BCR-ABL1 Kinase: Hunting an Elusive Target with New Weapons

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Tyrosine kinase inhibitors such as imatinib, dasatinib, and nilotinib interfere with ATP-binding pocket to inhibit BCR-ABL1 kinase. A recent report in *Cell* by Grebien et al. paves the way for a new approach to target BCR-ABL1 kinase by interfering with its SH2-kinase domain interface.

BCR-ABL1 fusion tyrosine kinase results from a reciprocal chromosomal translocation between chromosomes 9 and 22, producing the Philadelphia chromosome. BCR-ABL1 is expressed in chronic myeloid leukemia (CML) and a cohort of acute lymphocytic leukemia. BCR-ABL1 kinase triggers CML in chronic phase (CML-CP) when expressed in hematopoietic stem cells, but uncontrolled proliferation of BCR-ABL1-positive progenitors is responsible for manifestation of the disease (Marley and Gordon, 2005). The ability of BCR-ABL1 kinase to induce a CML-like disease in mouse models and the anti-leukemia effect of BCR-ABL1-specific antisense oligonucleotides indicated that BCR-ABL1 kinase should be targeted in CML patients (Daley et al., 1990; Skorski et al., 1994).

Imatinib, a small molecule tyrosine kinase inhibitor (TKI) of the ABL1 kinase revolutionized the treatment of CML (Druker et al., 1996). Imatinib, an ATP-competitive inhibitor, is the first choice drug in CML-CP, because of its high efficacy, low toxicity and ability to maintain durable remissions. However, a significant number of patients initially treated with imatinib will develop drug resistance, which is often caused by the appearance of clones expressing mutant forms of BCR-ABL1. More than 50 imatinib-resistant BCR-ABL1 kinase domain mutations (for example, Y253F/H, E255K, T315I, M351T, H396R) were detected in CML patients who relapsed after initial response to imatinib (Shah et al., 2002). These mutations directly prevent imatinib binding or affect the ability of the kinase to achieve the conformation required to bind imatinib. This spurred the research to generate TKIs, which would also inhibit imatinib-resistant BCR-ABL1 kinase mutants.

Dasatinib and nilotinib, the two representatives of ATP-competitive second-generation TKIs (2G-TKIs), have been successfully applied in patients resistant to imatinib (Santos and Quintás-Cardama, 2011). However, none of the 2G-TKIs were able to inhibit the BCR-ABL1 T315I gatekeeper mutant. Third-generation TKIs (3G-TKIs) such as DCC-2036, which binds to the "switch pocket" that govern the transition between the active and the inactive states of ABL1 kinase, and AP24534, which avoids the interaction with the side chain of T315, retain the efficacy against the clinically relevant BCR-ABL1 kinase mutants, including T315I, and are currently in clinical trials. In addition, the myristoyl pocket located near the carboxy-terminal lobe of the ABL1 kinase domain could be targeted by GNF-2 and its analogs to inhibit BCR-ABL1 kinase activity through an allosteric non-ATP-competitive mechanism (Zhang et al., 2010). Moreover, GNF-2, when used in combination with the ATP-competitive inhibitors, displayed an additive inhibitory effect against T315I mutant.

However, BCR-ABL1 kinase mutants resistant to the 3G-TKIs will likely develop (Eide et al., 2011), and targeting other sites on BCR-ABL1 itself may improve future therapeutic options. BCR-ABL1 kinase consists of numerous domains regulating its leukemogenic activity, including an SH2 domain. In addition to their role in protein-protein interaction, SH2 domains in certain kinases, like ABL1, were shown to activate the adjacent tyrosine kinase domain. The ability of the SH2 domain to stimulate ABL1 kinase activity depended on the establishment of a tight interface between the SH2 domain and the N-terminal lobe of the kinase domain. In the recent paper in *Cell*, Florian Grebien, Oliver Hantschel and colleagues from Giulio Superti-Furga's laboratory (Grebien et al., 2011) showed that an intramolecular interaction between the SH2 domain and the kinase domain in BCR-ABL1 triggers high catalytic activity of the kinase. Interestingly, the T231R mutation in the SH2 domain of the BCR-ABL1 was implicated in imatinib resistance. This mutation may stabilize the

