

# Attacking cancer at its foundation

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Foundational discoveries connecting the fields of viral oncogenes and human protein kinases stimulated a number of groups to begin drug discovery programs targeting these kinases. The clinical success of some of these programs has created much interest in protein kinase inhibitors in almost all pharmaceutical companies. Looking back on the development of imatinib, it would be easy to view its evolution as a relatively straightforward task. However, for those working in the protein kinase inhibitor field in the 1980s, the challenges were not insignificant. A strong basic scientific foundation, combined with a productive collaboration between industry and academia, and a mixture of skill, luck and perseverance was required to deliver imatinib from the laboratory to the clinic.

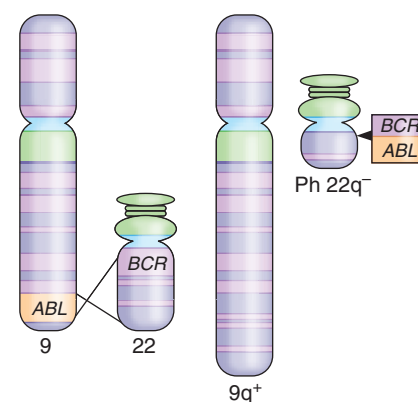
## The foundation on which imatinib discovery was built

Back in the mid-1980s, when the program that yielded imatinib began, the understanding of the structure and function of protein kinases and the availability of medicinal chemical tools to modulate their activity were in their infancy. Research from a number of different avenues, however, had converged to strongly suggest that the field of protein kinase inhibitors would be a fruitful area of pharmacology research. Having studied as a graduate student at the University of Dundee, and having been influenced by the pioneering work of Philip Cohen on the role of reversible protein phosphorylation in cell regulation, I was highly sensitized to the evolving connection between deregulated protein kinases and cancer pathogenesis.

This strong link is particularly well exemplified by the Rous sarcoma virus<sup>1</sup>, originally identified by Peyton Rous in 1911. This ultimately led to the discovery of v-SRC, the

founding member of the tyrosine kinase family<sup>2</sup>. The link between retroviral oncogenes and the oncogenic potential of deregulated protein kinases in the pathogenesis of human cancers was further enhanced by the findings that the v-erbB oncoprotein is a deregulated version of the epidermal growth factor receptor (EGFR)<sup>3</sup> and that v-sis is a ligand for the platelet-derived growth factor receptor (PDGFR)<sup>4,5</sup>. In chronic myeloid leukemia (CML), a similar link between these fields became evident, beginning with the identification of v-ABL from a mouse leukemia virus. The pioneering work of Nowell and Hungerford<sup>6</sup> and Rowley<sup>7</sup> identified a specific chromosomal abnormality in CML that subsequently led to elucidation of the central role of the BCR-ABL tyrosine protein kinase in Philadelphia chromosome-positive CML and acute lymphoblastic leukemia (Fig. 1). In addition, work on protein kinase C (PKC), a serine-threonine protein kinase, revealed it to be the target of phorbol esters, well-known tumor promoters<sup>8</sup>. The stage was thus set for a number of research groups from academia and industry to attempt to translate these findings into drug discovery programs.

Alex Matter and I, who had arrived at Ciba-Geigy in the mid-1980s, were convinced that protein kinase pharmacology could be a novel, targeted approach in the oncology field. If selective protein kinase inhibitors could be developed to target central pathogenetic cancer defects, they could potentially have considerable advantages over existing nonselective cytotoxic chemotherapies. At that time, the protein kinase family had a relatively small number of known members<sup>9</sup> (Fig. 2), and the task of identifying selective inhibitors did not seem overly challenging to a naive biochemist with little experience in the pharmaceutical industry. Within a short period of time, the protein kinase gene family began an exponential expansion. By 1987, a review of the gene family<sup>10</sup> classified about 65 members of the family, and it was predicted that the gene family could contain 1,000 members<sup>11</sup>. On the basis of data



**Figure 1** Diagram of the translocation that creates the Philadelphia chromosome. The *ABL* and *BCR* genes reside on the long arms of chromosomes 9 and 22, respectively. As a result of the (9;22) translocation, a *BCR-ABL* fusion gene is formed on the derivative chromosome 22 (Philadelphia chromosome).

