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Research Article

Constitutive Activation of Tyrosine Kinase by Oligomerization of BCR-ABL1, Allosteric Effect and Adaptor Proteins in Chronic Myeloid Leukemia and Novel Therapeutic Strategies

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Abstract

Introduction of orthosteric tyrosine kinase inhibitors (TKIs) targeting BCR-ABL1 has drastically improved prognosis of the patients with CML at chronic phase (CML-CP). However, once blast crisis occurs, conventional therapies including TKIs become ineffective to leukemia stem cells (LSCs) of CML. Thus substantially different therapeutics strategies are required. This review article is aimed at summarizing the mechanisms of BCR-ABL1 oligomerization, allosteric effect on its conformation and adaptor proteins around BCR-ABL1. Then, novel therapeutic strategies against CML LSCs based on these three upstream activation mechanisms of the BCR-ABL1 were shown. Oligomerization of BCR-ABL1 is required for constitutive activation of tyrosine kinase. Allosteric effect on conformation change of BCR-ABL1 induces autophosphorylation of BCR-ABL1 that is required for binding of adaptor proteins that mediate signal transduction. Thus inhibitors targeting the BCR coiled-coil (CC) domains that induce oligomerization, allosteric inhibitors and inhibitors against adaptor proteins are the promising therapeutic strategies to overcome various types of therapy resistance in CML. However, detailed investigations and trials are further required.

Keywords: BCR-ABL1, CML, therapy resistance, oligomerization, allostery, adaptor protein.

Abbreviations

ABL1=Abelson protooncogene 1, **ALL**=acute lymphoblastic leukemia, **AML**=acute myeloid leukemia, **AP**=accelerated phase, **BCR**=breakpoint cluster region, **BMM**=bone marrow microenvironment, **BP**=blast phase, **CC**=coiled-coil, **CML**=chronic myeloid leukemia, **CML-N**=neutrophilic chronic myeloid leukemia, **CP**=chronic phase, **ERK**=extracellular signal-regulated kinase, **GAB2**=GRB2-associated binding protein 2, **GRB2**=growth factor receptor-binding protein-2, **JAK**=Janus kinase, **KD**=kinase domain, **LSC**=leukemia stem cell, **MAPK**=mitogen-activated protein kinase, **mTOR**=mechanistic target of rapamycin, **PI3K**=phosphatidylinositol-3 kinase, **PR**=proline rich, **RasGEF**=Ras-specific guanine nucleotide exchange factor, **SH**=src homology, **SHP2**=src-homology 2 domain-containing phosphatase 2, **SOS**=Son of sevenless, **STAT**=signal transducers and activators of transcription, **TKI**=tyrosine kinase inhibitor,

Introduction

Introduction of orthosteric tyrosine kinase inhibitors (TKIs) targeting the *breakpoint cluster region (BCR)-Abelson protooncogene 1 (ABL1) fusion gene* products of chronic myeloid leukemia (CML) has drastically improved prognosis of the patients with CML at chronic phase (CML-CP) [1-5]. However, during TKI treatment, CML gradually progresses into blast phase (CML-BP) due to various resistance mechanisms, including secondary mutations within the sequences that these orthosteric TKIs target [6-8], activation of the downstream signaling effectors [9-13], induction of blast transformation [14-18] or other resistance mechanisms [19] such as *BCR-ABL1 gene* amplification [20], membrane transporter dysfunction [21] and new chromosomal aberrations (+8, +19, +21, +Y, second Ph+, or i(17)q) [22,23]. On the one hand, numerous reports on the therapy resistance to TKIs by secondary mutations have accumulated and new TKIs have been developed.

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However, once blast crisis occurs, TKIs become ineffective to leukemia stem cells (LSCs) of CML. On the other hand, the mechanisms of blast transformation are highly complicated, including additional genetic alterations [15,16], epigenetic dysfunctions [24-26], differentiation arrest [27-31], alternative splicing/mis-splicing [32-34] and aberrantly regulated factors in bone marrow microenvironment (BMM) [35,36]. Certainly there are many target points, but studies on the therapies against CML LSCs based on the mechanisms of blast transformation remain at an initial stage [17,18,37-40]. In addition, therapies against CML LSCs after BC caused by BCR-ABL1-dependent and -independent mechanisms have been intensively investigated [6,38,40-42]. However, we have not yet obtained satisfactory therapeutic strategies against CML LSCs probably due to persistence of CML LSCs after treatment[41-43]. Thus substantially different therapeutics strategies are required.

The constitutive activation of BCR-ABL1 tyrosine kinase are dependent on oligomerization of BCR-ABL1 [44-47], allosteric effect on conformational change of BCR-ABL1 [13,47-50] or adaptor proteins that mediate signal transduction from BCR-ABL1 [51-55]. There are many adaptor proteins around BCR-ABL1, but growth factor receptor-binding protein-2 (GRB2) is the most important in leukemogenesis of CML cells [54,55]. This review article is aimed at summarizing the mechanisms of BCR-ABL1 oligomerization, allosteric effect on its conformational change and adaptor proteins around BCR-ABL1. Then novel therapeutic strategies against CML LSCs based on these three upstream activation mechanisms of the BCR-ABL1 are shown.

Variants of BCR-ABL1 proteins and TKIs

Variants of BCR-ABL1

CML is characterized by the Philadelphia chromosome (Ph) generated by the reciprocal translocations t(9;22)(q34;q11.2) that generates the *BCR-ABL1* fusion gene. There are three types of BCR-ABL1 fusion gene products, p190, p210 and p230. Of these three variants, p210 is typically found in CML [56] and rarely in acute myeloid leukemia (AML) [57]. p190 is frequently observed in acute lymphoblastic leukemia (ALL) [58] and occasionally in AML [59], while p230 is specifically found in neutrophilic CML (CML-N) [60]. We focus on the p210 BCR-ABL1 that is substantially involved in CML leukemogenesis, because it is found in almost all the cases of CML and its activation of downstream signaling pathways is active at both CML-CP and CML-BP.

Constitutive activation of the tyrosine kinase in BCR-ABL1 further activates downstream signaling pathways including phosphatidylinositol-3 kinase (PI3K)/AKT/mechanistic target of rapamycin (mTOR), Ras/extracellular signal-regulated kinase (ERK) or Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signaling. These activated signaling pathways are involved in leukemogenesis of CML [6,38,40,42].

