*Review*

# Chronic myeloid leukemia: Attributes of break point cluster region-abelson (BCR-ABL)

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**Chronic myeloid leukemia is a molecular fault from neoplastic transformation of hematopoietic stem cells. It is elicited by an extensive spectrum of "fused oncoproteins" which are necessitated in the disease refractoriness. It docks a miscellany of fusion transcripts originating from chromosomal rearrangements. Additionally, vulnerability to genomic rearrangements is particularly enhanced in chronic myeloid leukemia which is triggered by BCR-ABL fusion gene. The hallmark genetic abnormality of chronic myeloid leukemia is at t (9; 22) (q34; q11) transformation fallouts into small Philadelphia chromosome. Three types of proteins are encoded by BCR-ABL oncogene such as p230, p210 and p190. 210 kilodalton dysregulated tyrosine kinase (p210) is indispensable and adequate for leukaemogenesis. BCR-ABL oncoprotein contains specific domains for the activation of signal transduction. Presently there are below par measures to demarcate this rapidly growing threat. The review will cover various mechanistic insights of the BCR-ABL genomic instability. Moreover effectiveness of therapeutic interventions recently designed keeping in view the molecular hierarchy will be evaluated.**

**Key words:** Chronic myeloid leukemia, imatinib, break point cluster region- c-Abelson (BCR-ABL).

## INTRODUCTION

Chronic myeloid leukemia (CML) is one of the most remarkable myeloproliferative disorders that emerges after the dysregulated clonal expansion and differentiation of totipotent hematopoietic stem cells

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**Abbreviations: AKT**: AKT protein, **Bcl-2**: Bcl-2 protein, **BclXL**: B-cell lymphoma-extra large, **BCR-ABL**: break point cluster region- c-Abelson, **CK-2**: casine kinase-2, **CML**: chronic myeloid leukemia, **c-Myc**: myelocytomatosis, **Cyc D**: cyclin D, **ERK**: extracellular signal regulated kinase, **GAB2**: GRB2-associated binding protein 2, **GRB2**: growth factor receptor-bound protein 2, **MAP-kinase**: mitogen activated protein-kinase, **Ph**: Philadelphia chromosome, **PI3K**: phosphoinositol-3 kinase, **Ras**: ras protein, **SH2**: SRC homology domain 2, **SH3**: SRC homology domain 3, **SOS**: Son of sevenless protein, **STAT**: Signal transducer and activator of transcription.

(Kramer et al., 2001; Goldman and Melo, 2001). CML is a biphasic or triphasic disease instigates with a chronic phase and then progressing through a variable accelerated phase into an acute phase (blastic phase) resembling an acute myeloid or lymphoid leukemia. A clinical description of chronic phase, CML includes an elevated white blood cell count predominately of well-differentiated cells of the granulocytes series and hepatosplenomegaly as an outcome of myeloidinfilteration of the liver and spleen (Melo et al., 2001).

The different fusion proteins encoded by BCR-ABL vary in size depend on the breakpoint in the BCR gene but share a high tyrosine kinase activity in part responsible for the leukemogenesis (Jacques et al., 2004). Almost all patients carry a specific translocation t (9;22) (q34;q11.2) and derivative der-(22) the Philadelphia chromosome (Ph) that results in the juxtaposition of the DNA sequence from the BCR-ABL genes and encodes a 210 kilodalton dysregulated tyrosine kinase which is necessary and

sufficient for leukaemogenesis. Although this Ph chromosome is thought to be the initial event in CML, the acquisition of additional cytogenetic abnormalities are likely responsible for disease progression (Naveen et al., 2008; Wittor et al., 2000). About one third of the patients with CML who appear to have normal karyotypes actually have a cytogenetically occult BCR-ABL gene usually located on a normal appearing chromosome 22 but very occasionally on chromosome 9 (Goldman and Melo, 2003).

ABL is a non-receptor tyrosine kinase that is voiced in most tissues. In cells, the ABL protein is distributed in both the nucleus, cytoplasm or can shuttle between the two compartments while BCR-ABL oncoprotein remains in the cytoplasm and the actual signal transduction pathways that the oncoprotein uses to alter gene expression cause the phenotype of CML (Goldman and Melo, 2008). In this review we will bring to limelight the key points involved in disease progression, signal transduction cascades and stimuli leading to chromosomal rearrangements.

