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Lessons learned from the development of imatinib

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1. Introduction

It is now 5 years since clinical trials with imatinib (Gleevec, Glivec, previously STI571 or CGP 57148B) began. In January 2000, we published a perspective entitled "Lessons learned from the development of an Abl tyrosine kinase inhibitor for chronic myelogenous leukemia" [1]. This perspective looked at the transition of imatinib from preclinical to clinical development and discussed issues that would have to be addressed for the success of protein kinase inhibitors in the clinic. In this article, we update our thinking on the development of kinase inhibitors as anti-cancer agents in the context of the volumes of new data on both the preclinical and clinical activity of imatinib. A number of recent reviews have been published on the preclinical and clinical profile of imatinib [2–4]. Other articles in this special issue of *Leukemia Research* review specific aspects regarding imatinib. Additional details can be found in the following sources: (1) original publications on the discovery of imatinib [5–8]; (2) pivotal, early clinical trials in CML [9,10]; and (3) registration and phase III trials in CML [11–14].

2. Development of imatinib

Given the success of imatinib and the enormous interest in protein kinase inhibitors, it is easy to forget the degree of skepticism that kinase inhibitors faced from the scientific community and the pharmaceutical industry in the 1980s and 1990s. One area of skepticism was whether compounds with specificity amongst protein kinases could be developed. The other reflected the belief that targeting of a single molecular defect with a selective agent would not be sufficient to treat highly heterogeneous cancers. Despite this skepticism, by the early 1990s, the 2-phenylaminopyrimidines were first reported as kinase inhibitors with selectivity for the protein kinase C (PKC), Abelson (ABL) and platelet-derived growth factor receptor (PDGFR) kinases [5–7,15,16]. As is the case with many drugs currently in clinical trials, an initial lead compound was identified by testing compound libraries for inhibition of protein kinases in vitro. The activity of the 2-phenylaminopyrimidine series was subsequently optimized for inhibition of ABL and PDGFR, by synthesizing a series of chemically related compounds and analyzing the relationship between their structure and activity in a variety of assays (Fig. 1). An important finding was that methyl substitution of the anilino-phenyl ring at the 6-position (Fig. 1, (2)) led to potent inhibition of the ABL and PDGFR kinases, but abolished activity on the PKC family. The 2-phenylaminopyrimidine class was finally optimized for absorption, distribution, metabolism and excretion properties by the introduction of the *N*-methyl piperazine group (Fig. 1, (3)). The most potent molecules in the series were inhibitors of both the ABL and the PDGFR kinases. Imatinib emerged from these efforts as the lead compound for preclinical development based on its selectivity against CML cells in vitro and its drug-like attributes, including pharmacokinetic and formulation properties.

The in vitro screening employed a panel of isolated protein kinase enzyme assays for initial chemical optimization. Although this sounds simple now, this was not the case in the late 1980s, when methods for the recombinant expression of active tyrosine protein kinases were in their infancy (e.g. Foulkes et al. [17] and Lydon et al. [18]). It was only with the development of baculovirus expression systems that enzymatically pure kinases with high specific activity could be obtained [19–21]. Such high quality enzymes were essential for effective high throughput screening, inhibitor characterization, and chemical optimization of lead compounds.