TKI resistance

The tyrosine kinase activity in BCR-ABL1 is effectively inhibited by orthosteric TKIs. The first TKI imatinib drastically improved prognosis of the patients with CML-CP [1,2]. However, secondary mutations in the tyrosine kinase domain of BCR-ABL1 induce resistance to TKIs. Thus the second generation TKIs dasatinib (sensitive to the mutations Y253H, E255V/K, F359V/I/C), nilotinib (sensitive to F317L/VLI/C, T315A, V299L), bosutinib (sensitive to Y253F/H, E279K, M351T, H396P/R) and the third generation TIK ponatinib (sensitive to T315I and others) have been successively developed [4,5,19,61-63]. The orthosteric inhibitors, i.e., type I, II, are ATP-competitive inhibitors that block the binding of ATP to the catalytic site of the Abl kinase domain (KD) [50]. Unfortunately these orthostetic inhibitors except for pnatinib are ineffective to the gatekeeper mutation T315I. Certainly PKIs are quite effective at CML-CP, but CML-CP progresses to accelerated phase (CML-AP) and finally CML-BP due to clonal evolution of resistant clones. To overcome this resistance to TKIs, novel therapies effectively targeting the critical points of BCR-ABL1-dependent and -independent resistance have been vigorously studied [6,19,38,40-42,61-64]. However, there remain various problems to overcome. Probably other therapeutic strategies should be pursued [47,50,65,66].

Allosteric effect on BCR-ABL1 conformation

Structure of BCR-ABL1

The p210 BCR-ABL1 protein consists of the following domains: the coiled-coil (CC) oligomerization domain in BCR (BCR-CC), GRB2-binding domain at Thy177 (Y177) in the Ser/Thr kinase (STK) domain, Dbl homology (DH) domain and pleckstrin homology (PH) domain in the BCR region, and the src homology (SH) domains 3, SH2, SH1 (tyrosine kinase domain), nuclear localization signal (NLS), DNA-binding (DB) domain and actin-binding (AB) domain in the ABL1 region [10,12,13,19,44,48,50,55,61] (Figure 1). The tyrosine kinase domain that TKIs target is in the SH1 domain [12,13,48,50,61]. BCR-CC is crucial for oligomerization of BCR-ABL1, leading to leukemogenesis of CML [44,45,46,66].

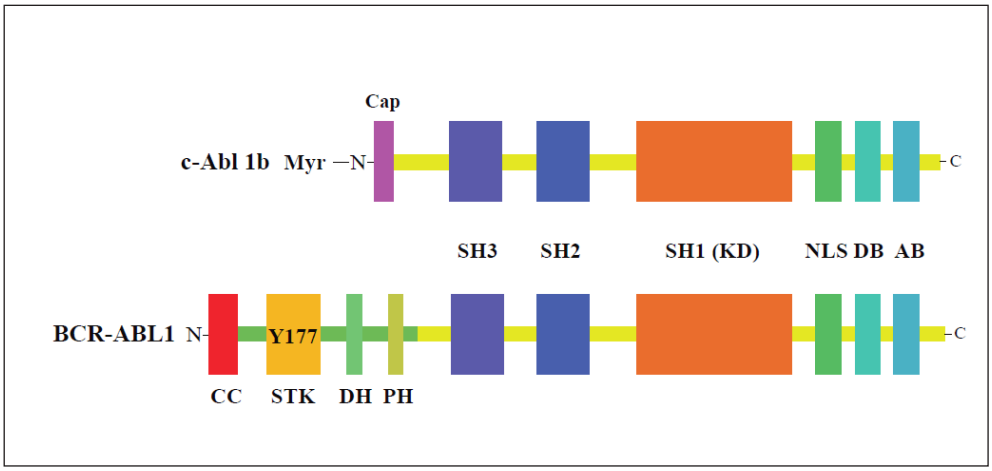

Figure 1. *Structure of c-Abl 1b and BCR-ABL1. c-Abl 1b has a myristoyl (Myr) and N-cap, which lack in BCR-ABL1. The c-Abl 1b and the BCR-ABL1 commonly contain src homology domain 3 (SH3), SH2, SH1 (tyrosine kinase domain[KD]), nuclear localization signal (NLS), DNA-binding (DB) domain and actin-binding (AB) domain, while the BCR retains coiled-coil (CC) oligomerization domain, growth factor receptor-binding protein-2 (GRB2)-binding domain at Tyr177 (Y177) in the Ser/Thr kinase (STK) domain, Dbl homology (DH) domain and pleckstrin homology (PH) domain.*

Conformational Change of BCR-ABL1

The c-Abl is a cytoplasmic, non-receptor tyrosine kinase, encoded by a protooncogene *c-abl* [50]. c-Abl has two isoforms 1a and 1b. The c-Abl 1b has an N-terminal myristoyl while c-Abl 1a lacks it [48,67]. Both isoforms have N-cap that contains about 80 residues with a critical role in auto-inhibition [47-50] (Figure 1). This N-cap is broken off from the BCR-ABL1 fusion protein. Thus the fusion between *BCR* and *ABL1* causes allosteric effect on its conformation [47,49]. This conformational change induces switch from a closed inactive state of c-Abl to an open active state of BCR-ABL1 [13,47-50], leading to activated oligomerization [65,66] and increased tyrosine kinase activity [44,45,48,61]. Oligomerization of BCR-ABL1 via BCR-CC is required for leukemogenesis of CML [44-47]. Due to the allosteric effect, autophosphorylation at Y177 in the BCR region of BCR-ABL1 is induced by the tyrosine kinase in the ABL1 region of BCR-ABL1 [13,61]. This phosphorylated Y177 (pY177) in BCR/ABL1 is the crucial binding site of an adapter protein GRB2 [44,68] that mediates several critical signaling pathways.