## CONFIGURATION BCR-ABL FUSION PROTEIN

The different fusion proteins encoded by BCR-ABL diverge in size depending on the breakpoint in the BCR gene but contribute to a high tyrosine kinase activity in part responsible for the leukemogenesis (Jacques et al., 2004). Depending on the particular breakpoints in the translocation and RNA splicing different forms of BCR-ABL protein with different molecular weights can be generated in patients (Ruibao, 2005). Three breakpoint cluster regions in the BCR gene have been described to date: major (M-BCR) minor (m-BCR) and micro (BCR). More than 95% of Ph-positive CML patients present a breakpoint in the M-BCR region. Two major breakpoints are found after the 13th exon resulting in an e13a2 fusion (e for BCR exon and a for ABL exon) or after the 14th exon resulting in an e14a2 fusion. Both fusion mRNAs are translated into p210BCR-ABL protein. Other junctions coding for p210BCR-ABL are rare. The breakpoint in the m-BCR region results in an e1a2 junction, which is translated into a p190BCR-ABL protein. It is possible to observe a third BCR-ABL protein which is p230BCR-ABL. It consists of more than 90% of p160BCR because the breakpoint is located in the 3' end of the BCR gene in the BCR region and its transcript contains an e19a2 junction (Brian et al., 2000; Jacques et al., 2004). BCR-ABL transduces signals from cell-surface growth factor and adhesion receptors to regulate cytoskeleton structure. The fusion of BCR sequences to ABL during the translocation associated with CML increases the tyrosine kinase activity of ABL and brings new regulatory domains/motifs to ABL such as GRB2 (Ruibao, 2005; Chalandon et al., 2004).

There are a number of important domains that make up ABL and BCR proteins. Two isoforms of ABL (human types 1a and 1b) are generated by alternative splicing of the first exon, one of them (1b) contains a myristoylation modification site (Myr). Apart from the alternatively spliced sequences, the amino terminal half of ABL contains tandem SH3, SH2 and the tyrosine-kinase domains. These domains can amass into an auto-inhibitory structure, in which the SH3 and SH2 domains function as a 'clamp' that holds the kinase in the 'off' state 119, 120 (Oliver and Giulio, 2004; Ruibao, 2005). In ABL1b, the myristoyl group at the extreme end of the amino-terminal segment also binds to the tyrosine-kinase domain and functions as a bolt that keeps the SH3–SH2 clamp in place119 and121. In its carboxy-terminal region, ABL contains four proline-rich SH3 binding sites (PPs), three nuclear localization signals (NLSs), one nuclear exporting signal (NES), a DNA-binding domain (DBD) and an actin-binding domain (ABD). BCR contains a coiled-coil oligomerization domain (CCD), a serine/threonine (S/T) kinase domain, Dbl/CDC24 guanine-nucleotide exchange factor homology domain (DHD), pleckstrin homology domain (PHD), a putative calcium-dependent lipid-binding site (CaLB) and a RAC guanosine triphosphatase-activating protein domain (RAC-GAPD). BCR also contains binding sites for growth factor receptor-bound protein 2 (GRB2) at tyrosine 177 (Y177) (Ruibao, 2005) Figure 1.