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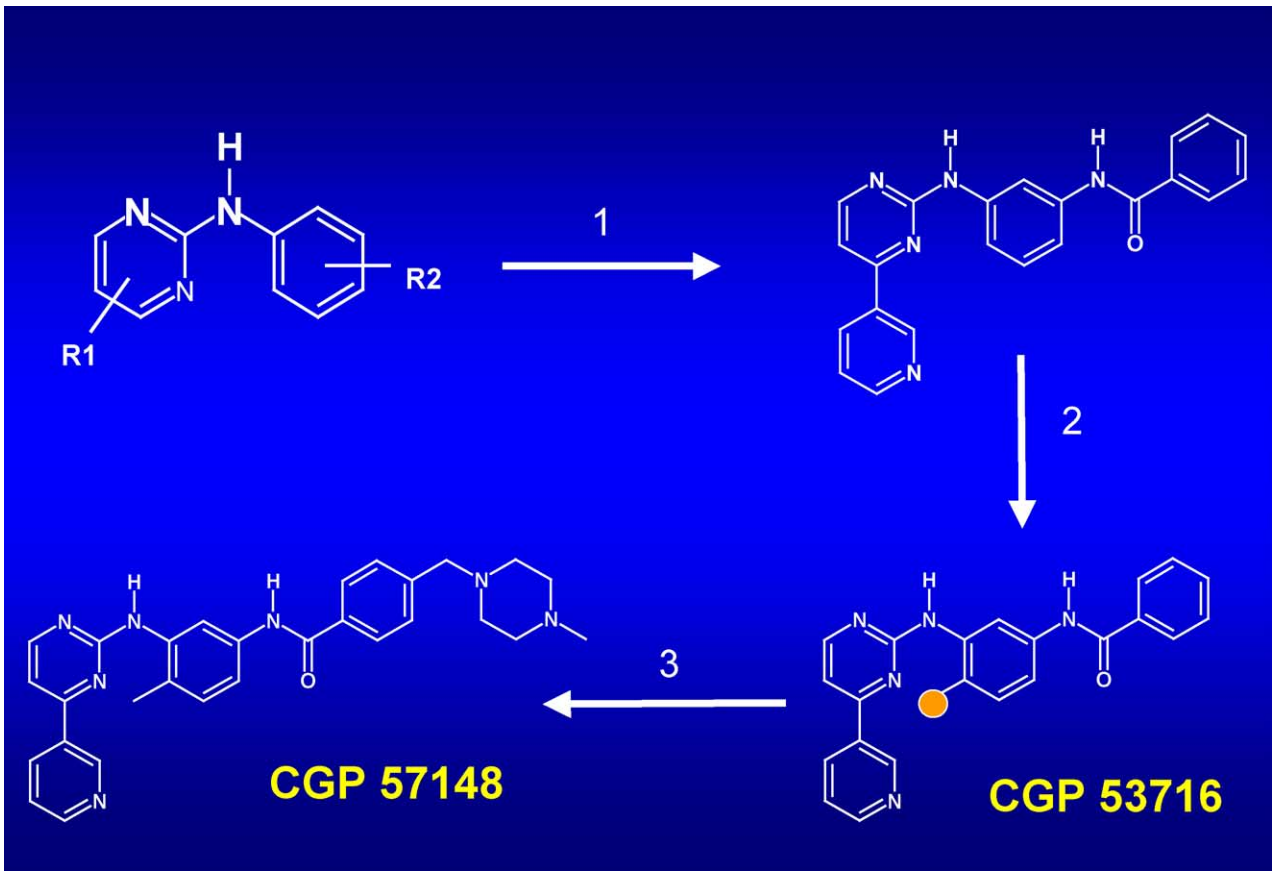

Fig. 1. Optimization of the 2-phenylaminopyrimidine class: initial derivatization of the 2-phenylaminopyrimidine chemical class (1) led ATP competitive inhibitor of serine/threonine protein kinases [6]. Methyl substitution of the anilino-phenyl ring at the 6-position (2) led to potent inhibition of the ABL and PDGFR kinases, but abolished activity on the PKC family [16]. The 2-phenylaminopyrimidines class was finally optimized for ADME properties (3) by the introduction of the *N*-methyl piperazine group [7].

2.1. Structural basis of inhibition

After much medicinal chemistry effort, we were unable to separate a number of activities from the inhibition profile of imatinib. Thus, imatinib is not a specific inhibitor of ABL but also inhibits PDGFRs, KIT, ARG (*abl*-related gene) and potentially other enzymes, which have not yet been tested. We were initially confused by the profile of kinases inhibited by imatinib. From a primary sequence analysis, our initial expectation was that enzymes closely related to ABL in the tyrosine protein kinase phylogenetic tree [22] would be inhibited and those distantly related would not [22]. This was clearly not the case (Fig. 2). Secondly, we believed that imatinib inhibited the "activated" form of the enzyme [1]. Much clarity has been brought to the mechanism of action of imatinib by Kuriyan and co-workers' elegant crystallographic studies of imatinib and related compounds bound to ABL [23,24]. These studies revealed that imatinib binds to the inactive conformation of ABL (Fig. 3A). Although the catalytic domains of active protein kinases are structurally very similar, the crystal structures of inactive kinases reveal a remarkable plasticity that allows the adoption of distinct inactive confirmations [25–27]. By inference, we would predict that the inactive conformation of KIT and the PDGFRs are structurally similar to ABL, and that imatinib is able to bind and stabilize the inactive conformations of these enzymes. Due to steric constraints, the inactive conformation of ABL is not compatible with ATP binding (Fig. 3C, see DGF region of activation loop). It is therefore likely that this binding mode provides the unique selectivity profile and resulting favorable therapeutic index of imatinib.