Inhibitors Targeting Molecules of Allostery

Inhibitors Targeting BCR-CC

BCR-CC is required for oligomerization of BCR-ABL1 and leukemogenesis of CML via compositional change of BCR-ABL1 [44-46,65,66]. Thus, inhibitors against BCR-CC is expected to inhibit cell proliferation and induce apoptosis of CML cells (Table 1). N-terminal BCR-CC (residues 1-71) contains α1-helix (residues 5-17) and α2-helix (residues 28-67) [45,65,69], and via interaction between these two helices BCR-ABL1 forms dimer and tetramer [65,70]. To disrupt the oligomerization of BCR-ABL1, introduction of the mutated CC peptide with the N-terminal CC deletion (Δ1-61 or Δ1-63) into CML cell lines succeeded in reducing cell proliferation and inducing apoptosis [44,70,71] (Table 1). Then, several CC mutated inhibitors have been elaborated to effectively reduce the tyrosine kinase activity of BCR-ABL1, including CCmut2 [45], CCmut3 [46,69], leukemia-specific cell-penetrating peptide (CPP)-conjugated CCmut3 (CPP-CCmut3) [72], and CPP-CCmut3-st [66] (Table 1). In addition, HSP90AB1 in combination with 17AAG (tanespimycin) induces nuclear transport of BCR-ABL1, resulting in induction of apoptosis by activating pro-apoptotic genes and reduction of cell proliferation by decreased cytoplasmic oncogenic signaling transduction [73] (Table 1).

Allosteric Inhibitors

The c-Abl 1b is composed of myristoyl, N-cap (residues 1-80), SH3 domain (residues 85-138), linker-H3/H2 (residues 149-152), SH2 domain (residues 153-237), linker-H2/KD (residues 238-250), KD (residues 255-534, made by N-lobe, ATP-binding site, catalytic domain and C-lobe), and last exon region (NLS, DB and AB) [48,50,74-77]. The compact conformation of the autoinhibited state of c-Abl 1b shows (a) binding of the myristoyl group at the hydrophobic pocket (i.e., the myristoyl pocket) of the C-lobe KD, (b) juxtaposition between the SH3 domain and N-lobe KD, and (c) direct contact of the SH2 domain with C-lobe KD [13,47-50,74-77]. In turn, the extended active conformation of c-Abl 1b is characterized by the SH2 docking onto the top of the N-lobe KD [13,47,48,75,76].

Table 1. *Inhibitors targeting BCR-CC in CML.*

Inhibitors	Mechanisms	Effect	References
Deletion of CC (Δ1-61)	Inhibition of oligomerization of BCR-ABL1	Inhibition of tyrosine kinase activity of BCR-ABL1	[71]
Deletion of CC (Δ1-63)	Inhibition of oligomerization of BCR-ABL1	Inhibition of tyrosine kinase activity of BCR-ABL1	[44,70]
CCmut2	Inhibition of oligomerization of BCR-ABL1	Inhibition of tyrosine kinase activity of BCR-ABL1	[45]
CCmut3	Inhibition of oligomerization of BCR-ABL1	Inhibition of tyrosine kinase activity of BCR-ABL1	[46,69]
CPP-CCmut3	Inhibition of oligomerization of BCR-ABL1	Inhibition of tyrosine kinase activity of BCR-ABL1	[72]
CPP-CCmut3-st	Inhibition of oligomerization of BCR-ABL1	Inhibition of tyrosine kinase activity of BCR-ABL1	[66]
HSP90AB1 + 17AAG	Nuclear transport of BCR-ABL1	Reduced tyrosine kinase activity and signal transduction	[73]

Abbreviations: *ABL1=Abelson protooncogene 1, BCR=breakpoint cluster region, CC=coiled-coil, CPP=cell-penetrating peptide.*

In BCR-ABL1, due to the allosteric effect by deletion of the myristoyl and N-cap of c-Abl 1b, its tyrosine kinase is constitutively activated. Thus the myristoyl pocket and the interaction between SH2 and KD are two target points of allosteric inhibitors to inhibit constitutively activated tyrosine kinase of BCR-ABL1. Accordingly, there are two types of allosteric inhibitors (Table 2). First, the allosteric inhibitors GNF-2 (Coi et al., 2009)[78,79], GNF-5 [80], BO1 [81,82], asciminib (ABL001) [83-85], BCR.lib_01, BCR.lib_02 and BCR.lib_03 [50] bind to the myristoyl pocket site at the C-terminus of Abl KD, because these allosteric inhibitors mimic the myristate substrate. This binding to the myristoyl pocket induces a compact inactive conformation of KD, leading to inhibition of the tyrosine kinase activity of BCR-ABL1 (Table 2). In particular, asciminib is active against all catalytic ATP site mutations including the gatekeeper T315I mutation [85]. Second, 7c12 [86,87] and i7c12-HA4 [88] are allosteric inhibitors that interfere with the interaction between the SH2 and KD. These allosteric inhibitors switch an extended active conformation to a compact inactive conformation of KD, resulting in the tyrosine kinase inhibition of BCR-ABL1 (Table 2).

Table 2. *Allosteric inhibitors in CML.*

Inhibitors	Mechanisms	Effect	References
GNF-2	Binding to the myristoyl pocket site of KD	Induction of a compact inactive conformation of KD	[78,79]
GNF-5	Binding to the myristoyl pocket site of KD	Induction of a compact inactive conformation of KD	[80]
BO1	Binding to the myristoyl pocket site of KD	Induction of a compact inactive conformation of KD	[81,81]
Asciminib	Binding to the myristoyl pocket site of KD	Induction of a compact inactive conformation of KD	[83-85]
BCR.lib_01, 02,03	Binding to the myristoyl pocket site of KD	Induction of a compact inactive conformation of KD	[50]
7c12	Interference with interactions between SH2 and KD	Switch from an extended active to a compact inactive conformation of KD	[86,87]
7c12-HA4	Interference with interactions between SH2 and KD	Switch from an extended active to a compact inactive conformation of KD	[88]

Abbreviations: BCR=breakpoint cluster region, KD=kinase domain, SH2=src homology 2.

