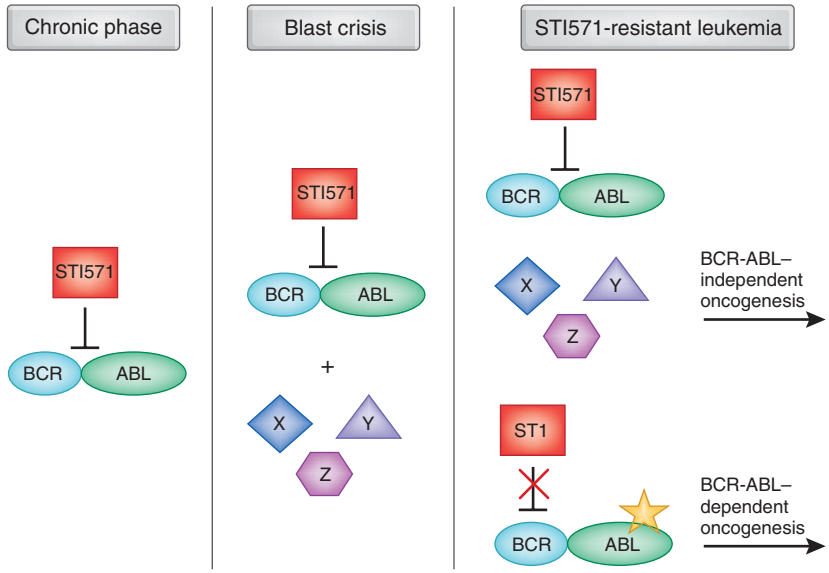

Figure 1 Driver mutations in chronic phase versus blast crisis. Chronic-phase CML is driven solely by the BCR-ABL kinase, so the responses to ABL kinase inhibitors such as imatinib (shown here using its earlier name, STI571) seem straightforward. Blast crisis is characterized by multiple secondary cytogenetic and molecular abnormalities in addition to the BCR-ABL translocation that contribute to disease progression, so responses to imatinib were unanticipated. When considering explanations for relapse, we postulated two potential scenarios: imatinib continues to inhibit BCR-ABL but the leukemia is no longer dependent on BCR-ABL activity (top right), or BCR-ABL activity is restored by a change in the leukemia cell or host that prevents imatinib from reaching its target (bottom right). Analysis of BCR-ABL kinase activity revealed that the second scenario is correct.

My fascination with CML began during my clinical residency while I was caring for young patients undergoing allogeneic bone marrow transplantation (BMT). Although BMT offered a chance of cure, complications from graft-versus-host disease were substantial. BMT could be made safer by removal of T cells from the donor marrow, but the lack of graft-versus-host disease was accompanied by a higher relapse rate, establishing a crucial role for the donor immune system in eliminating residual CML cells[2]. In parallel with these immunological insights into the mechanism of CML cure, several laboratories reported that the Philadelphia chromosome, originally linked to CML by Peter Nowell and his colleagues[3] and characterized as a reciprocal translocation by Janet Rowley[4], targeted the Abelson tyrosine kinase[5,6]. In 1985, the year I began my internal medicine training, Owen Witte's group at the University of California–Los Angeles (UCLA)

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Figure 2 Original sequence trace revealing the T315I mutation. Sequencing of cDNAs spanning the kinase domain of BCR-ABL revealed a single-nucleotide substitution from ACT to ATT, resulting in a change from threonine to isoleucine at position 315. The right panel shows an example of the press coverage after we reported these results.

showed greatly enhanced tyrosine kinase activity in anti-ABL immunoprecipitates from blood cells of CML patients, and they isolated the first full-length BCR-ABL[7,8]. Three years later, I was working in Owen's laboratory characterizing the oncogenic properties of BCR-ABL in cell-culture models while caring for patients with CML in the UCLA oncology clinic.

The scientific questions at that time were riveting. Foremost among them was whether a single gene could cause cancer, or whether a chain reaction of oncogenic changes was required. CML was an ideal human cancer to explore this question. Compared with other tumors, the cytogenetic profile of people with CML in the chronic phase of the disease was simple—the Philadelphia chromosome was the sole abnormality until the disease progressed to blast crisis, when multiple additional genomic alterations were present (**Fig. 1**). With the BCR-ABL complementary DNA in hand, it was now possible to ask whether BCR-ABL is sufficient to cause CML. In 1990, Daley *et al.*[9] showed that mice transplanted with marrow exposed to BCR-ABL–expressing retroviruses develop a CML-like illness within weeks, providing clear evidence that BCR-ABL is a genetic driver of CML. The field next turned to the question of how, and I was one of many investigators characterizing the signaling pathways that BCR-ABL usurped to cause leukemia. At that time, little thought was given to the idea of inhibiting BCR-ABL as a treatment for CML. There were no chemical tools available to test the hypothesis, and there was no compelling reason to think it would work. Once a disease was established, it was assumed either that the initiating event was no longer required for disease maintenance or that a multidrug cocktail targeting multiple lesions would be needed.