3. Is a selective kinase inhibitor necessary or desirable?

The project team spent considerable time debating the potential effects of blocking kinases besides ABL in the context of CML. The bottom line was that we could not predict whether these activities were an advantage or a disadvantage. On one hand, off target activities were a concern as it was assumed that they would narrow the therapeutic index of imatinib due to their potential for side effects. On the other hand, it was possible that off target activities, particularly KIT inhibition, might contribute to the clinical efficacy of imatinib in CML [28,29]. Fortunately, the side effect pro-

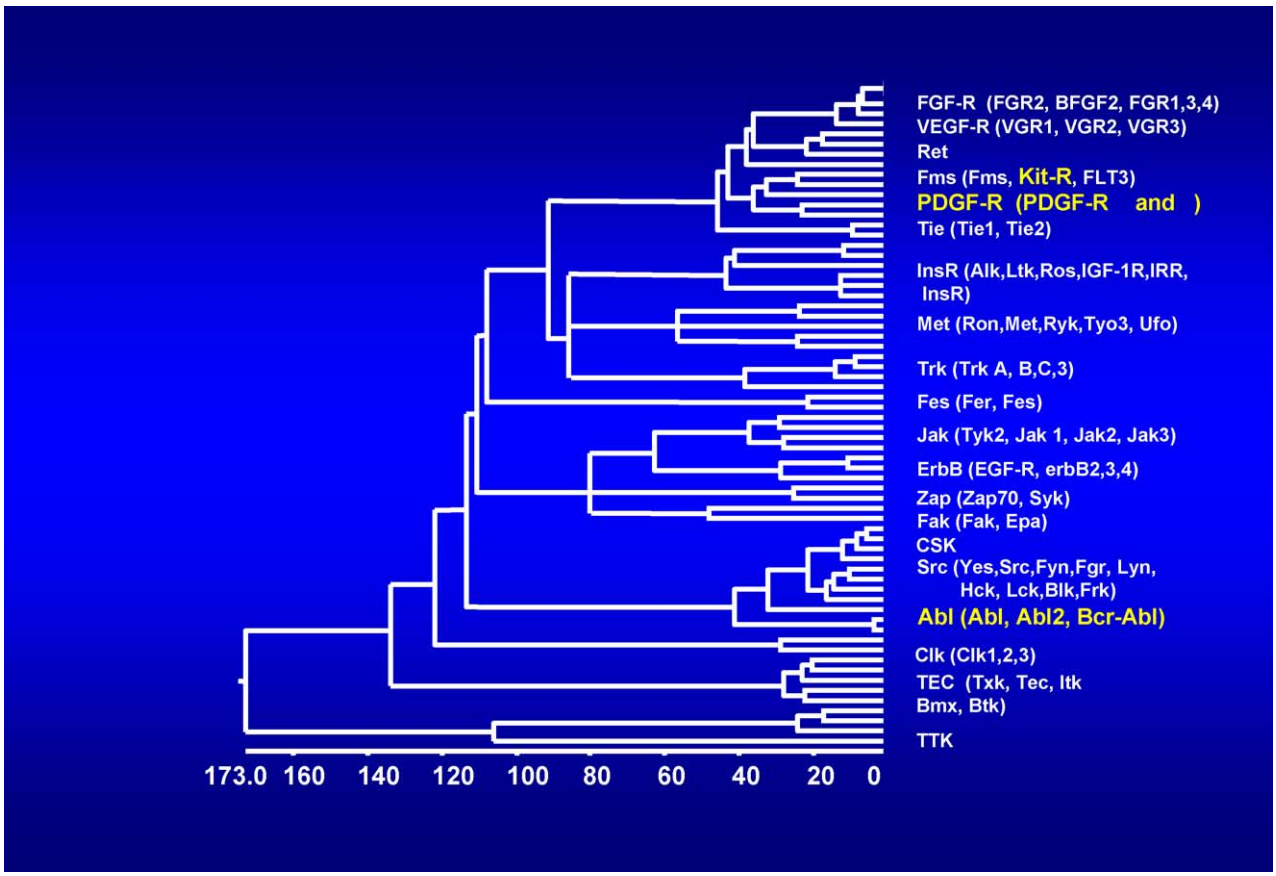

Fig. 2. Tyrosine protein kinases phylogenetic tree: kinases known to be inhibited by imatinib are shown in yellow. Adapted from Hunter [83]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

file of imatinib has been quite mild [30]. Whether activities other than ABL inhibition contribute to imatinib's clinical activity can only be determined through clinical testing of a mono-specific ABL inhibitor. Until there is additional experience with inhibitors of numerous other kinases, the issue of specificity of kinase inhibitors and their potential for toxicity versus clinical benefits will need to be addressed empirically. However, the targeting of KIT and PDGFR presented an opportunity to broaden the clinical use of imatinib to diseases that also involved deregulated PDGFR or KIT kinases. Given the costs and risks involved in clinical development, the potential for increasing the market size for imatinib became a good argument for initiating clinical development of imatinib.