Adaptor Proteins

GRB2-SOS1 complex

There are several adaptor proteins around BCR-ABL1 such as GRB2, Son of sevenless 1 (SOS1), GRB2-associated binding protein 2 (GAB2), src-homology 2 domain-containing phosphatase 2 (SHP2) and CRKL [51-53]. Of these, GRB2 plays the central role in signal transduction and leukemogenesis by BCR-ABL1 [54,55]. GRB2 contains a single SH2 and two SH3 domains (an N-terminal SH3 [nSH3] and a C-terminal SH3 [cSH3]) [54,89]. The SH2 domain of GRB2 recognizes and binds high affinity specific phosphorylated tyrosine in the pYXN motif [55], whereas the SH3 domains of GRB2 bind to proline rich (PR) domains containing the PXXP motif [55]. The SH2 domain of GRB2 binds to pY177 [68,90]. Then both n-SH3 and cSH3 of the active GRB2 recruits SOS1 that is one of the Ras-specific guanine nucleotide exchange factors (RasGEFs) [54], and SOS1 binds to the SH3 domains of GRB2 via its C-terminal PR domain [54]. GRB2 translocates SOS1 to the cell membrane [91]. Active SOS1 exchanges GDP by GTP and induces conformational change of the inactive GDP-bound state of Ras (Ras-GDP) to its active GTP-bound form (Ras-GTP) [47,54,55,92] (Figure 2). The activated Ras then activates Raf [92], leading to activation of the Ras/Raf/extracellular signal-regulated kinase (ERK) signaling pathway[54,55,93].

GRB2-GAB2 Complex

Binding of GAB2 to GRB2 is another important mechanism of signal transduction from BCR-ABL1 [52,94,95]. GAB2 has binding sites for other SH2-containing signal relay proteins such as p85α regulatory subunit of type Ia PI3K and SHP2 [95]. The GAB2 bound to pY177-GRB2 via the GRB2 SH3 binding site in GAB2 is tyrosine phosphorylated [94,96]. The pY177-GRB2-GAB2 complex further activates signaling pathways (Figure 2). On the one hand, the GAB2 interacts with p85α-PI3K and activates the PI3K signaling pathway, leading to activation of AKT [94] and induction of cell proliferation, cell survival and leukemogenesis of CML (Figure 2). On the other hand, the GAB2 binds to SHP2 [96] and the activated SHP2 further activates ERK [94], resulting in leukemogenesis of CML (Figure 2). As a result, GRB2-GAB2 complex activates the PI3K/AKT signaling via binding to p85α-PI3K and the Ras/ERK signaling via binding to SHP2 [94,95]. In this regard, the Ras/ERK signaling pathway is activated by both the GRB2-SOS1 complex and the GRB2-GAB2-SHP2 complex [94,95] (Figure 2).

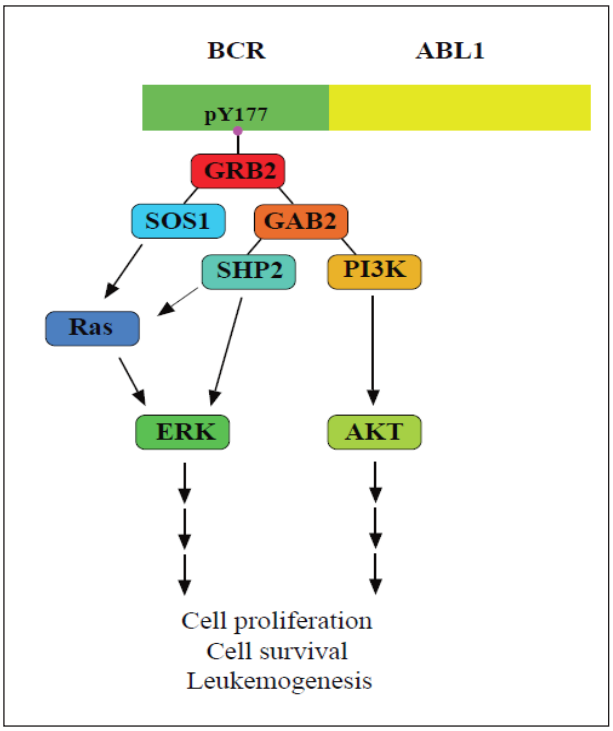

Figure 2. *Activation of the Ras/ERK signaling by the GRB2-SOS1 complex and the GRB2-GAB2-SHP2 complex, and activation of the PI3K/AKT signaling by the GRB2-GAB2-PI3K complex. Abbreviations: ABL1=Abelson protooncogene 1, BCR=breakpoint cluster region, ERK=extracellular signal-regulated kinase, GAB2=GRB2-associated binding protein 2, GRB2=growth factor receptor-binding protein-2, PI3K=phosphatidylinositol-3 kinase, SHP2=src-homology 2 domain-containing phosphatase 2, SOS=Son of sevenless.*

Inhibitors Against Adaptor Proteins

Inhibitors Against pY177-GRB2 Binding

There are several target points around GRB2. First, a Y177 mutant (Y177F) targets the interaction between pY177 and GRB2. The BCR-ABL1 mutant (Y177F) reduces the downstream effector of the Ras/ERK signaling, resulting in inhibition of cell proliferation and leukemogenesis [93,94,97] (Table 3). This is thought to be caused by the reduced activity of both the GRB2-SOS1 and the GRB2-GAB2-SHP2 (Table 3, Figure 2). In addition, the mutant Y177F induces suppression of the activity of PI3K/AKT via the reduced activity of the GRB2-GAB2-PI3K [94] (Table 3, Figure 2).

Inhibitors Against GRB2-SOS1 Complex

Modulation of the interaction between GRB2 and SOS1 is another therapeutic target point. On the one hand, deletion of the GRB2 SH3 blocks the interaction between the GRB2 nSH3/cSH3 and the SOS1 PR domain, and the recruitment of SOS1 to the cell membrane is inhibited, leading to suppression of the Ras activity [55,98] (Table 3, Figure 2). On the other hand, the polypeptides that disrupt the GRB2-SOS1 interaction also have effect on inhibition of the GRB2-SOS1 binding and recruitment of SOS1. This finally inhibits the Ras/ERK signaling pathway [54,99] (Table 3, Figure 2).