The ideal tool to test the BCR-ABL and CML maintenance hypothesis emerged from the kinase inhibitor program led by Nick Lydon at Ciba-Geigy Pharmaceuticals in Basel, Switzerland. In experiments that today would typically be pursued first by RNA interference, Brian Druker and his colleagues showed that imatinib inhibits BCR-ABL kinase activity and selectively impairs the growth of Philadelphia-positive, but not Philadelphia-negative, leukemia cells[10]. These preclinical results led Brian and Nick to think hard about a clinical trial of imatinib in individuals with CML. In 1995, they invited me to Basel to help design the trial and lobby the Ciba-Geigy leadership to move the project forward. Working on the imatinib project as a young assistant professor changed my scientific life, and I am forever grateful to Brian and Nick for this invitation.

The phase 1 clinical study of imatinib began in 1998 at Oregon Health & Science University, UCLA and The University of Texas M.D. Anderson Cancer Center. Moshe Talpaz, who led the development of interferon for CML several years earlier, joined Brian and me and provided important clinical trial expertise. As this was the first human study of imatinib, ethical considerations required that we only test the drug in patients who had exhausted all other treatment options. Because the trial was officially designed only to test imatinib safety, we could have included any cancer patients whose tumors had progressed on standard chemotherapy, as is typical in most phase 1 studies. However, we were anxious to learn whether imatinib might also confer some clinical benefit, and we therefore enrolled only subjects with Philadelphia chromosome–positive CML. Furthermore, we restricted eligibility to patients still in the chronic phase of the disease, and we intentionally excluded individuals who had progressed to blast crisis. Although patients with end-stage blast crisis might be the more typical choice for a first-in-human trial, we reasoned that only patients in the chronic phase had a chance of benefit (as BCR-ABL was the only known abnormality), and that the infections, bleeding and fatigue commonly associated with blast crisis would be impossible to distinguish from potential side effects of imatinib. We satisfied the ethical requirements for a phase 1 study by including only chronic-phase patients who were ineligible for BMT and had failed interferon, the only approved therapy for CML at the time. Although they all had leukemia, these initial volunteers were relatively healthy. But it was only a matter of time until they progressed to blast crisis.

Subjects were treated according to a typical, dose-escalation schedule as follows: three individuals (one each in Portland, Houston and Los Angeles) received the same daily dose and were followed for 28 days for side effects. Each month, Brian, Moshe and I reviewed the progress of these patients by teleconference and, after seeing no side effects, proceeded to the next higher dose. The white blood cell counts of these early subjects continued to rise,

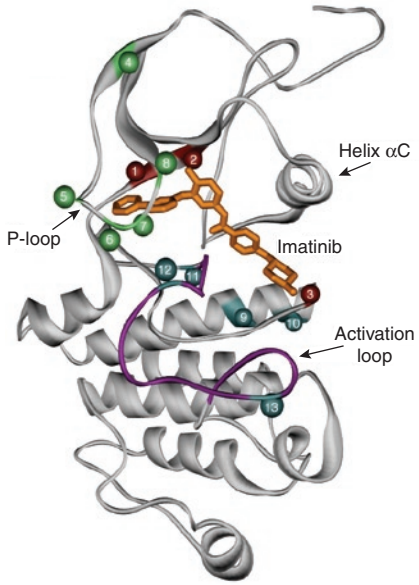

Figure 3 ABL kinase domain structure bound to imatinib, with locations of 13 resistance mutations indicated. Ribbon representation of the kinase domain of c-ABL depicting imatinib-resistant mutations. Imatinib structure is shown in gold. Positions 1–3 (red) are mutations that directly affect imatinib binding through steric hindrance. All other positions are likely to affect the ability of the kinase to achieve the conformation required to bind imatinib, including those in the P-loop (4–8; green) and those in the vicinity of the activation loop (9–13; cyan). The activation loop is colored purple. The positions of amino acids found to be mutated are depicted by spheres. Reprinted from ref. 13.