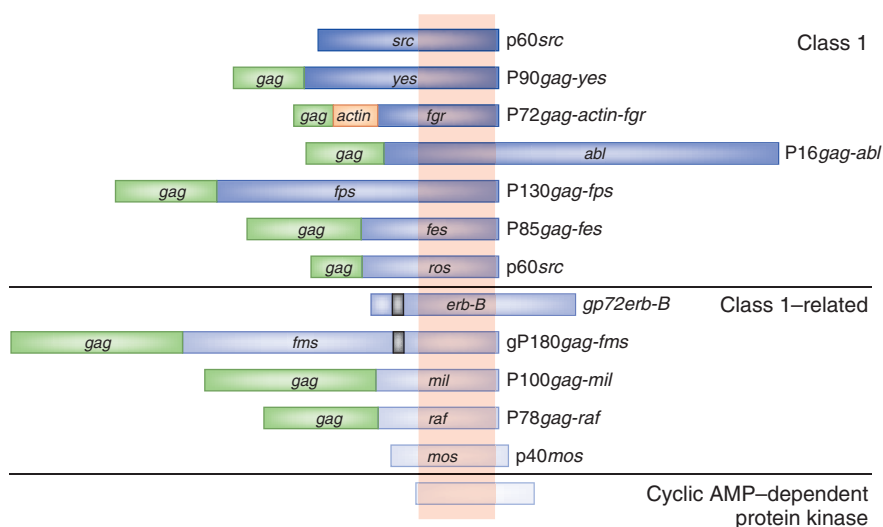
from the Human Genome Project, researchers now know that there are about 500 members of the protein kinase family<sup>12</sup>. The task of generating selective inhibitors had rapidly evolved to become a daunting technical challenge, and a crucial question we and others in the field needed to address was whether it would be possible to develop selective inhibitors.

On the basis of sequence alignment, members of the kinase family were known to share a conserved ATP binding site. As a consequence, there were many skeptics (quite reasonably at the time) who argued that it would be impossible to develop selective protein kinase inhibitors directed to the ATP binding site. However, given the sequence differences within the aligned kinase domains, and the knowledge that they bind and phosphorylate different target substrates, we hypothesized that selectivity should be possible by targeting the variable residues within the active site.

## Discovery of imatinib

The initial challenge we faced at Ciba-Geigy was the development of biochemical assays

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**Figure 2** Status of the protein kinase family in the mid-1980s, when the kinase program at Ciba-Geigy began. The following text is from the original legend in reference 9: “Structures of two groups of viral oncogene products and a normal serine-specific protein kinase (bottom) are given by the bars, whose length is proportionate to the number of amino acids in each protein: the NH<sub>2</sub> end of each chain is on the left. The class 1 products display clear tyrosine-kinase activity: the class 1-related products do not. Analysis of the amino acid sequences of the proteins shows they have a common 250 amino acid region related to the protein-kinase domain of p60src (color). In most cases part of the protein encoded by the viral gene *gag* is synthesized with the oncogene protein as single product. Two proteins extend outside the cell and have identifiable transmembrane domains (black).” Figure redrawn from reference 9.

robust enough for compound screening. The concept of high-throughput screening as it is viewed today was not conceivable with the technology available at that time. Given the need to obtain enzymatically pure protein kinases, we embarked on the systematic expression of protein kinases using recombinant expression systems. Our first attempt at producing recombinant enzyme in *Escherichia coli* worked relatively well for the kinase domain of v-ABL<sup>13,14</sup>, but it did not translate to other kinases. Fortunately, following the advice of Chuck Stiles, we started collaborating with Tom Roberts at the Dana-Farber Cancer Institute, who had pioneered the use of baculoviruses for tyrosine kinase expression. This collaboration proved to be extremely fruitful, and most of our enzymes for screening were subsequently expressed using this system<sup>15–18</sup>.

Notably, the interaction with the Roberts lab led to my meeting Brian Druker and to our future collaboration on the development of what became known as imatinib. An additional important tool that Brian developed in the Roberts lab was the 4G10 antibody to phosphotyrosine<sup>19</sup>. This was a crucial reagent for profiling tyrosine protein kinase activity in cells by western blotting and for the development of ELISA-based high-throughput screening assays<sup>20</sup>.