Table 3. *Inhibitors against adaptor proteins in CML.*

Inhibitors	Mechanisms	Effect	References
Y177 mutant (Y177F)	Inhibition of GRB2-SOS1 binding	Suppression of Ras/ERK signaling	[93,97]
Y177 mutant (Y177F)	Inhibition of GRB2-GAB2-SHP2 binding	Suppression of Ras/ERK signaling	[94]
Y177 mutant (Y177F)	Inhibition of GRB2-GAB2-PI3K binding	Suppression of PI3K/AKT signaling	[94]
Deletion of GRB2 SH3	Inhibition of SOS1 binding to GRB2	Suppression of SOS1 recruitment and Ras activity	[55,98]
GRB2-SOS1 interaction inhibitors	Inhibition of SOS1 binding to GRB2	Suppression of SOS1 recruitment and Ras activity	[54,99]
GAB2 mutant lacking binding sites for PI3K and SHP2	Inhibition of GAB2-PI3K and GAB2-SHP2 binding	Suppression of PI3K/AKT and Ras/ERK signaling	[95]

Abbreviations: ERK=extracellular signal-regulated kinase, GAB2=GRB2-associated binding protein 2, GRB2=growth factor receptor-binding protein-2, PI3K=phosphatidylinositol-3 kinase, SH3=src homology 3, SHP2=src-homology 2 domain-containing phosphatase 2, SOS=Son of sevenless

Inhibitors against GAB2-PI3K binding and GAB2-SHP2 binding

The Y177 mutant (Y177F) also induces reduction of the GRB2-GAB2 activity, as indicated above. This affects both the PI3K/ AKT signaling via the Y177-GRB2-GAB2-PI3K complex and the Ras/ERK signaling via the Y177-GRB2-GARB2-SHP2 complex (Table 3). Inhibition of both bindings is also a possible therapeutic strategy. GAB2 has the binding sites for PI3K and SHP2 [94]. The GAB2 mutant that lacks both binding sites blocks the GAB2-PI3K binding and the GAB2-SHP2 binding [95]. Thus this GAB2 mutant simultaneously suppresses the PI3K/AKT signaling and the Ras/ERK signaling pathways [95] (Table 3, Figure 2). This can be a promising therapeutic strategy against CML LSCs.

Conclusion

In spite of the success of orthosteric PKIs in management of CML-CP, therapies against CML LSCs in CML-BP are difficult due to the persistence of therapy-resistant LSCs. In this regard, substantially different therapeutic strategies based on the mechanisms of oligomerization, allosteric effect and adaptor proteins, including inhibitors targeting BCR-CC, allosteric inhibitors and inhibitors against adaptor proteins, are the promising strategies that can overcome various types of therapy resistance in CML. However, detailed investigations and trials are further required.

References

1. Druker, B.J., Guilhot, F., O'Brien, S,G., et al. (2006) Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med, 355(23): 2408-2417.
2. Fausel, C. (2007) Targeted chronic myeloid leukemia therapy: seeking a cure. J Manag Care Pharm, 13(8 Suppl A): 8-12.

3. Hochhaus, A., Baccarani, M., Silver, R.T., et al. (2020) European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia, 34(4): 966-984.

4. Ciftciler, R. and Haznedaroglu, I.C. (2021) Tailored tyrosine kinase inhibitor (TKI) treatment of chronic myeloid leukemia (CML) based on current evidence. Eur Rev Med Pharmacol Sci, 25(24): 7787-7798.

5. Leak, S., Horne, G.A. and Copland, M. (2023) Targeting BCR-ABL1-positive leukaemias: a review article. Camb Prism Precis Med, 1: e21.

6. Soverini, S., De Santis, S., Monaldi, C., Bruno, S. and Mancini, M. (2021) Targeting leukemic stem cells in chronic myeloid leukemia: Is it worth the effort? Int J Mol Sci, 22(13): 7093.

7. Benjamin, E.S.B., Ravindra, N., Rajamani, B.M., et al. (2021) *BCR-ABL1* kinase domain mutation analysis by next generation sequencing detected additional mutations in chronic myeloid leukemia patients with suboptimal response to imatinib. Leuk lymphoma, 62(6): 1528-1531.

8. Brioli, A., Lomaia, E., Fabisch, C., et al. (2024) Management and outcome of patients with chronic myeloid leukemia in blast phase in the tyrosine kinase inhibitor era - analysis of the European LeukemiaNet Blast Phase Registry. Leukemia, 38(5): 1072-1080.

9. Calabretta, B. and Perrotti, D. (2004) The biology of CML blast crisis. Blood, 103(11): 4010-4022.

10. Quintás-Cardama, A. and Cortes, J. (2009) Molecular biology of bcr-abl1-positive chronic myeloid leukemia. Blood, 113(8): 1619-1630.

11. Perrotti, D., Jamieson, C., Goldman, J. and Skorski, T. (2010) Chronic myeloid leukemia: mechanisms of blastic transformation. J Clin Invest, 120(7): 2254-2264.

12. Kang, Z.J., Liu, Y.F., Xu, L.Z., et al. (2016) The Philadelphia chromosome in leukemogenesis. Chin J Cancer, 35: 48.

13. El-Tanani, M., Nsairat, H., Matalka, II., et al. (2024) The impact of the BCR-ABL oncogene in the pathology and treatment of chronic myeloid leukemia. Pathology Res Pract, 254: 155161.

14. Radich, J.P., Dai, H., Mao, M., et al. (2006) Gene expression changes associated with progression and response in chronic myeloid leukemia. Proc Natl Acad Sci U S A, 103(8): 2794-2799.

15. Branford, S., Wang, P., Yeung, D.T., et al. (2018) Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. Blood, 132(9): 948-961.

16. Ochi Y, Yoshida K, Huang YJ, et al. (2021) Clonal evolution and clinical implications of genetic abnormalities in blastic transformation of chronic myeloid leukaemia. Nat Commun, 12(1): 2833.

17. Wang, L., Li. L., Chen, R., Huang, X. and Ye, X. (2021) Understanding and monitoring chronic myeloid leukemia blast crisis: how to better manage patients. Cancer Manag Res, 13: 4987-5000.

18. Sakitani, M. (2025) Blast transformation of chronic myeloid leukemia caused by genetic alterations, epigenetic dysfunctions and aberrantly regulated factors in bone marrow environment. Int J Res Med Clin Sci, 3(1): 1-13.

19. Amarante-Mendes, G.P., Rana, A., Datoguia, T.S., Hamerschlak, N. and Brumatti, G. (2022) BCR-ABL1 tyrosine kinase complex signaling transduction: challenges to overcome resistance in chronic myeloid leukemia. Pharmaceutics, 14(1): 215.

20. Barnes, D.J., Palaiologou, D., Panousopoulou, E., et al. (2005) Bcr-Abl expression levels determine the rate of development of resistance. Caner Res, 65(19): 8912-8919.

21. Vasconcelos, F.C., Silva, K.L., Souza, P.S., et al. (2011) Variation of MDR proteins expression and activity levels according to clinical status and evolution of CML patients. Cytometry B Clin Cytom, 80(3): 158-166.