indicating that imatinib was having no effect on the disease. But when we reached higher dose levels, we witnessed a profound change. First, in patients taking 200 mg, then more consistently in patients taking 250 mg or 300 mg, we saw sustained declines in white blood cells that were maintained off all other chemotherapy. At that moment, the three of us knew that imatinib was working—it was just a matter of how well and for how long. The answer to the 'how well' question came several months later, when we observed cytogenetic remissions in many of our patients, indicating that Philadelphia chromosome–positive cells were no longer detectable in the bone marrow. Earlier studies of BMT and interferon had established that individuals with cytogenetic remissions live longer. Therefore, we knew in the summer of 1999, after treating just 83 patients, that imatinib would be a success. Patients with CML and clinicians rallied around this early data with remarkable speed, allowing Novartis to conduct four phase 2 studies in over 1,000 patients. These led to approval of imatinib by the US Food and Drug Administration by May 2001—in record time.

In the spring of 1999, after seeing white blood cell declines in chronic-phase patients, we reversed our earlier decision to enroll only patients in chronic phase and treated a few patients with blast crisis, primarily on ethical grounds that we should at least offer this encouraging, important, potentially life-sustaining new drug to end-stage patients. In some cases, the results were breathtaking—complete hematological and cytogenetic remissions within weeks of starting imatinib in patients who were near death from end-stage leukemia. Unlike in the chronic-phase patients, these miraculous responses were not seen in everyone. But the end-stage patients provided compelling proof of concept that genetically complex cancers can also remain dependent on an initiating driver mutation, serving as a foundation for the now widely accepted phenomenon called oncogene addiction[11].

But the euphoria of blast-crisis remissions was short lived. Despite continued imatinib treatment, relapses occurred in many patients, often in dramatic fashion, with florid return of leukemic blasts in the circulation just weeks after a complete cytogenetic response. In my laboratory, we turned our attention to understanding why the drug stopped working. The first clue came from experiments by Mercedes Gorre, a graduate student in my group, who showed that BCR-ABL kinase activity was fully restored at relapse and could no longer be inhibited by imatinib, even when leukemic blasts from these individuals were exposed to very high concentrations of the drug in culture.

Figure 4 Individuals who participated in the phase 1 clinical trial of imatinib at UCLA. Ron Vietti (top left) was one of three patients in the first cohort of the phase 1 trial receiving 25 mg of imatinib. This dose was ineffective, but he later changed to a higher dose and remains disease free 11 years later. Virginia Garner (top right, with arms raised) started imatinib in one of the higher-dose cohorts and responded immediately. She remains well and helps raise money for the Leukemia and Lymphoma Society as an active participant in the Race for the Cure campaign with her husband Van. Tony Huntimer (bottom left) also responded immediately to imatinib. He continues to pursue his hobby of racing collectible cars in the California desert at very high speeds (against our medical advice). David Lawyer (bottom right) responded to imatinib for several years but developed resistance from a G250E mutation, which was sensitive to dasatinib in the laboratory. He died on 15 July 2003 from progressive disease, just four months before the phase 1 study of dasatinib opened. He is shown here on his fifty-seventh birthday, on 18 May 2003, with his wife Tracy.

She zeroed in on the explanation: the CML cells from these patients had new mutations in the BCR-ABL kinase domain that prevented imatinib from binding but did not interfere with ATP hydrolysis or downstream substrate phosphorylation[12] (**Fig. 2**).

Mercedes' discovery suggested an immediate therapeutic strategy to overcome resistance. Her data argued that CML cells are dependent on BCR-ABL even at relapse. In theory, one could screen for drugs that inhibit imatinib-resistant BCR-ABL mutants, and these drugs should be effective in patients who relapse. At first, this seemed a tractable challenge, because the patients that Mercedes analyzed shared a common resistance mutation in which Thr315 was mutated to isoleucine (T315I). When Neil Shah joined the project and expanded the analysis, we soon realized that the problem was much more complex. The list of resistance mutations quickly expanded to 13 (ref. 13), and the prospect of designing mutation-specific inhibitors seemed hopeless.