4. Preclinical testing of imatinib

In vitro enzyme testing of imatinib was followed by cellular screening on IL-3 dependent cell lines and factor independent *bcr-abl*-transformed derivatives. In a pivotal set of cellular experiments, imatinib was shown to selectively suppress the proliferation of BCR-ABL-expressing cells in vitro [8]. This selectivity was confirmed using patient cells in colony-forming assays. 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 [8]. The efficacy and specificity of imatinib was confirmed and extended by several laboratories and reinforced the selectivity of imatinib for *bcr-abl* positive leukemias [31–34]. These cellular assays convinced us that imatinib could be useful in diseases involving deregulated ABL protein tyrosine kinase activity.

An important lesson that we can take from the preclinical profiling of imatinib is the importance of having a robust and clinically relevant panel of cellular tests that can predict both cellular activity and selectivity. These cellular experiments also defined the target concentration of imatinib that we predicted would be required in vivo for clinical activity to be seen. Our use of recombinant factor independent, *bcr-abl*-transformed lines and factor dependent controls, followed by testing on CML and normal patient samples, correctly revealed the potential of imatinib and gave us the confidence that imatinib should be moved into clinical development. This was despite data that only showed inhibition of tumor growth as opposed to eradication of tumors using in vivo animal models [8]. The reason for this modest in vivo activity became apparent from the murine