22. Wang, W., Cortes, J.E., Tang, G., et al. (2016) Risk stratification of chromosomal abnormalities in chronic myelogenous leukemia in the era of tyrosine kinase inhibitor therapy. Blood, 127(22): 2742-2750.

23. Alhuraiji, A., Kantarjian, H., Boddu, P., et al. (2018) Prognostic significance of additional chromosomal abnormalities at the time of diagnosis in patients with chronic myeloid leukemia treated with frontline tyrosine kinase inhibitors. Am J Hematol, 93(1): 84-90.

24. Yang, H., Liang, H., Yan, J.S., Tao, R., Hao, S.G. and Ma, L.Y. (2012) Down-regulation of hematopoiesis master regulator PU.1 via aberrant methylation in chronic myeloid leukemia. Int J Hematol, 96(1): 65-73.

25. Annamaneni, S., Kagita, S., Gorre, M., Digumarti, R.R., Satti, V. and Battini, M.R. (2014) Methylation status of *CEBPA* gene promoter in chronic myeloid leukemia.

Hematology, 19(1): 42-44.

26. Yin, X., Zhou, M., Fu, Y., et al. (2019) Histone demethylase RBP2 mediates the blast crisis of chronic myeloid leukemia through an RBP2/PTEN/BCR-ABL cascade. Cell Signal, 63: 109360.

27. Perrotti, D., Cesi, V., Trotta, R., et al. (2002) BCR-ABL suppresses C/EBPα expression through inhibitory action of hnRNP E2. Nat Genet, 30(1): 48-58.

28. Guerzoni, C., Bardini, M., Mariani, S.A., et al. (2006) Inducible activation of CEBPB, a gene negatively regulated by BCR/ABL, inhibits proliferation and promotes differentiation of BCR/ABL-expressing cells. Blood, 107(10): 4080-4089.

29. Chang, J.S., Santhanam, R., Trotta, R., et al. (2007) High levels of the BCR/ABL oncoprotein are required for the MAPK-hnRNP-E2 dependent suppression of C/EBPα-driven myeloid differentiation. Blood, 110(3): 994-1003.

30. Soliera, A.R., Lidonnici, M.R., Ferrari-Amorotti, G., et al. (2008) Transcriptional repression of c-Myb and GATA-2 is involved in the biologic effects of C/EBPα in p210BCR/ABL-expressing cells. Blood, 112(5): 1942-1950.

31. Zhang, S.J., Ma, L.Y., Huang, Q.H., et al. (2008) Gain-of-function mutation of GATA-2 in acute myeloid transformation of chronic myeloid leukemia. Proc Natl Acad Sci U S A, 105(6): 2076-2081.

32. Abrahamsson, A.E., Geron, I., Gotlib, J., et al. (2009) Glycogen synthase kinase 3beta missplicing contributes to leukemia stem cell generation. Proc Natl Acad Sci U S A, 106(10): 3925-3929.

33. Iacobucci, I., Storlazzi, C.T., Cilloni, D., et al. (2009) Identification and molecular characterization of recurrent genomic deletions on 7p12 in the IKZF1 gene in a large cohort of BCR-ABL1-positive acute lymphoblastic leukemia patients: on behalf of Gruppo Italiano Malattie Ematologiche dell'Adulto Acute Leukemia Working Party (GIMEMA AL WP). Blood, 114(10): 2159-2167.

34. Dong, Y., Liu, F., Wu, C., et al. (2016) Illegitimate RAG-mediated recombination events are involved in IKZF1 **Δ**3-6 deletion in BCR-ABL1 lymphoblastic leukaemia. Clin Exp Immunol, 185(3): 320-331.

35. Honda, H., Ushijima, T., Wakazono, K., et al. (2000) Acquired loss of p53 induces blastic transformation in p210(bcr/abl)-expressing hematopoietic cells: a transgenic study for blast crisis of human CML. Blood, 95(5): 1144-1150.

36. Ravi, R., Mookerjee, B., Bhujwalla, Z.M., et al. (2000) Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1α. Genes Dev, 14(1): 34-44.

37. Cheloni, G., Tanturli, M., Tusa, I., et al. (2017) Targeting chronic myeloid leukemia stem cells with the hypoxia-inducible factor inhibitor acriflavine. Blood, 130(5): 655-665.

38. Mojtahedi, H., Yazdanpanah, N. and Rezaei, N. (2021) Chronic myeloid leukemia stem cells: targeting therapeutic implications. Stem Cell Res Ther, 12(1): 603.

39. How, J., Venkataraman, V. and Hobbs, G.S. (2021) Blast and accelerated phase CML: room for improvement. Hematology Am Soc Hematol Educ Program, 2021(1): 122-128.

40. Pamuk, G.E. and Ehrlich, L.A. (2024) An overview of myeloid blast-phase chronic myeloid leukemia. Cancers (Basel), 16(21): 3615.

41. Holyoake, T.L. and Vetrie, D. (2017) The chronic myeloid leukemia stem cell: stemming the tide of persistence. Blood, 129(12): 1595-1606.

42. Muselli, F., Peyron, J.F. and Mary, D. (2019) Druggable biochemical pathways and potential therapeutic alternatives to target leukemic stem cells and eliminate the residual disease in chronic myeloid leukemia. Int J Mol Sci, 20(22): 5616.

43. Sakitani, M. (2024) Induction of cancer stem cells in tumor microenvironment and involvement of HIFs and carbonic anhydrase IX in oncogenesis of solid tumors and hematological malignancies including adult T-cell leukemia/lymphoma (ATL). Int J Res Med Clin Sci, 2(1): 52-76.

44. He, Y., Wertheim, J.A., Xu, L., et al. (2002) The coiled-coil domain and Tyr177 of bcr are required to induce a murine chronic myelogenous leukemia-like disease by bcr/abl. Blood, 99(8): 2957-2968.

45. Dixon, A.S., Pendley, S.S., Bruno, B.J., et al. (2011) Disruption of Bcr-Abl coiled coil oligomerization by design. J Biol Chem, 286(31): 27751-27760.

46. Woessner, D.W., Eiring, A.M., Bruno, B.J., et al. (2015) A coiled-coil mimetic intercepts BCR-ABL1 dimerization in native and kinase-mutant chronic myeloid leukemia. Leukemia, 29(8): 1668-1675.