## CONSEQUENCE OF BCR-ABL IN CHRONIC MYELOID LEUKEMIA (CML)

BCR-ABL plays a fundamental role in the regulation cell proliferation, apoptosis, differentiation and adhesion. In addition BCR-ABL can provoke resistance to cytostatic drugs, irradiation by modulation of DNA repair mechanisms, cell cycle checkpoints and Bcl-2 protein family members (Tomasz, 2002; Chalandon et al., 2004). BCR-ABL oncogenic tyrosine kinase displays two complementary characters in CML. The first and best notorious is stimulation of signaling pathways that render cells independent of their environment. BCR-ABL allocates cells to proliferate in the absence of growth factors, protects them from apoptosis in the absence of external survival factors and promotes invasion and metastasis. The second role of BCR-ABL in hematological malignancies, which is only just beginning to be fully recognized that it can render cells resistant to genotoxic therapies (Brian et al., 2000; Tomasz, 2002). Therefore it is not surprising that dysregulated tyrosine kinase activity has a central role in malignant transformation (Michael and Brian, 2001). BCR-ABL tyrosine kinase activity leads to an activation of several signal transduction pathways that are also utilized by hematopoietic growth factors including steel factor thrombopoietin, interleukin-3 and granulocyte/macrophage-colony stimulating factor. In
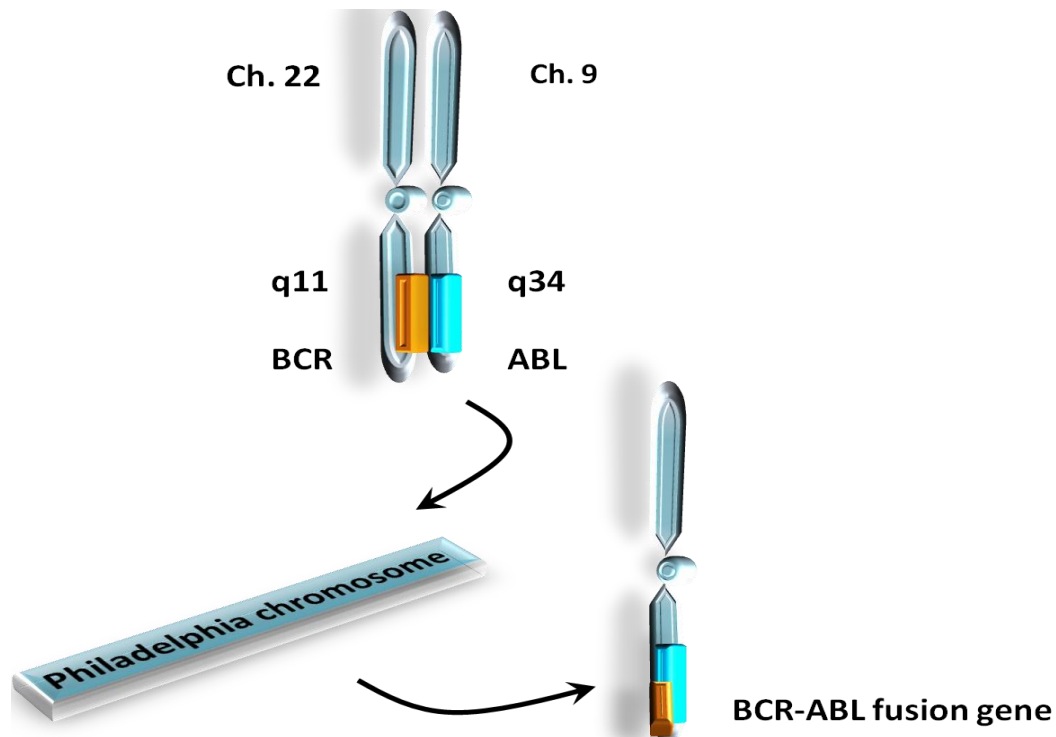
**Figure 1.** Chromosomal translocation: t (9;22) (q34;q11) transformation results into small Philadelphia chromosome and BCR-ABL fusion gene.

several model systems BCR-ABL has over-lapping biological effects with hematopoietic growth factors (Sattler and Salgia, 1997).

## BCR-ABL SIGNAL TRANSDUCTION IN CHRONIC MYELOID LEUKEMIA (CML)

BCR-ABL oncoprotein has a vital role in the CML progression by the activation of major signaling pathways such as Ras-MAP kinase, JAK-STAT and PI3K-AKT pathways. BCR-ABL fusion protein causes the activation of c-MYC, c-FOS and CDK genes, which have an important role in the CML development (Tomasz, 2002; Melo and Goldman, 2008). PI-3k was discovered as an activity that phosphorylates phosphoinositols at the D-39 position of the inositol ring and produces novel phosphoinositides. Activated PI3K with AKT is involved in the altered cell adhesion and migration. BCR-ABL causes the activation of PI3K-AKT pathway which in turn dampens the programmed cell death (Tomasz et al., 1997).

Phosphorylation at the Y177 residue of BCR-ABL engenders a high affinity-binding site for GRB2. GRB2 binds to BCR-ABL through its SH2 domain and binds to SOS and GAB2 through its SH3 domains. SOS in turn activates Ras, which is indispensable for cell survival and cell proliferation. BCR-ABL augments the cellular activation of Ras (Ruibao, 2005). CK-2 plays a critical

role in growth control and many of its substrates are growth regulators. The oncoprotein BCR-ABL causes the down regulation of CK-2 and ICSBP which regulate proliferation and survival of myeloid cells by inducing differentiation of monocytic cells (Heriche and Chambaz, 1998; Ruibao, 2005).