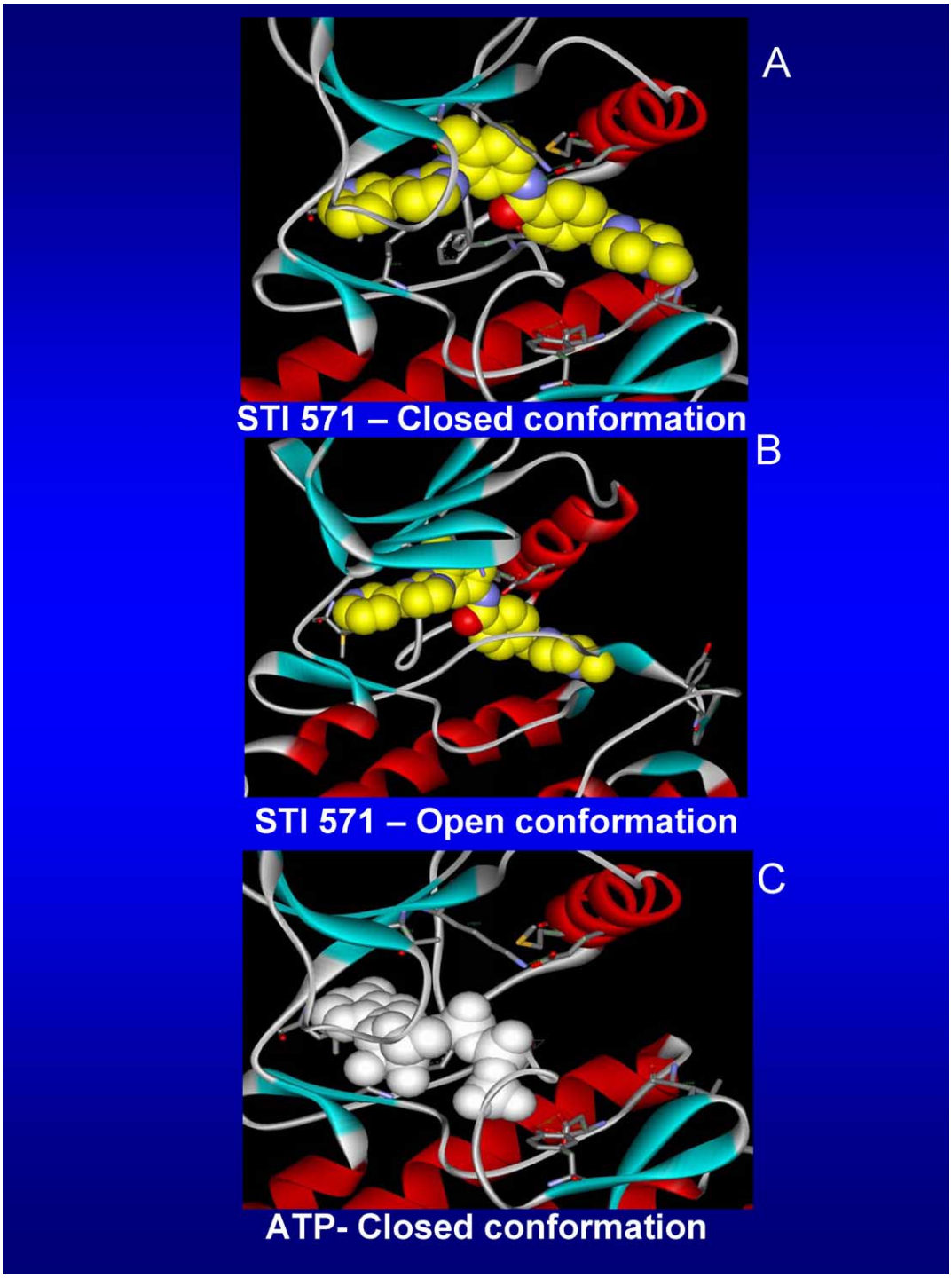

Fig. 3. The structure of imatinib bound to ABL. Imatinib bound to the inactive, closed confirmation of ABL (panel A) in which imatinib straddles the conserved activation loop DFG motif and its acid amine and piperazine (right hand side of inhibitor) pass under helix C [24]. Panel B shows a model of imatinib with ABL in the active confirmation. Note that this binding mode in not allowed due to the orientation of the activation loop. Panel C shows a model of ATP within the inactive confirmation of ABL. Again, this binding mode is not allowed due to the position of the DFG region of the activation loop.

pharmacokinetic profile of imatinib. This profiling revealed a short drug half-life in mice, which was not seen in other species (rat, dog, human). Thus, in nude mice, a single dose of imatinib inhibited BCR-ABL kinase activity for only 2–5 h. However, a three times per day dosing schedule results in a continual block of BCR-ABL kinase activity and results in eradication of tumors in 87% of imatinib-treated mice [35].