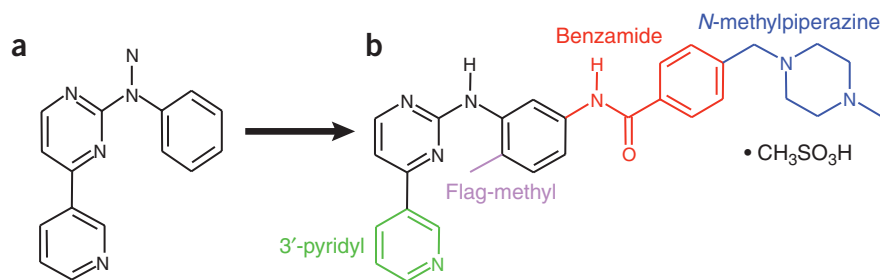
The major medicinal chemistry challenge we faced at the start of the program was to

determine which chemical starting points to use. A number of compounds had been reported<sup>21–23</sup> as inhibitors of protein kinases in the early 1980s. Much of our early work focused on exploring these early leads and screening the Ciba-Geigy compound collection. Under the leadership of Peter Traxler (who taught me most of what I know about the challenges and excitement of medicinal chemistry), we started work on the synthesis of kinase inhibitors, using inhibitors such as erbstatin<sup>24</sup>, flavones and isoflavones<sup>25</sup>, and staurosporin<sup>26</sup> as starting points. We were not alone in our approach, as others in the field were following similar strategies. Although our early efforts generated interesting ABL inhibitors<sup>27</sup>, they were far from being drug like. However, our early medicinal chemistry work gave the discovery team encouragement by showing that specificity could be achieved by targeting the ATP binding site of protein kinases. Although our selectivity panel was pitifully small when viewed by today’s standards<sup>28</sup>, we considered it to be state of the art at that time! A key event in stimulating heightened interest in the field was the report by Levitzki and colleagues<sup>29</sup> of convincing selectivity attributes obtained using a series of benzylidene-malononitrile compounds based on erbstatin.

Despite our interest in BCR-ABL as a target, our initial screening activities identified mainly EGFR hits, and we concentrated much

of our early efforts on optimizing a number of these compounds<sup>23</sup>. It was not until the early 1990s that we finally identified the phenylamino-pyrimidine class of inhibitors that eventually gave rise to imatinib. Thomas Meyer and Juerg Zimmermann, who were working on PKC, discovered this inhibitor class when screening anti-inflammatory molecules, which, they hypothesized, could be protein kinase inhibitors. The initial screening hit had low potency and poor specificity, inhibiting both serine-threonine and tyrosine kinases. From this starting point, a series of derivatives were synthesized that showed good selectivity for PKC- $\alpha$ . The addition of a 3-pyridyl (Fig. 3a) group at the 3-position of the pyrimidine enhanced the cellular activity of this class<sup>30</sup>. During further optimization of this class for PKC- $\alpha$  inhibition, we found that introduction of a benzamide group at the phenyl ring (Fig. 3b) led to modest activity against tyrosine protein kinases, including ABL and PDGFR. On the basis of biochemical profiling data generated by Helmut Mett, Elisabeth Buchdunger and Thomas Meyer, Juerg Zimmermann<sup>31</sup> made the key observation that substitutions of a methyl group at the 6-position of the anilino phenyl ring abrogate PKC inhibition but enhance ABL and PDGFR inhibition (Fig. 3b). The attachment of a highly polar side chain, *N*-methylpiperazine, markedly improved solubility and oral bioavailability (Fig. 3b), resulting in imatinib<sup>32</sup> (originally named CGP 57148B, then renamed STI571 and now known as imatinib mesylate, imatinib or its trade names Gleevec or Glivec).

