Allosteric BCR-ABL inhibitors in Philadelphia chromosome-positive acute lymphoblastic leukemia: novel opportunities for drug combinations to overcome resistance

Oliver Hantschel

Ecole Polytechnique Fédérale de Lausanne, School of Life Sciences, Swiss Institute for Experimental Cancer Research (ISREC), Lausanne, Switzerland

E-mail: oliver.hantschel@epfl.ch *doi:10.3324/haematol.2012.061812*

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The t(9;22) translocation that results in the formation of the Philadelphia chromosome (Ph) causes expression of the BCR-ABL tyrosine kinase fusion protein. Depending on the translocation breakpoint within the *BCR* gene, a protein of 210 kDa (termed BCR-ABL p210) or 185 kDa (termed BCR-ABL p185) can be expressed (Figure 1). Expression of p210 is the molecular hallmark of chronic myelogenous leukemia, whereas in Ph$^+$ acute lymphoblastic leukemia (Ph$^+$ ALL) expression of either p210 or p185 can be found. In contrast to its proto-oncogenic counterpart c-ABL, BCR-ABL displays constitutive tyrosine kinase activity.[1] The ATP-competitive BCR-ABL tyrosine kinase inhibitor imatinib leads to durable remissions in the majority of patients with chronic myelogenous leukemia in the chronic phase and converted chronic myelogenous leukemia to a chronic disease. Imatinib also improved the previously devastating outcome in Ph$^+$ ALL patients. However, the occurrence of imatinib resistance mutations in the ABL kinase domain became clinically problematic and led to the development of nilotinib and dasatinib, both also ATP-competitive inhibitors, which target most imatinib-resistant BCR-ABL mutations.[2] Still, short-lived responses in advanced phase chronic myelogenous leukemia and Ph$^+$ ALL, resistance caused by the T315I mutation, compound mutations (two or more mutations in the same clone) and foreseeable problems with long-term tolerability of all three BCR-ABL inhibitors remain challenging problems.[3]

The unsuspected identification of an allosteric site on the kinase domain with a location distinct from the catalytic cleft, to which imatinib and its successors bind, offered an alternative possibility to target imatinib-resistant BCR-ABL. Seminal work revealed molecular structures of autoinhibited c-ABL and showed intramolecular binding of the N-terminal myristate moiety of c-ABL to a deep hydrophobic pocket in the kinase domain.[4,5] Myristoylation is a co-translational covalent modification of the N-terminus of approximately 100 different human proteins, including c-ABL. Myristate binding causes a 90° bending of the α-I helix in the kinase domain that is required for the assembly of the catalytically inactive (autoinhibited) conformation of c-ABL.[4-6] Removal of the myristate or blocking its binding pocket led to the disassembly of the autoinhibited c-ABL conformation by unbending the α-I helix and resulted in a dramatic increase of kinase activity.[6] In the BCR-ABL fusion protein, the very N-terminus of ABL (including the myristoylation site) is missing because of the translocation event (Figure 1), whereas the myristate pocket in the kinase domain is preserved (and supposedly not occupied). This observation implied that chemical compounds that bind to the myristate pocket could re-establish some of the c-ABL autoinhibitory constraints that are lost in BCR-ABL (Figure 2).[5] As a consequence BCR-ABL activity would be allosterically inhibited. Furthermore, such compounds should be able to inhibit imatinib resistance mutations, as an alternative site is being targeted.

How to find such allosteric inhibitors targeting the myristate pocket?