Activation of JAK-STAT signaling pathway by BCR-ABL oncoprotein confers resistance to programmed cell death (Ralph and Tong, 2004). ABL is a protein kinase that is involved in the phosphorylation of various downstream molecules but myristoylation in the hydrophobic region of the protein makes it silent by auto inhibition. Contrarily, if there is a fusion of BCR and ABL, it de-represses the activity of ABL. The activation of ABL is restored as soon as there is a fusion of BCR with ABL and this fusion protein is hyper-activated to disturb the spatial-temporal behavior of the signaling (Mian et al., 2009). Two point mutations C944T and T932C have been screened in ABL gene, which causes complete/partial Imatinib resistance. The agitated transgenic activity of mutated BCR-ABL, resistant to Imatinib, encloses an essential role in the CML progression (Catherine et al., 2002; Zafar et al., 2004; Aamir et al., 2011) Figures 2 and 3.

## CONCLUSION AND FUTURE DIRECTIONS

ABL kinase that is implicated in the phosphorylation of various downstream molecules but  myristoylation  in  the
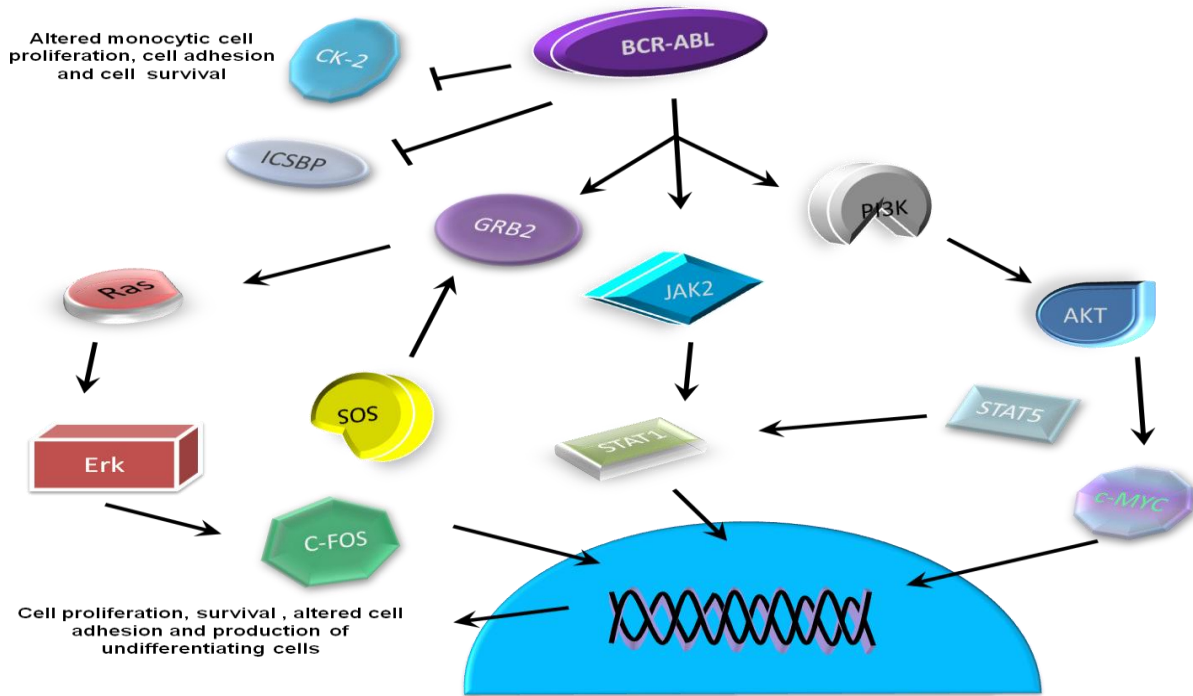
**Figure 2.** BCR-ABL Signaling in CML: BCR-ABL oncoprotein causes the recruitment of growth regulators like GRB2, JAK and PI-3K. PI-3K activates downstream molecules AKT and c-MYC which instigate antiapoptotic tricks. SOS protein facilitates the hyper activation of GRB2 which in turn activates the Ras. Ras further stimulates the ERK, which assists in the activation of growth enhancer c-FOS, take part in cell proliferation and survival. Cell adhesion, monocytic cell proliferation and cell survival are altered by the inhibition of ICSBP and CK-2.
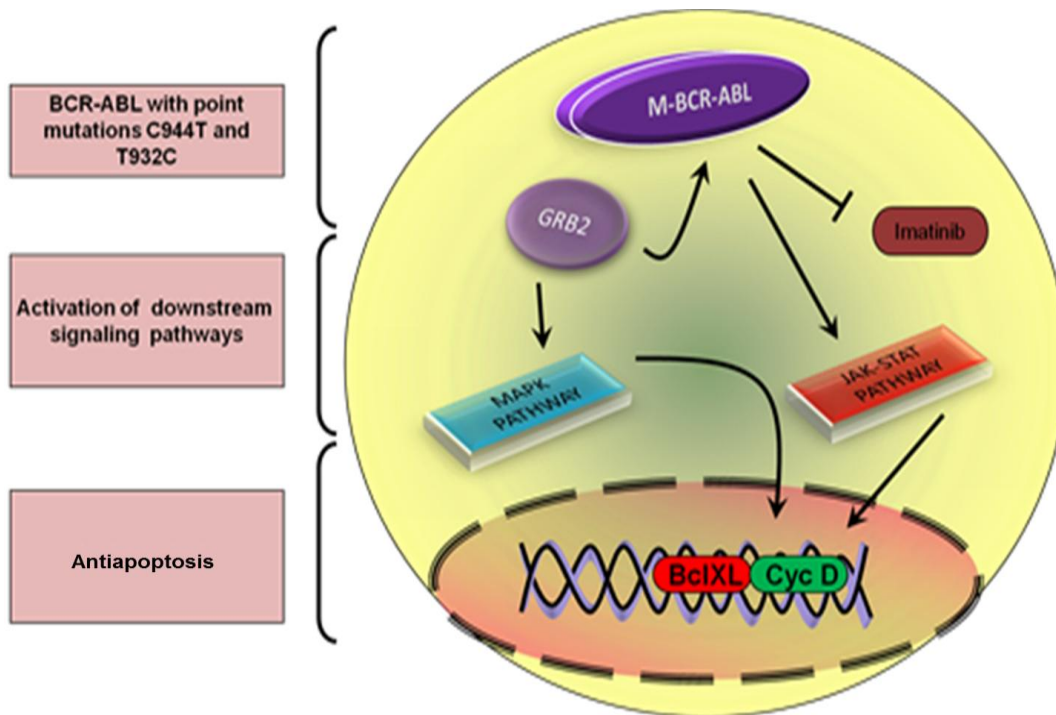