After the publication of the crystal structures of the cAMP-dependent protein kinase<sup>33</sup>, we made major efforts to understand the molecular basis of imatinib’s selectivity using molecular modeling. However, we could never rationalize the molecular basis of the observed selectivity. In fact, our hypothesized binding mode for imatinib was completely wrong, as we believed it was binding to the active form of the enzyme<sup>34</sup>. Enlightenment came when John Kuriyan’s lab published the X-ray structure of the ABL kinase in complex with imatinib<sup>35</sup> (Fig. 4). The key finding of these studies was that imatinib binds to the inactive conformation of ABL. The catalytic domains of various kinases adopt a strikingly similar structure in the active conformation<sup>36</sup>. By contrast, crystal structures of inactive kinases reveal marked catalytic-domain plasticity that results in distinct inactive conformations. By exploiting this distinct inactive conformation of the ABL kinase domain (which is presumably also found in the PDGFR and KIT kinases), imatinib can achieve its high specificity within the kinase family. Although



**Figure 3** Lead optimization. (a,b) Synthetic evolution of imatinib from a 2-phenylaminopyrimidine backbone (a). Introducing a 3'-pyridyl group (b; green) at the 3-position of the pyrimidine improved activity in cellular assays. Activity against tyrosine kinases was enhanced by addition of a benzamide group (red) to the phenyl ring and the attachment of a 'flag-methyl' group (purple) to the diaminophenyl ring, which abolished activity against PKC. Adding *N*-methylpiperazine (blue) increased water solubility and oral bioavailability.

we did not understand the true selectivity of imatinib at the time of its discovery, this became apparent with the development of the KINOMEScan assay technology (<http://www.kinomescan.com>). Luckily, the initial selectivity we saw with our limited array of enzymes<sup>32</sup> was essentially maintained when profiled against the kinome array<sup>28,37</sup> (Fig. 5a).

By 1993, Brian Druker had moved to Oregon Health & Science University and established various BCR-ABL-driven models. He was interested in finding BCR-ABL kinase inhibitors and fortuitously contacted me in 1993. We quickly established a collaboration to test our two best compounds, imatinib and CGP 53716 (ref. 30), a closely related precursor with similar specificity. Brian's initial studies<sup>38,39</sup> confirmed and extended the findings of Elisabeth Buchdunger<sup>32</sup> that imatinib had inhibitory activity against ABL and its activated derivatives v-ABL, BCR-ABL and TEL-ABL. Notably, Brian and Elisabeth also found inhibition of KIT<sup>40,41</sup>, which subsequently led to clinical testing of imatinib in gastrointestinal stromal tumors (GIST)<sup>42</sup>. At the time, however, this finding caused considerable concern, as we did not know whether this activity would be an advantage (enhance the killing of CML myeloid cells) or a disadvantage (result in myelosuppression by blocking bone marrow recovery). A pivotal experiment of Brian's used cells from individuals with CML in colony-forming assays<sup>38</sup>. In these studies, imatinib caused a 92–98% decrease in the number of BCR-ABL-positive colonies formed, with minimal inhibition of normal colony formation. This suggested that imatinib is selective for BCR-ABL-transformed cells while allowing the growth of normal cells, allaying our fears that imatinib would be myelosuppressive. These exciting results, using an *ex vivo* model of CML, had a major influence on the selection of CML as the best initial clinical indication for developing ima-

tinib. In further experiments, imatinib was shown to selectively suppress the proliferation of BCR-ABL-expressing cells *in vitro* and *in vivo*<sup>38</sup>.

### Preclinical development

On the basis of the biological profile of imatinib and its attractive drug-like properties, we began preclinical development of imatinib in early 1994. However, after relatively smooth progress during the discovery phase, we soon ran into a major problem. Our initially strategy for CML was to develop imatinib as a parenteral formulation for intravenous infusion. The envisaged target population for our phase 1 study was patients with late-stage CML treated in a hospital setting. However, the intravenous formulation failed in late-stage infusion toxicology studies owing to drug precipitation during infusion. In retrospect, this setback was fortunate, as it allowed us to switch to an oral form of imatinib. On the basis of pharmacokinetics studies, which were rapidly generated by Peter Graff, we quickly changed track and began developing an oral formulation of imatinib. This turned out to be extremely attractive (good pharmaceutical and absorption, distribution, metabolism and excretion properties) and allowed subsequent testing of imatinib in chronic-phase CML in an outpatient setting.