Nathanael Gray and his team pursued a clever approach by using a high-throughput cell-based screen identifying numerous compounds that inhibited the growth of Ba/F3 cells expressing BCR-ABL p210, but not of parental Ba/F3 cells, thereby enabling the identification of BCR-ABL-selective compounds.[7] Structural inspection of the screening hits and discrimination of compounds belonging to known ATP-competitive inhibitor scaffolds resulted in the identification of a lead scaffold, of which one derivative, termed GNF-2, was chosen for further follow-up. Binding experiments with recombinant ABL showed that GNF-2 did indeed act as a non-ATP competitive (allosteric) inhibitor and that its binding to the ABL kinase domain could be blocked by previously described mutants in the myristate binding pocket which prevented myristate binding.[5,7] Some BCR-ABL forms carrying imatinib resistance mutations were sensitive to GNF-2, whereas others, including the T315I mutation that is resistant to imatinib, nilotinib and dasatinib, were also resistant to GNF-2.[7] This was a puzzling observation as none of the imatinib resistance mutations is in proximity to the myristate binding pocket. Evidence of 'gain-of-function' properties, such as increased kinase activity or oncogenic transformation, has been obtained for some imatinib resistance mutations and in particular for the T315I mutation.[8-10] It has been argued that some BCR-ABL kinase domain mutants causing imatinib resistance are able to stabilize a more active conformation of BCR-ABL to which imatinib binds less well. As the proposed mechanism of action of GNF-2 is to re-establish an inactive (autoinhibited) conformation of BCR-ABL, the observed cross-resistance to GNF-2 would fit this model (Figure 2). Recent elegant structural work, combining evidence from crystallography, nuclear magnetic resonance and hydrogen-exchange mass spectrometry experiments confirmed the proposed binding mode and mechanism of action of GNF-2 (and its close derivative GNF-5).[11] Furthermore, a combination of nilotinib and GNF-5 was shown to prolong survival of mice in a BCR-ABL T315I xenograft model, whereas each drug alone had no effect.[11] This work and additional observations from *in vitro* drug combination studies[12,13] provided convincing evidence that the combined application of ATP-competitive and allosteric BCR-ABL inhibitors can overcome resistance to either agent alone. Nevertheless, the efficacy of GNF-2 on primary human Ph$^+$ leukemia cells has not been demonstrated.

In this issue of Haematologica, Mian *et al.* explored the efficacy and the clinical feasibility of allosteric inhibition in the treatment of Ph$^+$ ALL.[14] The authors compared the sensitivity of BCR-ABL p210 and BCR-ABL p185 to GNF-2 inhibition, as

it was not known whether p185 is inhibited by GNF-2 and, if so, how strong the inhibition is in comparison to that of p210. The authors used primary cells from Ph+ ALL patients expressing either p185 or p210, as well as Ba/F3 cells that were retrovirally transduced to express p185 or p210 and other established ALL cell lines. Consistently, the sensitivity of p210 to GNF-2 was 3-5 fold higher than that of p185 when assaying inhibition of cell proliferation, BCR-ABL autophosphorylation and phosphorylation of the canonical BCR-ABL substrate CrkL. This differential sensitivity was also preserved when imatinib-resistant kinase domain point mutants in the P-loop of p185 and p210 were assayed. In contrast, both p210 and p185 carrying the T315I mutation were insensitive to GNF-2. The molecular basis for the observed differential sensitivity is unclear, as both BCR-ABL isoforms carry the identical