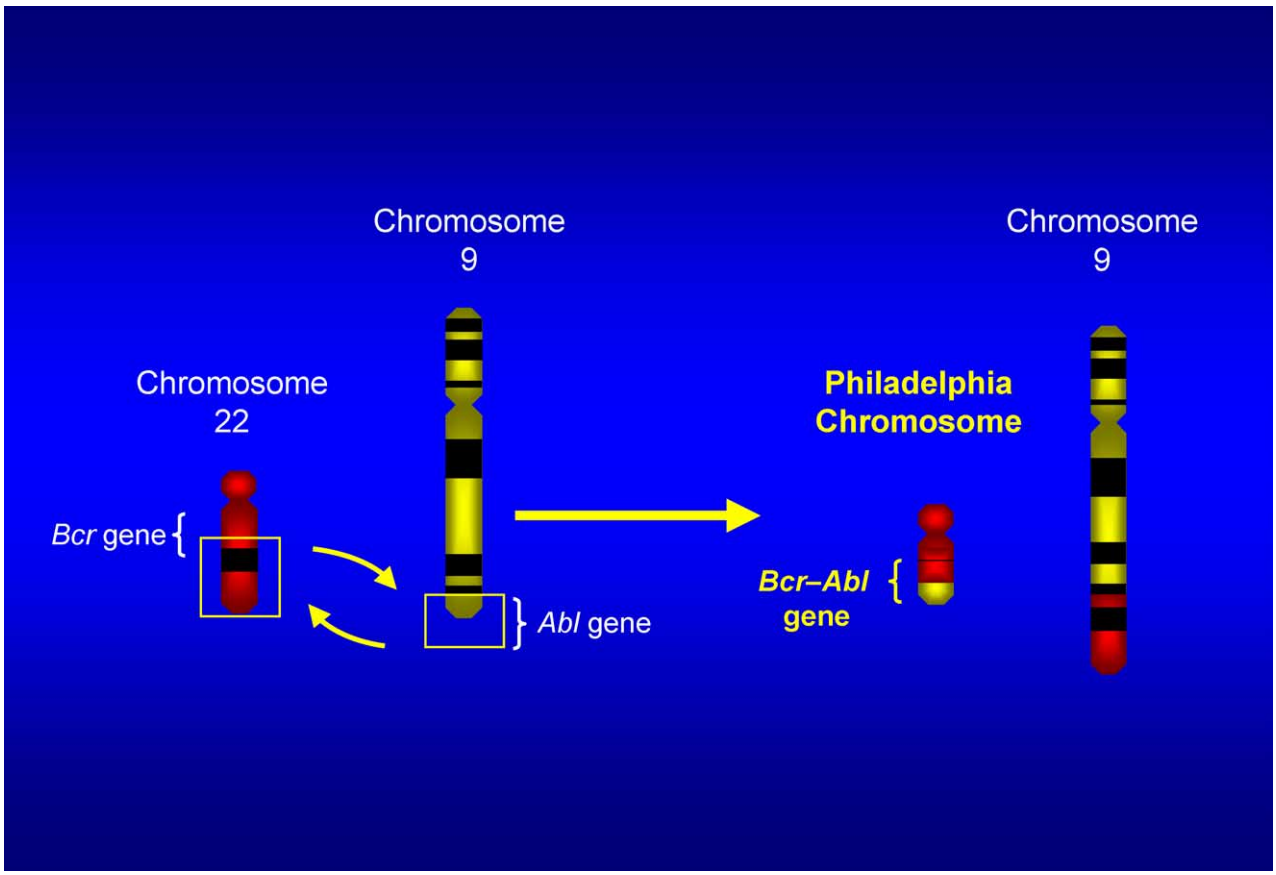

Fig. 4. The Philadelphia chromosome: the (9;22)(q34:11) translocation results in the generation of the *bcr-abl* oncogene whose product has constitutive kinase activity.

5. Bcr-Abl as a target

Over the last two decades, the evolving oncogene field has revolutionized our understanding of cancer (reviewed in references [36–38]). The scientific rational behind the selection of BCR-ABL as a target for the development of protein kinase inhibitors has always strong. Pioneering basic science work from numerous laboratories established the pivotal role for BCR-ABL kinase activity in CML (Fig. 4) [3,39,40]. The presence of *bcr-abl* in the majority of CML patients, and the dependence of BCR-ABL function on its kinase activity made this a particularly attractive target for the design of selective kinase inhibitors. Further, the ability to identify patients carrying the *bcr-abl* oncogene by the presence of the Philadelphia chromosome enabled us to readily select patients who were likely to benefit from imatinib therapy for the early trials.

Selection of patients who are likely to respond to targeted therapies could significantly reduce the risk and therefore the cost of clinical development, both of which are currently high. As our understanding of the molecular pathogenesis of cancer improves, it is likely that the cancer market will become significantly more fragmented. Therefore, a reduction in development risk and cost will become even more critical factors. In the case of CML, the clinical development of imatinib has shown that a combination of the right drug, and the appropriate target disease and patient population can reveal the true potential of such targeted agents.

Another unique advantage that assisted in the clinical development of imatinib was the ability to monitor response with surrogate end points, specifically, hematological and cytogenetic responses. Also, it was possible to incorporate a pharmacodynamic endpoint of inhibition of BCR-ABL kinase activity by assaying CRKL phosphorylation [9]. Lastly, from preclinical studies, we had a good indication that target blood levels for clinical activity were 1 μM trough levels of imatinib, and this prediction was confirmed in clinical trials [41]. Thus the parameters for efficient clinical testing of a kinase inhibitor were met: validation of the target and selection of patients highly likely to respond to therapy. In addition, the clinical trials analyzed kinase inhibition in the tumor, and pharmacokinetics, so that both positive and negative clinical results could be interpreted with confidence.

6. Activity in other tumors

According to the paradigm established above, imatinib would be expected to have activity against tumors where it has been established that a target of imatinib (i.e. ABL,

ARG, KIT or PDGFRs) is critical to the pathogenesis of the cancer.

The most striking example of this prediction being borne out has been the clinical results for imatinib in gastrointestinal stromal tumor (GIST). GISTs are mesenchymal neoplasms that can arise from any organ in the gastrointestinal tract or from the mesentery or omentum. More than 90% of GISTs express KIT [42], and biochemical evidence of KIT activation can be found in almost all GISTs [43]. In approximately 90% of cases, this activation is linked to somatic mutations of KIT, usually involving exons 9 or 11 [43]. Response rate of GISTs to single- or multi-agent chemotherapy is less than 5% [44]. In contrast, in phase I and II trials of imatinib in GIST, 53–65% of patients had objective responses, using a minimum dose of 400 mg per day [45,46].

Translocations involving the *PDGFRB* gene have been identified in several myeloproliferative and myelodysplastic syndromes. The most common of these translocations, (5;12)(q33;p13), is seen in a subset of patients with chronic myelomonocytic leukemia (CMML) and results in fusion of the *EVT6* (*TEL*) and *PDGFRB* genes [47,48]. Several patients with CMML containing the (5;12)(q33;p13) translocation have been treated with imatinib and all achieved complete hematologic remissions [49,50]. The PDGFRB pathway is also a target in dermatofibrosarcoma protuberans (DFSP), a low-grade sarcoma of the dermis that often recurs after surgical excision. These tumors are characterized by a (17;22) translocation involving the *COL1A1* and *PDGF-B* genes, which results in over-production of fusion COL1A1-PDGF-BB ligand and consequent hyperactivation of PDGFRB [51]. It has been shown that imatinib inhibits the growth of DFSP cells both in culture and in immunodeficient mice [52], and preliminary results in patients look promising [53,54].