Despite the promising preclinical data, there were still hurdles to overcome before clinical trials commenced. The most noteworthy issue concerned the safety profile of imatinib. The initial chronic preclinical toxicology studies revealed liver and kidney effects in dogs. To further investigate these findings and determine whether a safe phase 1 starting dose for initiation of clinical trials could be found, we initiated additional toxicology studies, including extensive rat and monkey toxicology. Luckily, the results of these studies allowed the drug to move forward into

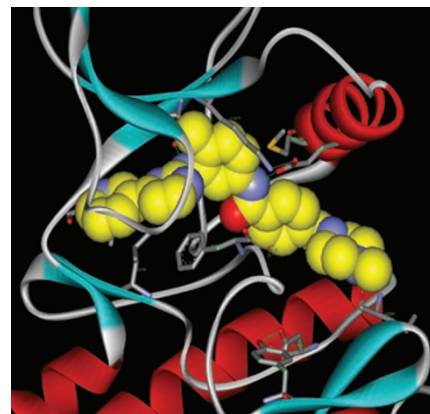
clinical trials (see NDA 21-335 at <http://www.foiservices.com> for the preclinical data file).

A second issue with which we were constantly faced was marketing concerns regarding CML. There were many perceived risks in developing a new drug class targeting a clinically unvalidated target in a small disease population. However, key supporters of the program—Alex Matter, head of oncology research at Ciba-Geigy, who had nurtured the program over many years; Graham Brown, the head of clinical development; and George Haas, the head of R&D—actively urged its promotion into clinical development.

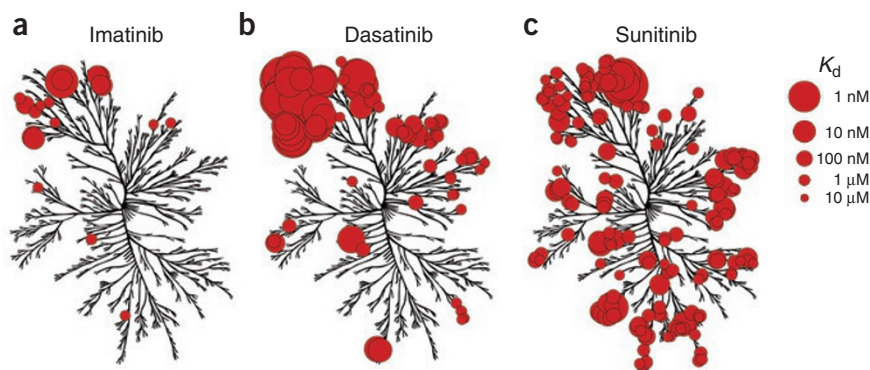
By 1997, Ciba and Sandoz were in the process of merging to form Novartis. The resulting organizational changes inevitably caused some delays. I left Novartis at that time to found Kinetix Pharmaceuticals in Boston, and Elisabeth Buchdunger took over leadership of the imatinib program. Elisabeth Buchdunger, Alex Matter and the development team, along with Brian Druker, helped shepherd imatinib toward clinical trials. Their efforts ultimately prevailed, and imatinib entered phase 1 trials in June 1998.

### Clinical development

Brian and his colleagues, Charles Sawyers and Moshe Talpaz, started the dose-escalation phase 1 study of imatinib in June 1998. This was a very exciting time, as I followed the



**Figure 4** Imatinib bound to the inactive, closed conformation of ABL. Imatinib (space-filling model) straddles the conserved activation loop DFG motif (the Phe382 side chain is shown below the imatinib benzamide oxygen), and its acid amine and piperazine (right-hand side of inhibitor) pass under helix C. Important side chains in the upper lobe (Lys271 and Glu286 ion pair) and activation loop (Tyr393) are shown. Tyr393 of the activation loop is the major site of phosphorylation of ABL. The activation loop is folded into the active site, with Tyr393 making a hydrogen bond with Asp363 (bottom). Alpha helices are shown in red and beta sheets in blue. Adapted from reference 35.