A potential solution emerged from our collaboration with John Kuriyan. John's group had solved the first crystal structure of ABL bound to imatinib, and they were now working with us to study the impact of each new resistance mutation on BCR-ABL structure. What emerged was a two-part model. Some mutations, such as T315I, cause resistance through steric hindrance: the additional mass provided by the new amino acid occupies more space in the drug binding pocket such that imatinib can no longer bind. But many resistance mutations could not be explained by this model. Like all kinases, BCR-ABL cycles through distinct changes in conformation as it carries out its kinase function. These changes can be considered as 'on' and 'off' states on the basis of the position of the activation loop, which has a role in substrate phosphorylation. Curiously, imatinib binds BCR-ABL only when it reaches the 'off' state with the activation loop closed. We proposed that many imatinib resistance mutations alter the flexibility of BCR-ABL such that it can no longer achieve the fully 'off' conformation. In CML cells bearing one of these resistance mutations, we surmised that BCR-ABL never assumes the shape required by imatinib for optimal binding (**Fig. 3**). The conformation model suggested a new strategy: search for BCR-ABL inhibitors that bind the 'on' conformation, because these drugs should

remain active against most imatinib-resistant mutants. In collaboration with Francis Lee from Bristol-Myers Squibb, Neil Shah in my group showed that the dual ABL-SRC inhibitor dasatinib had these properties and was highly active in imatinib-resistant laboratory models[14]. The phase 1 clinical trial of dasatinib conducted at UCLA and M.D. Anderson was highly positive[15], and dasatinib was approved in 2006 as treatment for imatinib-resistant CML, just five years after Mercedes identified the first imatinib resistance mutation.

Today, patients with CML are still treated with single-agent BCR-ABL kinase inhibitors, starting with imatinib and followed by dasatinib or nilotinib if they develop resistance. But studies of relapse in sequentially treated patients have shown that multidrug-resistant subclones can emerge, with multiple kinase domain mutations in the same BCR-ABL molecule[16]. Although the relapse rate in newly diagnosed patients with CML treated with BCR-ABL inhibitors remains low (about 20% at five years), the residual CML cells found in most patients might be a reservoir for further resistance. On the basis of the success of triple-drug therapy for HIV and AIDS, carefully selected combinations of kinase inhibitors could prevent resistant subclones from emerging by blocking all avenues of escape. A crucial missing piece is an effective inhibitor of the T315I mutant that is resistant to all of the currently approved BCR-ABL inhibitors.

The satisfaction of knowing that your work has contributed to the lives of thousands of CML patients is impossible to put into words (**Fig. 4**). It has been gratifying to see the application of the principles learned from imatinib to so many other cancers. In 2009, people with sarcomas, breast cancer, lung cancer, kidney cancer and melanoma all benefit from treatment with kinase inhibitors. These more recent clinical successes all share themes first observed with imatinib. The individuals most likely to benefit are those whose tumors have genetic perturbations in the gene or pathway targeted by the inhibitor. These genetically complex cancers can still be dependent on a single driver mutation, as first shown so clearly in blast crisis. Finally, resistance to these newer kinase inhibitors also commonly occurs through mutation of the drug target. The path forward seems clear: as researchers discover new drug targets, they must also discover a cocktail of inhibitors to prevent relapse when used in appropriate combinations.

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1. Kuhn, T.S. *The Structure of Scientific Revolutions*. (University of Chicago Press, Chicago, 1962).
2. Goldman, J.M. *et al. Ann. Intern. Med.* **108**, 806–814 (1988).
3. Nowell, P.C. & Hungerford, D.A. *J. Natl. Cancer Inst.* **25**, 85–109 (1960).
4. Rowley, J.D. *Nature* **243**, 290–293 (1973).
5. de Klein, A. *et al. Nature* **300**, 765–767 (1982).
6. Shtivelman, E., Lifshitz, B., Gale, R.P. & Canaani, E. *Nature* **315**, 550–554 (1985).
7. Konopka, J.B., Watanabe, S.M., Singer, J.W., Collins, S.J. & Witte, O.N. *Proc. Natl. Acad. Sci. USA* **82**, 1810–1814 (1985).
8. Mes-Masson, A.M., McLaughlin, J., Daley, G.Q., Paskind, M. & Witte, O.N. *Proc. Natl. Acad. Sci. USA* **83**, 9768–9772 (1986).
9. Daley, G.Q., Van Etten, R.A. & Baltimore, D. *Science* **247**, 824–830 (1990).
10. Druker, B.J. *et al. Nat. Med.* **2**, 561–566 (1996).
11. Weinstein, I.B. *Science* **297**, 63–64 (2002).
12. Gorre, M.E. *et al. Science* **293**, 876–880 (2001).
13. Shah, N.P. *et al. Cancer Cell* **2**, 117–125 (2002).
14. Shah, N.P. *et al. Science* **305**, 399–401 (2004).
15. Talpaz, M. *et al. N. Engl. J. Med.* **354**, 2531–2541 (2006).
16. Shah, N.P. *et al. J. Clin. Invest.* **117**, 2562–2569 (2007).