47. Liu, Y., Zhang, M., Tsai, C.J., Jang, H. and Nussinov, R.

(2022) Allosteric regulation of autoinhibition and activation of c-Abl. Comput Struct Biotechnol, 20: 4257-4270.

48. Nagar, B., Hantschel, O., Young, M.A., et al. (2003) Structural basis for the autoinhibition of c-Abl tyrosine kinase. Cell, 112(6): 859-871.

49. Corbi-Verge, C., Marinelli, F., Zafra-Ruano. A., Ruiz-Sanz, J., Luque, I. and Faraldo-Gómez, J.D. (2013) Two-state dynamics of the SH3–SH2 tandem of Abl kinase and the allosteric role of the N-cap. Proc Natl Acad Sci U S A, 110(36): E3372-80.

50. Carofiglio, F., Trisciuzzi, D., Gambacorta, N., Leonetti, F., Stefanachi, A., Nicolotti, O. (2020) Bcr-Abl allosteric inhibitors: where we are and where we are going to. Molecules, 25(18): 4210.

51. Johnson, K.J., Griswold, I.J., O'Hare, T., et al. (2009) A BCR-ABL mutant lacking direct binding sites for the GRB2, CBL and CRKL adapter proteins fails to induce leukemia in mice. PLoS One, 4(10): e7439.

52. Titz, B., Low, T., Komisopoulou, E., Chen, S.S., Rubbi, L. and Graeber, T.G. (2010) The proximal signaling network of the BCR-ABL1 oncogene shows a modular organization. Oncogene, 29(44): 5895-5910.

53. Gregor, T., Bosakova, M.K., Nita, A., et al. (2020) Elucidation of protein interactions necessary for the maintenance of the BCR-ABL signaling complex. Cell Mol Life Sci, 77(19): 3885-3903.

54. Liao, T.J., Jang, H., Fushman, D. and Nussinov, R. (2020) SOS1 interacts with Grb2 through regions that induce closed nSH3 conformations. K Chem Phys, 153(4): 045106.

55. Liu, Y., Jang, H., Zhang, M., Tsai, C.J., Maloney, R. and Nussinov, R. (2022) The structural basis of BCR-ABL recruitment of GRB2 in chronic myelogenous leukemia. Biophys J, 121(12): 2251-2265.

56. Ross, T.S. and Mgbemena, V.E. (2014) Re-evaluating the role of BCR/ABL in chronic myelogenous leukemia. Mol Cell Oncol, 1(3): e963450.

57. Nacheva, E.P., Grace, C.D., Brazma, D., et al. (2013) Does BCR/ABL1 positive acute myeloid leukaemia exist? Br J Haematol, 161(4): 541-550.

58. Hovorkova, L., Winkowska, L., Skorepova, J., et al. (2024) Distinct pattern of genomic breakpoints in CML and BCR::ABL1-positive ALL: analysis of 971 patients. Mol Cancer, 23(1): 138.

59. Rosenberg, M.W., Savage, S.L., Eide, C.A., et al. (2022) Comprehensive molecular characterization of a rare case of Philadelphia chromosome-positive acute myeloid leukemia. Cold Spring Harb Mol Case Stud, 8(6): a006218.

60. Pane, F., Frigeri, F., Sindona, M., et al. (1996) Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). Blood, 88(7): 2410-2414.

61. Braun, T.P., Eide, C.A., Druker, B.J. (2020) Response and resistance to BCR-ABL1-targeted therapies. Cancer Cell, 37(4): 530-542.

62. Poudel, G., Tolland, M.G., Hughes, T.P. and Pagani, I.S. (2022) Mechanisms of resistance and implications for treatment strategies in chronic myeloid leukaemia. Cancers (Basel), 14(14): 3300.

63. Alves, R., Gonçalves, A.C., Rutella, S., et al. (2021) Resistance to tyrosine kinase inhibitors in chronic myeloid leukemia — from molecular mechanisms to clinical relevance. Cancers (Basel), 13(19): 4820.

64. Sun, J., Hu, R., Han, M., et al. (2024) Mechanisms underlying therapeutic resistance of tyrosine kinase inhibitors in chronic myeloid leukemia. Int J Biol Sci, 20(1): 175-181.

65. Liu, Y., Zhang, M., Jang, H. and Nussinov, R. (2023) Higher-order interactions of Bcr-Abl can broaden chronic myeloid leukemia (CML) drug repertoire. Protein Sci, 32(1): e4505.

66. Lima, M.C.P., Hornsby, B.D., Lim, C.S. and Cheatham, T.E. 3rd. (2024) Molecular Modeling of Single- and Double-Hydrocarbon-Stapled Coiled-Coil Inhibitors against Bcr-Abl: toward a treatment strategy for CML. J Phys Chem B, 128(27): 6476-6491.

67. Renshaw, M.W., Capozza, M.A. and Wang, J.Y. (1988) Differential expression of type-specific c-abl mRNAs in mouse tissues and cell lines. Mol Cell Biol, 8(10): 4547-4751.

68. Pendergast, A.M., Quilliam, L.A., Cripe, L.D., et al. (1993) BCR-ABL-induced oncogenesis is mediated by direct interaction with the SH2 domain of the GRB-2 adaptor protein. Cell, 75(1): 175-185.

69. Dixon, A.S., Miller, G.D., Bruno, B.J., et al. (2012) Improved coiled-coil design enhances interaction with Bcr-Abl and induces apoptosis. Mol Pharm, 9(1): 187-195.

70. Beissert, T., Puccetti, E., Bianchini, A., et al. (2003) Targeting of the N-terminal coiled coil oligomerization interface of BCR interferes with the transformation

potential of BCR-ABL and increases sensitivity to STI571. Blood, 102(8): 2985-2993.

71. Zhang, X., Subrahmanyam, R., Wong, R., Gross, A.W. and Ren, R. (2001) The NH(2)-terminal coiled-coil domain and tyrosine 177 play important roles in induction of a myeloproliferative disease in mice by Bcr-Abl. Mol Cell Biol, 21(3): 840-953.

72. Bruno, B.J. and Lim, C.S. (2015) Inhibition of bcr-abl in human leukemic cells with a coiled-coil protein delivered by a leukemia-specific cell-penetrating Peptide. Mol Pharm, 12(5): 1412-1421.