**Figure 5** Kinase dendrogram interaction maps for imatinib, dasatinib and sunitinib. (a–c) Kinases that bind imatinib (a), dasatinib (b) and sunitinib (c) are shown in red circles; larger circles indicate higher-affinity binding. Interactions with  $K_d < 10$  are shown. Details of methodology used to generate these data can be found in references 28 and 37, and full data sets are available at <http://www.Ambitbio.com/technology/publications>. Figure kindly provided by P. Zarrinkar.

dose escalation toward drug levels at which we thought we should see a therapeutic effect (on the basis of pharmacokinetic studies). However, the rapid and marked hematological responses that were seen at imatinib doses of 300 mg and above far exceeded our most optimistic expectations<sup>43</sup>. Ninety-eight percent of chronic-phase patients treated at 300 mg and above achieved a complete hematologic response<sup>43</sup>. Despite dose escalation to 1,000 mg, a maximally tolerated dose of imatinib could not be defined. These phase 1 findings showed that imatinib is well tolerated at efficacious doses and has attractive pharmacokinetic properties<sup>43</sup>. Brian presented these exciting results at a plenary session of the American Society of Hematology in December 1999.

Phase 2 clinical trials confirmed and extended the results seen in the phase 1 studies and led to approval of imatinib by the US Food and Drug Administration in May 2001, less than three years after the start of the clinical program. It is very instructive to look at the imatinib New Drug Application document (NDA 21-335); it gives a realistic picture of the complexity of the drug development process and the interactions between the company and the Food and Drug Administration that resulted in its rapid approval (using Fast-Track Designation and Accelerated Approval, which allowed surrogate markers to be used in the phase 2 registration trial).

In addition to inhibiting the ABL tyrosine kinase, imatinib inhibits the PDGFR and KIT tyrosine kinases<sup>32,38,40,41</sup>. This has made it possible for imatinib to be tested in a number of other cancers in which mutated kinases have been identified. One such disease is GIST, which is driven mainly by KIT mutations<sup>44,45</sup>. Imatinib has also shown substantial activity in patients with translocations involving

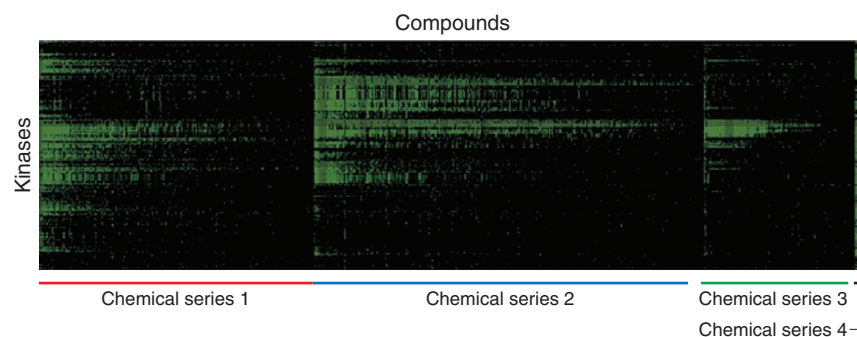
PDGFRB (chronic myelomonocytic leukemia<sup>46–48</sup>) and PDGFRA (hypereosinophilic syndrome<sup>49,50</sup>).

#### Translating the success of imatinib to other malignancies

It is not uncommon now to see imatinib-treated individuals with CML who have been in remission for eight years or more. The imatinib clinical trials were a dramatic demonstration of the potential of targeting a central pathogenetic event in a malignancy. Recently, findings from the EGFR inhibitor field<sup>51</sup> have converged with those in CML and GIST to reinforce this paradigm. Although the phase 3 studies of gefitinib in combination with chemotherapy were disappointing, as no increased patient survival was observed<sup>51</sup>, some subjects had profound responses in the phase 2 studies<sup>52,53</sup>. Studies have now found somatic mutations in the *EGFR* gene in patients that had a durable clinical response to gefitinib or erlotinib<sup>54–56</sup>, and these mutations confer drug sensitivity to EGFR inhibi-

tors. As summarized by Arteaga<sup>52</sup>, 93% of patients who had a durable response have these EGFR-activating mutations. However, despite the initial response to gefitinib or erlotinib, disease eventually progressed in all cases despite continued treatment. Similarly, the molecular basis for resistance in non-small-cell lung cancer has been shown<sup>57,58</sup> in a substantial number of cases to result from a drug-resistance mutation located in the ATP binding site of EGFR (T790M). This change corresponds to the gatekeeper mutation, which is observed at a high frequency in advanced CML with acquired imatinib resistance (T315I in ABL; ref. 59) and in imatinib-resistant GIST (T670I in KIT and T674I in PDGFRA). Thus, in a variety of advanced malignancies, the mechanism of drug resistance is almost identical. This further reinforces the importance of these deregulated kinases as central pathogenetic events responsible for driving malignancy. A future challenge for this class of drug will therefore be to address acquired drug resistance.