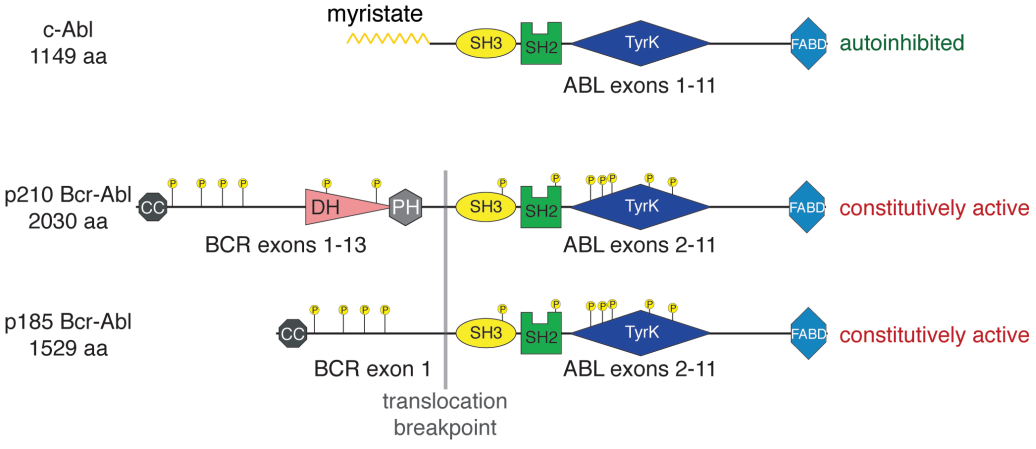

Figure 1. Schematic domain representation of c-ABL, p185 and p210 BCR-ABL. The fusion site between *BCR* and *ABL* exons is represented as a gray vertical line. The exons of the *BCR* and *ABL1* genes that are included in the fusion proteins and the number of amino acids of the full-length proteins are indicated.