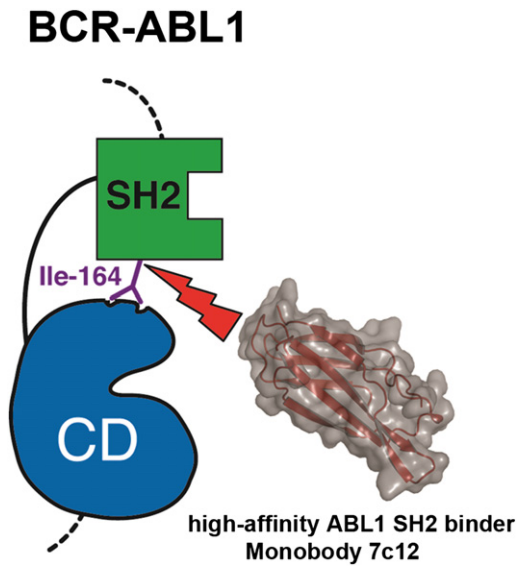

Figure 1. Targeting the SH2-Kinase Domain Interface in BCR-ABL1
Cartoon diagram of the SH2-kinase domain interface underlining the critical role of Ile164 (I164). The 7c12 monobody interferes with the I164 to inhibit BCR-ABL1 kinase activity.

SH2-kinase domain interface. Conversely, the I164E mutation located in the αA-βB-loop of the SH2 domain negated the allosteric activation of the BCR-ABL1 kinase by disrupting the SH2-kinase domain interface. Therefore, the SH2-kinase domain interface appears to play a critical structural role in adopting and maintaining of the active conformation of BCR-ABL1 kinase. In addition, the BCR-ABL I164E mutant did not induce leukemia in mice, revealing a critical role of the BCR-ABL1 SH2-kinase interface in leukemogenesis. This effect might depend on the almost exclusive lack of activation of STAT5, but not PI3k-Akt and Ras-MAPK pathways, by the BCR-ABL I164E mutant. Importantly, disruption of the SH2-kinase domain interface by I164E not only sensitized BCR-ABL1 to imatinib and nilotinib but also enhanced inhibition of TKI resistant BCR-ABL1 mutants including T315I.

To test if the SH2-kinase domain interface is "druggable," a single-domain binding protein (monobody) called 7c12, which interacts with the interface, was generated by phage-display library sorting (Figure 1). 7c12 exerted an inhibitory effect on BCR-ABL1 and T315I mutant kinase activities; the inhibition was improved when 7c12 was fused with a previously generated HA4 monobody, which interacts with the SH2 phosphotyrosine-binding pocket. The HA4-7c12 tandem monobody inhibited BCR-ABL1-mediated transformation and induced apoptosis in CML patient cells.

In conclusion, the SH2-kinase domain interface appears to play an essential role in the achievement and maintenance of the active conformation of BCR-ABL1 kinase and is also critical for BCR-ABL1-mediated leukemogenesis probably due to activation of STAT5. Moreover, interference with the SH2-kinase domain interface induced apoptosis in primary CML cells. Thus, in addition to the ATP- and myristate-binding sites, the SH2-kinase domain interface in BCR-ABL1 represents a potential "druggable" target for novel small molecule inhibitors. However, given the high mutagenic activity in CML cells, drug resistant mutants may emerge during therapy with the use of new compounds disrupting the SH2-kinase domain interface (Koptyra et al., 2006). Therefore, one could ask if anti-BCR-ABL1 kinase small molecule inhibitor therapy should be combined with drugs that inhibit genomic instability and prevent the appearance of resistant BCR-ABL1 mutants.

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