**Figure 3.** BCR-ABL and Apoptosis: mutated BCR-ABL fusion protein inhibits the binding of Imatinib (a tyrosine kinase inhibitor). The uncontrolled inauguration of signaling cascades, activate anti-apoptotic proteins, BclXL and Cyc D, restrain vital role in the CML progression.

hydrophobic region of the protein makes it silent by auto inhibition. Contrarily, if there is a fusion of BCR and ABL, it de-represses the activity of ABL. The activation of ABL is restored as soon as there is a fusion of BCR with ABL and this fusion protein is hyperactivated to bother the spatial-temporal behavior of the signaling (Mian et al., 2009). To treat this pathology, imatinib is used to extinguish and diminish the effect of BCR-ABL. According to obtainable findings there is paradigm shift from sensitivity towards resistance. The amendment in the BCR-ABL fusion gene by the point mutations causes the creation of mutated oncoprotein which inhibits the binding of Imatinib and starts uncontrolled CML progression. The pattern of genomic rearrangement is paramount to outline the key players mediating oncogenesis. The underpinnings of chromosomal reorganizations encouraged a variety of unexplored aspects. Even though there are escalating highways and byways of chimeric transcripts but fraught desire for effectual drug design cannot be overlooked. Keeping in view the preliminary teething stages of CML therapy, there is a burning need to clutch and lengthen with positive clinical outcomes. Despite the fact that CML is a detrimental inconsistency, interventions to address this indomitable disease are not impressive. The pattern of genomic rearrangement is paramount to delineate the key players mediating oncogenesis.

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