Taken together, the above examples indicate that effective treatment of advanced malignancies with activating kinase mutations is likely to require a combination of agents that target key, distinct deregulated processes in the target cell<sup>60</sup>. For a rational combination therapy to be clinically applied, progress must be made on understanding the pathogenetic events that occur during tumor progression. Empirically, such approaches are already being used in the clinic. For example, the adjuvant use of trastuzumab (Herceptin) with tamoxifen in HER2-positive, estrogen receptor-positive breast cancer is one example of a situation where two separate signaling pathways are targeted by noncytotoxic signal transduction modulators, resulting in well-tolerated and effective therapy. Other examples are likely to follow,



**Figure 6** Characterization and annotation of compound libraries. An example of a library of several thousand compounds screened against several hundred kinases. The heat-map representation of the results, with compounds in columns and kinases in rows, shows binding interactions in green. Compounds are sorted by chemical series. The representation reveals the relationship between chemical structure and kinase interaction pattern. Reproduced from ref. 64.

as the pathophysiology of cancer progression becomes better understood.

A second question has emerged from an ongoing discussion regarding the desired selectivity profile of kinase inhibitors. At one end of the spectrum are drugs such as imatinib (Fig. 5a)—type II inhibitors that target the inactive kinase conformation<sup>61</sup>—which show an impressive degree of selectivity. At the other extreme are relatively nonselective drugs such as dasatinib (Fig. 5b) and sunitinib<sup>28,36</sup> (Fig. 5c), which target the active conformations of their target kinases. However, despite having less selectivity, dasatinib and sunitinib have distinct patterns of kinase inhibition, with dasatinib preferentially targeting the tyrosine kinase family<sup>28</sup> (Fig. 5b). Our initial hypothesis was that the better the selectivity, the fewer off-target side effects would be observed during chronic therapy. However, an unplanned result of lower selectivity may be that, in addition to the primary kinase target, an unknown combination of additional kinases are targeted in the tumor, resulting in improved antitumor activity. The downside of this approach is that off-target dose-limiting toxicities may prevent sufficient dosing to effectively block the intended target.

Examples of a more rational approach to this multikinase inhibitor strategy are molecules such as ZD6474 (refs. 62,63) that target EGFR and VEGFR2, two validated, independent contributors to malignancy and tumor progression. Although the development of such agents has encountered clinical concerns, owing to mechanism-based adverse events such as hypertension, this approach is clearly attractive. Given the plasticity of kinases, it is hard to design such selectivity *de novo*. However, new approaches to the discovery of molecules with a combination of useful activities, using kinome array assays of large chemical libraries (Fig. 6), have recently been described<sup>64</sup>. Such broad profiling methods will undoubtedly become the norm in the future.

## Conclusion

Imatinib revolutionized the oncology field in one important respect. It showed that, by targeting an oncogene that is the molecular cause of CML, one can kill the defective cells without major side effects on normal cells. The recent findings with EGFR inhibitors have further reinforced the importance of

targeting drugs against foundational cancer mutations. In both cases, disease relapse during therapy is associated with the acquisition of drug-resistant escape mutations in the target kinase, reinforcing the central driver role of these targets in the pathophysiology of the tumor. Despite the success of imatinib in chronic-phase CML, the challenge for the future will be to develop rational combination therapies based on an understanding of cancer pathogenesis. This will be especially difficult in the more complex solid tumors, which may have accumulated multiple oncogenic mutations and thus have greater heterogeneity. However, identification of the key drivers in the malignant process, combined with molecular diagnostics and biomarkers, will be essential steps in developing the appropriate drug combination for use in the selected patient population.

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## COMPETING INTERESTS STATEMENT

The author declares competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/naturemedicine/>.

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