The PDGF receptors and KIT are expressed in many common tumors and have been reported to be activated by both autocrine and paracrine mechanisms. However, it is unclear whether monotherapy with imatinib would be useful in any of these disorders. Imatinib may have a role in the treatment of such cancers, but meaningful conclusions will only be derived from carefully designed clinical trials that incorporate proteomic and genomic assessment of target activation status, with careful evaluation of responding patients.

Thus far, there is one example where an empiric clinical trial of imatinib has shown remarkable success. Initial reports demonstrated that patients with hypereosinophilic syndrome (HES) achieved complete hematologic responses to single agent imatinib, often with relatively low doses [55]. Molecular evaluations of these patients revealed an intrachromosomal deletion on chromosome 4 that fuses a gene of unknown function, *FIP1L1*, to the *PDGFRA*, resulting in activation of the PDGFRA [56]. This fusion protein is likely the causative molecular abnormality of a subset of patients with HES and accounts for the sensitivity to imatinib.

7. Analyzing subgroups of responding patients

The HES example demonstrates how the knowledge of the molecular mechanism of action of a drug, coupled with an empiric observation of clinical benefit, led to the rapid identification of the molecular pathogenesis of this disorder. An even better demonstration of some of the issues facing targeted therapies comes from the clinical trials in GIST. Virtually all GISTs express KIT, and for this reason, might be expected to be a good target for imatinib. However, a closer inspection of the data shows that KIT mutational status correlates with response. Specifically, patients with mutated KIT have a 72% response rate while patients with no mutations in KIT have only an 18% response rate [57]. Thus, the overall response rate in patients with GIST is highly dependent of the frequency of mutated KIT. For example, if 90% of patients with GIST have mutated KIT, then the response rate would be in excess of 60%. In contrast, if 90% of GISTs expressed wild-type KIT, then the response rate would be 20% or less. However, examination of the responding patients would identify those who responded as having KIT mutations in their tumors. As it turns out, the majority of GISTs have KIT mutations, which account for the high response rate. But what accounts for the 18% of patients with wild-type KIT who responded? Careful examination of these patients' tumors revealed that one-third of them had PDGFRA mutations that accounted for their response [58]. Similar to the HES experience, examination of responding patients coupled with an understanding of the molecular mechanism of action of a drug can yield important insights.

In reflecting on the experience with inhibitors of the epidermal growth factor receptor (EGFR), it will be of interest to see if similar information regarding subsets of responding patients will be forthcoming. It is certainly possible that the negative results of the phase III studies combining Iressa with chemotherapy were due to the fact that the majority of patients would not be expected to respond to Iressa as their EGFR signaling pathway was not critical to the growth or survival of the tumor. If, however, a molecular marker or mechanism to define responding patients was identified (e.g. activating mutations of the EGFR such as type III receptor deletions [59]), it might have been possible to select patients with a much greater likelihood of responding to this therapy. For the future, therapy will depend on the tumor type being treated, the appropriate use of diagnostic methods to identify potential responders and the appropriate selection of combination agents for clinical use.

8. Improved efficiency of drug design and specificity of inhibition

New methodologies in drug discovery, optimization and profiling of protein kinase inhibitors are likely to have a significant impact on the next generation of protein kinase

inhibitors. A selection of such technologies include the following.