73. Peng, Y., Huang, Z., Zhou, F., Wang, T., Mou, K. and Feng, W. (2021) Effect of HSP90AB1 and CC domain interaction on Bcr-Abl protein cytoplasm localization and function in chronic myeloid leukemia cells. Cell Commun Signal, 19(1): 71.

74. Nagar, B., Hantschel, O., Seeliger, M., et al. (2006) Organization of the SH3-SH2 unit in active and inactive forms of the c-Abl tyrosine kinase. Mol Cell, 21(6): 787-798.

75. Skora, L., Mestan, J., Fabbro, D., Jahnke, W. and Grzesiek, S. (2013) NMR reveals the allosteric opening and closing of Abelson tyrosine kinase by ATP-site and myristoyl pocket inhibitors. Proc Natl Acad Sci U S A. 110(47): E4437-E4445.

76. Saleh, T., Rossi, P. and Kalodimos, C.G. (2017) Atomic view of the energy landscape in the allosteric regulation of Abl kinase. Nat Struct Mol Biol, 24(11): 893-901.

77. Xie, T., Saleh, T., Rossi, P. and Kalodimos, C.G. (2020) Conformational states dynamically populated by a kinase determine its function. Science, 370(6513): eabc2754.

78. Adrián, F.J., Ding, Q., Sim, T., et al. (2006) Allosteric inhibitors of Bcr-abl-dependent cell proliferation. Nat Chem Biol, 2(2): 95-102.

79. Choi, Y., Seeliger, M.A., Panjarian, S.B., et al. (2009) N-myristoylated c-Abl tyrosine kinase localizes to the endoplasmic reticulum upon binding to an allosteric inhibitor. J Biol Chem, 284(42): 29005-29014.

80. Zhang, J., Adrián, F.J., Jahnke, W., et al. (2010) Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. Nature, 463(7280): 501-506.

81. Radi, M., Crespan, E., Botta, G., et al. (2008) Discovery and SAR of 1,3,4-thiadiazole derivatives as potent Abl tyrosine kinase inhibitors and cytodifferentiating agents. Bioorg Med Chem Lett, 18(3): 1207-1211.

82. Crespan, E., Radi, M., Zanoli, S., Schenone, S., Botta, M. and Maga, G. (2010) Dual Src and Abl inhibitors target wild type Abl and the AblT315I Imatinib-resistant mutant with different mechanisms. Bioorg Me Chem, 18(11): 3999-4008.

83. Wylie, A.A., Schoepfer, J., Jahnke, W., et al. (2017) The allosteric inhibitor ABL001 enables dual targeting of BCR-ABL1. Nature, 543(7647): 733-737.

84. Schoepfer, J., Jahnke, W., Berellini, G., et al. (2018) Discovery of Asciminib (ABL001), an allosteric inhibitor of the tyrosine kinase activity of BCR-ABL1. J Med Chem, 61(18): 8120-8135.

85. Hughes, T.P., Mauro, M.J., Cortes, J.E., et al. (2019) Asciminib in chronic myeloid leukemia after ABL kinase inhibitor failure. N Engl J Med, 381(24): 2315-2326.

86. Filippakopoulos, P., Kofler, M., Hantschel, O., et al. (2008) Structural coupling of SH2-kinase domains links Fes and Abl substrate recognition and kinase activation. Cell, 134(5): 793-803.

87. Grebien, F., Hantschel, O., Wojcik, J., et al. (2011) Targeting the SH2-kinase interface in Bcr-Abl inhibits leukemogenesis. Cell, 147(2): 306-319.

88. Wojcik, J., Hantschel, O., Grebien, F., et al. (2010) A potent and highly specific FN3 monobody inhibitor of the Abl SH2 domain. Nat Struct Mol Biol, 17(4): 519-527.

89. Lowenstein, E.J., Daly, R.J., Batzer, A.G., et al. (1992) The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. Cell, 70(3): 431-442.

90. Puil, L., Liu, J., Gish, G., et al. (1994) Bcr-Abl oncoproteins bind directly to activators of the Ras signalling pathway. EBMO J, 13(4): 764-773.

91. Buday, L., Egan, S.E., Rodriguez Viciana, P., Cantrell, D.A. and Downward, J. (1994) A complex of Grb2 adaptor protein, Sos exchange factor, and a 36-kDa membrane-bound tyrosine phosphoprotein is implicated in ras activation in T cells. J Biol Chem, 269(12): 9019-9023.

92. Chardin, P., Cussac, D., Maignan, S. and Ducruix, A. (1995) The Grb2 adaptor. FEBS Lett, 369(1): 47-51.

93. Chu, S., Li, L., Singh, H. and Bhatia, R. (2007) BCR-tyrosine 177 plays an essential role in Ras and Akt activation and in human hematopoietic progenitor transformation in chronic myelogenous leukemia. Cancer Res, 67(14): 7045-7053.

94. Sattler, M., Mohi, M.G., Pride, Y.B., et al. (2002) Critical role for Gab2 in transformation by BCR/ABL. Cancer Cell, 1(5): 479-492.

95. Gu, S., Chan, W.W. and Mohi, G., et al. (2016) Distinct GAB2 signaling pathways are essential for myeloid and lymphoid transformation and leukemogenesis by BCR-ABL1. Blood, 127(14): 1803-1813.

96. Gu, H., Pratt, J.C., Burakoff, S.J. and Neel, B.G. (1998) Cloning of p97/Gab2, the major SHP2-binding protein in hematopoietic cells, reveals a novel pathway for cytokine-induced gene activation. Mol Cell, 2(6): 729-740.

97. Million, R.P. and Van Etten, R.A. (2000) The Grb2 binding site is required for the induction of chronic myeloid leukemia-like disease in mice by the Bcr/Abl tyrosine kinase. Blood, 96(2): 664-670.

98. Gishizky, M.L., Cortez, D. and Pendergast, A.M. (1995) Mutant forms of growth factor-binding protein-2 reverse BCR-ABL-induced transformation. Proc Natl Acad Sci U S A, 92(24): 10889-10893.

99. Kardinal, C., Konkol, B., Lin, H., et al. (2001) Chronic myelogenous leukemia blast cell proliferation is inhibited by peptides that disrupt Grb2-SoS complexes. Blood, 98(6): 1773-1781.

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