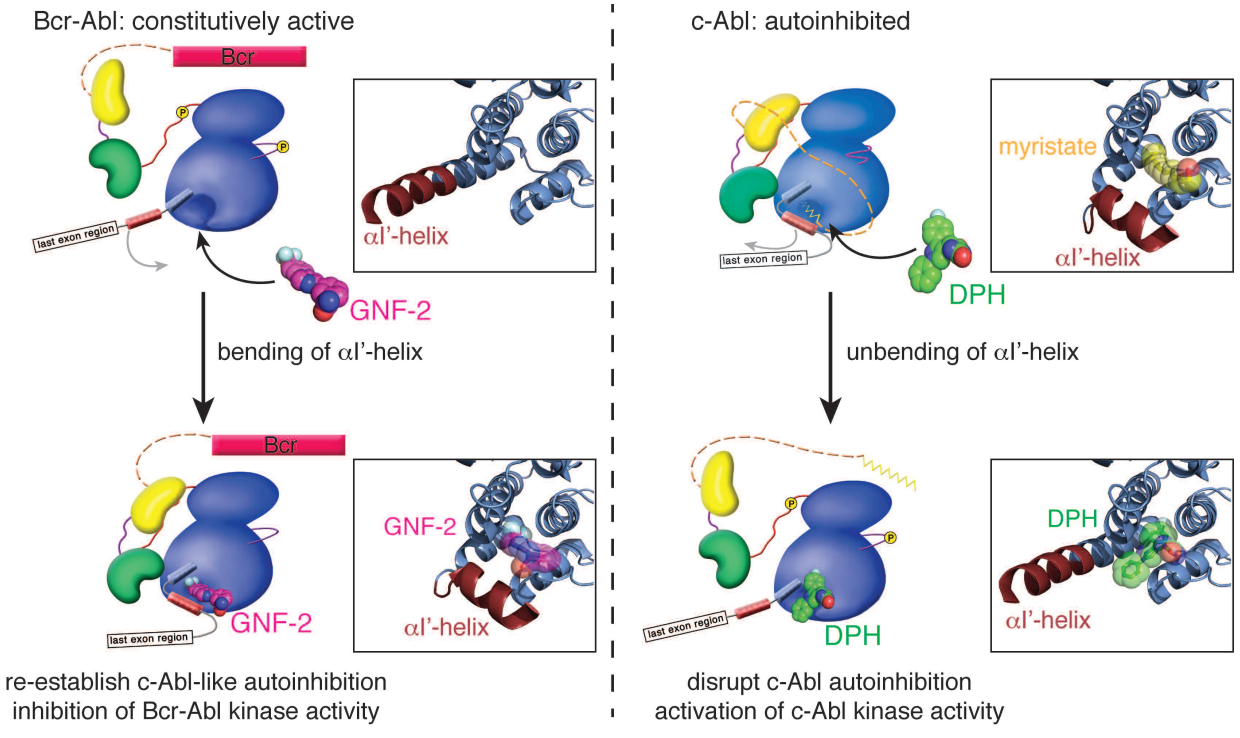

Figure 2. Schematic representation of the mechanism of action of BCR-ABL/c-ABL myristate pocket binders. The SH3, SH2 and kinase domains are shown in yellow, green and blue, respectively. The BCR-part and the C-terminal last exon region downstream of the ABL kinase domain is shown without further details on domains or sequence motifs. The myristoyl group that is attached to the N-terminus of c-ABL binds to a deep hydrophobic pocket in the kinase domain and induces bending of the C-terminal αI-helix (shown in dark red). BCR-ABL is not myristoylated, but retains the myristate pocket. GNF-2 binds to the myristate pocket (left panel), bends the αI-helix (as myristate binding does) and induces a c-ABL-like autoinhibited conformation of BCR-ABL that has reduced kinase activity. In contrast, binding of DPH to the myristate pocket (right panel) is not compatible with the inactive conformation and leads to activation of ABL kinase activity by un-bending the αI-helix and disruption of autoinhibitory constraints. Next to the schematic representations, a blow-up shows the myristate pocket from the crystal structures of the ABL kinase domain alone and in complex with myristate,[19] GNF-2[11] or DPH[15].

ABL-part (Figure 1). A possible explanation could be that p185 can less readily conduct the conformational changes that commence with GNF-2 binding (Figure 2). Alternatively, differences in the downstream signaling networks of the two BCR-ABL isoforms might be responsible. The presented data not only further establishes allosteric inhibition as a possible alternative targeted approach for the treatment of Ph^+ ALL, but also underlines the notion that the BCR-ABL breakpoint needs to be accurately defined in order to choose the optimal treatment regimen. Given the observed lower GNF-2 sensitivity of p185 one may predict that patients expressing p210 respond better than patients expressing p185 in possible future clinical studies involving GNF-2.

It is interesting to note that another class of compounds that target the ABL myristoyl pocket was recently identified in a high-throughput screen. In contrast to GNF-2, the compound DPH, potently activated (rather than inhibited) c-ABL kinase activity.[15] DPH was able to displace a myristoylated peptide that corresponded in sequence to the N-terminus of c-ABL from the myristoyl pocket. Importantly, DPH is not able to induce the bent conformation of the α-I helix that is seen in the autoinhibited ABL structures upon binding of myristate and induced by binding of GNF-2 (Figure 2). Therefore, although GNF-2 and DPH both bind to the myristate pocket, GNF-2 inhibits BCR-ABL activity, whereas DPH activates c-ABL activity. These differences are due to the ability of GNF-2 to enable and of DPH to prevent bending of the α-I helix in the kinase domain. The identification of DPH and other c-ABL activators targeting the myristoyl pocket[16] provides very valuable tools for studying the physiological functions of endogenous c-ABL.

In conclusion, during the past 10 years we have witnessed the ascent of first- (imatinib), second- (nilotinib and dasatinib) and several third-generation ATP-competitive BCR-ABL inhibitors. Together with the identification of allosteric inhibitors of BCR-ABL targeting the myristate pocket, the N-terminal coiled-coil oligomerization domain,[17] or the SH2-kinase domain interaction,[18] we are provided with a large tool-kit to study the regulation of wild-type and mutated BCR-ABL. This will enable us to make rational decisions for the best possible combination of two or more of these highly potent targeted agents and to assess their efficacy in pre-clinical models and ultimately in patients with Ph^+ leukemias.

Oliver Hantschel is an Assistant Professor for Translational Oncology at the Swiss Institute for Experimental Cancer Research (ISREC), which is integrated in the School of Life Sciences at the Swiss Federal Institute of Technology in Lausanne, Switzerland. His work is dedicated to the molecular understanding of the signaling pathways and the regulation of ABL fusion oncoproteins and the study of the mechanism of action of ATP-competitive and allosteric kinase inhibitors.

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