1. *Target validation*: The use of RNA interference (RNAi) [60,61] gene knockout (loss of function) and cDNA expression (gain of function) in conjunction with expression profiling will establish "molecular fingerprints" of target pathways and the result of their selective blockade. This will aid significantly in the profiling of drug candidates for on target and off target effects [62]. Chemical genetic approaches are also making an impact in this area (e.g. Bishop et al. [63]).
2. *High throughput screening (HTS) and ultra HTS (UHTS)*: Automation of compound archiving, handling, preparation, and analytics, coupled with improved automation of HTS and UHTS, have resulted in the ability to rapidly generate larger amounts of high quality assay data in a parallel fashion. To be practically usable, such large data sets must be coupled with relational database storage and powerful methods for search, retrieval and visualization of large structure–activity relation data sets (e.g. versatile data mining and presentation tools such as Spotfire^{TM}). Once large compound profile database archives have been obtained, computational database mining methods will become increasingly important in the identification of interesting virtual hits for initiating exploratory chemistry projects.
3. *Affinity based screening methods*: Liquid chromatography–mass spectrometry (LC–MS [64] allows screening of inhibitor libraries for binding to different activation states of target protein kinases (i.e. inactive versus active conformation). Thus, screens can be performed in the absence of enzyme activity readout, allowing the identification of molecules that bind only to the inactive enzyme conformation. The screening application of this method can be dramatically increased with the use of mass encoded libraries [65].
4. *Compound libraries*: Many companies have made intensive efforts to improve the chemical diversity of their screening compound collections. Advances in computational methods for library diversity analysis and high throughput methods for the synthesis, purification and analytics of compounds are making a major impact in this area. Additionally, once biological results are available from screening such collections, and from follow-up chemistry projects on leads, the constructing of focused target class (e.g. phosphotransferases) inhibitor collections becomes possible. These focused compound collections can be profiled through larger panels of enzyme and cellular screens in an array manner. In the case of potential kinase inhibitors, this allows for rapid profiling of compounds from sublibraries, which have a higher probability of lead identification. Such focused collections allow screens to be front-loaded with compounds that are likely to have the highest potential hit rate within the kinase gene family.
5. *Medicinal chemistry methods*: Advances in high throughput chemistries (combinatorial synthesis such as multi-component reactions (reviewed in Weber [66]), solid phase parallel synthesis (e.g. Tan et al. [67]), and diversity oriented synthesis (e.g. Schreiber [68]) are aiding in library synthesis and early lead explosion. Other technical improvements in throughput include microwave chemistries [69], solid support reagents [70], purification (e.g. supercritical fluid chromatography) and analytical automation and methods.
6. *Structural biology*: The vast amount of X-ray data on protein kinases and the ability to carry out inhibitor design within a kinase family can reduce the resources needed for compound optimization and aid medicinal chemistry in the search for inhibitors of both the open and closed confirmation of protein kinase active sites [27,71]. However, to be useful in drug optimization, crystallography needs to be tightly coupled to the medicinal chemistry optimization program, rather than providing a retrospective explanation of binding mode after a drug candidate has been selected.
7. *Absorption, distribution, metabolism, and excretion (ADME) and physical/chemical profiling*: Rational in vitro and in vivo ADME screening has enabled compounds with unattractive ADME profiles to be eliminated earlier in the development process [72]. Additional predictive work on favorable physicochemical properties which influence ADME and formulation are aiding during the recovery phase in the selection of compounds which have the highest probability of development success [73]. Such work has traditionally been performed later in drug development, which results in difficulties in turnaround time if technical issues emerge at more advanced stages of the development path.
8. *Profiling of signal transduction pathways using phosphoproteomic methods*: Sensitive approaches based on LC–MS that enable the rapid identification of multiple sites of tyrosine phosphorylation on a number of different proteins have been developed [74,75]. Such methods have been used to follow changes in tyrosine phosphorylation patterns that occur during the inhibition of BCR-ABL by imatinib [76].
9. *Improved murine cancer models*: One of the major problems in the development of anti-cancer agents is the weakness of current xenograft animal tumor models to predict the utility of targeted agents or their combination with existing agents. For example, the use of EGFR inhibitors (e.g. Irresa) in a variety of carcinomas did not reveal significant patient benefit when combined with first line cytotoxic agents (phase III) as was predicted from numerous in vivo preclinical xenograph models. Novel mouse cancer models, using a variety of gene transfer techniques that can allow tissue-specific regulated expression of target oncogenes or tumor suppressor genes, are expected to have an impact on the development of targeted therapies [77] where the animal model has been

validated with respect to the molecular defects within the pathway of interest.

10. *Diagnostic methods*: The use of genomic methods including mutational analysis [78] and gene expression profiling should impact both diagnosis and therapy of cancers. Early examples of the potential of this approach were the identification of distinct types of diffuse large B-cell lymphoma (DLBCL) [79], pediatric acute lymphoblastic leukemia [80], and other cancers [81,82]. Future progress in this area is likely to have a significant impact on the design of clinical trials.

9. Conclusions

The last few years has been filled with tremendous excitement as the dramatic activity of imatinib has emerged from clinical trials to surpass even our most optimistic preclinical expectations. We believe that this success has paved the way for protein kinase inhibitors as a therapeutic class of drugs, with application in both malignant and non-malignant proliferative diseases. Despite the success of imatinib, the use of protein kinase inhibitors for the treatment of more complex solid tumors in which significant heterogeneity at the cellular and molecular levels exists will present additional technical hurdles. However, recent scientific advances in our understanding of the pathophysiology of cancers and novel discovery and diagnosis methodologies are likely to make a major impact in both identification of new drugs and their rational clinical development. In the case of CML, the clinical development of imatinib has shown that a combination of the right drug and the appropriate disease target and patient population can reveal the true potential of such targeted agents.

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