INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm with an incidence of 1-2 cases per100, 000 adults, and accounts for 15% of newly diagnosed cases of leukemia in adults ***(Jemal et al., 2010).***

CML is characterized by a balanced genetic translocation, (9; 22) (q34; q11.2), involving a fusion of the Abelson oncogene(ABL) from chromosome 9q34 with the breakpoint cluster region (BCR) gene on chromosome 22q11.2. This rearrangement is known as the Philadelphia chromosome ***(Jabbour and Kanterjian, 2014).***

BCR-ABL is a constitutively active tyrosine kinase that promotes growth and replication through downstream pathways such as RAS, RAF, JUN kinase, MYC, and STAT. This influences leukemogenesis by creating a cytokine-independent cell cycle with aberrant apoptotic signals in response to cytokine withdrawal ***(Jabbour and Kanterjian, 2018*).**

About 30-50% of patients diagnosed as chronic myeloid leukemia are asymptomatic, which is found on routine physical examination or blood tests. CML can be classified into three disease phases: chronic phase (CP), accelerated phase (AP), and blast phase (BP) ***(Jabbour and Kanterjian, 2014).***

Tyrosine kinase inhibitors are the mainstay of Chronic myeloid leukemia treatment, but some patients need additional

treatment modalities due to resistance and intolerance to TKIs, and allogeneic haematopoietic stem cell transplantation (alloHSCT) is still a treatment option in selected cases ***(Keskin and Eskazan, 2015).***

The first approved TK inhibitor (TKI) was Imatinib Mesylate (Glivec). Rapidly, other TKIs were developed, namely Dasatinib (Sprycel), Nilotinib (Tasigna), Bosutinib (Busulif) and Ponatinib (Iclusig). The current treatment goals include not only the prevention of the transformation to the advanced phases and the prolongation of survival, but also a good quality of life comparable to that of non-leukemic individuals ***(Baccarani et al., 2014).***

Imatinib mesylate (Glivec) is the first line of treatment for newly diagnosed chronic-phase chronic myeloid leukemia (CML-CP) patients. However, up to 15% to25% of newly diagnosed CML-CP patients have primary resistance (failure to achieve any level of cytogenetic response at 6 months, lack of major cytogenetic response at 12 months, and absence of complete cytogenetic response at 18 months) to imatinib at 400 mg/d ***(Shah, 2007).*** Currently, mechanisms of primary imatinib resistance are not well defined, but kinase domain mutations do not appear to be an important cause ***(Hughes and Branford, 2006).***

The Organic cation trasporter1 OCT-1 protein is a member of the largest superfamily of transporters, the solute carrier family, which transport in an electrogenic fashion a variety of organic

cations including drugs, toxins, and other xenobiotics. The transporter is predicted to have12 transmembrane domains and binding pockets with partially overlapping interaction domains for different substrates and inhibitors ***(White et al., 2007).*** Polymorphisms of *OCT-1* are known to affect substrate transportation and evidence exists for the entry of imatinib into cells by OCT-1 ***(Crossman et al., 2005).***

It was found that intracellular imatinib uptake correlates with OCT1 expression or activity and may thus determine the therapeutic outcome ***(Wang et al., 2008).***

AIM OF THE WORK

The aim of the work is to assess properly the relevance of organic cation transporter1on the response of adult patients with chronic myeloid leukemia to treatment.

Chapter 1

CHRONIC MYELOID LEUKEMIA

Introduction:

Chronic myeloid leukemia (CML) is a clonal bone marrow (BM) disease characterized by neoplastic overproduction of, mainly, granulocytes. The leukemogenic event in CML is thought to occur at the level of the hematopoietic stem cell (HSC). This explains why most hematopoietic lineages, including neutrophils, eosinophils, basophils, erythroid cells, and megakaryocytes, involved in the disease process ***(Zhou and Xu, 2015).***

Chronic myeloid leukemia (CML) is primarily an adult disease and its incidence is rare in children and adolescents, accounting for only 2% to 3% of all leukemias in this age group ***(Chandra et al., 2018).***

The Bcr-Abl oncogenic tyrosine kinase is responsible for initiating and maintaining the leukemia phenotype of CML cells. This oncoprotein is also responsible for the phosphorylation, activation and dysregulation of intracellular signaling proteins that regulate the survival and growth of progenitor cells in the bone marrow ***(Zhou and Xu, 2015).***

As the development of BCR-ABL1 targeting treatments has proceeded – first imatinib, then nilotinib and dasatinib – the

treatment of chronic myeloid leukaemia (CML) has been revolutionised, and most patients live longer. patients with chronic phase chronic myeloid leukaemia (CML‑CP) were shawn to have similar survival rates in all age groups to that of general population in the era of tyrosine kinase inhibitors (TKIs) ***(Sasaki et al., 2014).***

Pathophysiology of CML

CML-CP originates from an abnormal pluripotent hematopoietic stem cell (HSCs) that has acquired the Philadelphia Chromosome (Ph) resulting from a balanced reciprocal translocation t (9; 22) (q34; q11). This distinctive cytogenetic abnormality leads to the juxtaposition of c-abl oncogene1 (ABL1) gene with the breakpoint cluster region (BCR) encoding the p210 BCR-ABL1 protein with oncogenic activity. This capacity seems to be due to the continuously activated tyrosine kinase activity (TK) activity of BCR-ABL1 fusion protein compared to the native c-ABL ***(Greuber et al., 2013).***

The expression of such oncogene in a single hematopoietic stem cell induces a proliferative advantage and aberrant differentiation capacity of this cell over the normal counterpart and results therefore the sole oncogenic event sufficient to induce leukemia ***(Cea et al., 2013).***

Several BCR-ABL1 isoforms with different molecular weights have been reported, depending on the break point

occurring within the BCR gene. A major breakpoint cluster region (M-bcr) and a minor breakpoint cluster region (m-bcr) have been defined ***(Rumpold and Webersinke, 2011).***

The M-bcr occurs between exons 12 through 16 exons (also known as exons b1 to b5) and the resulting fusion transcripts with ABL generate a 210-kDa protein named p210 which is the most common BCR-ABL1 form. It is observed in 99% of the CML patients and in one-third of Ph-positive B cell acute lymphoblastic leukemia (Ph+ B-ALL) ***(Cea et al., 2013).***

The m-bcr localizes to an area sited downstream of exon 1 and gives rise to a fusion transcript with ABL named p190. It is rarely observed in CML but is the most frequent BCR-ABL1 isoform in Ph+ B-ALL. Finally, 3' breakpoints downstream of BCR exon 19 have also been described and they give rise to a 230-kDa fusion protein (p230 BCR-ABL), which is typically found in the rare chronic neutrophilic leukemia (CNL) ***(Rumpold and Webersinke, 2011)***

Although all three BCR-ABL1 fusion protein variants are equally effective in inducing CML–like myeloproliferative syndrome in mice, a significant difference in their tyrosine kinase activity has been demonstrated with P190 exhibiting a higher activity than P210 and p230, respectively ***(Cea et al., 2013).***

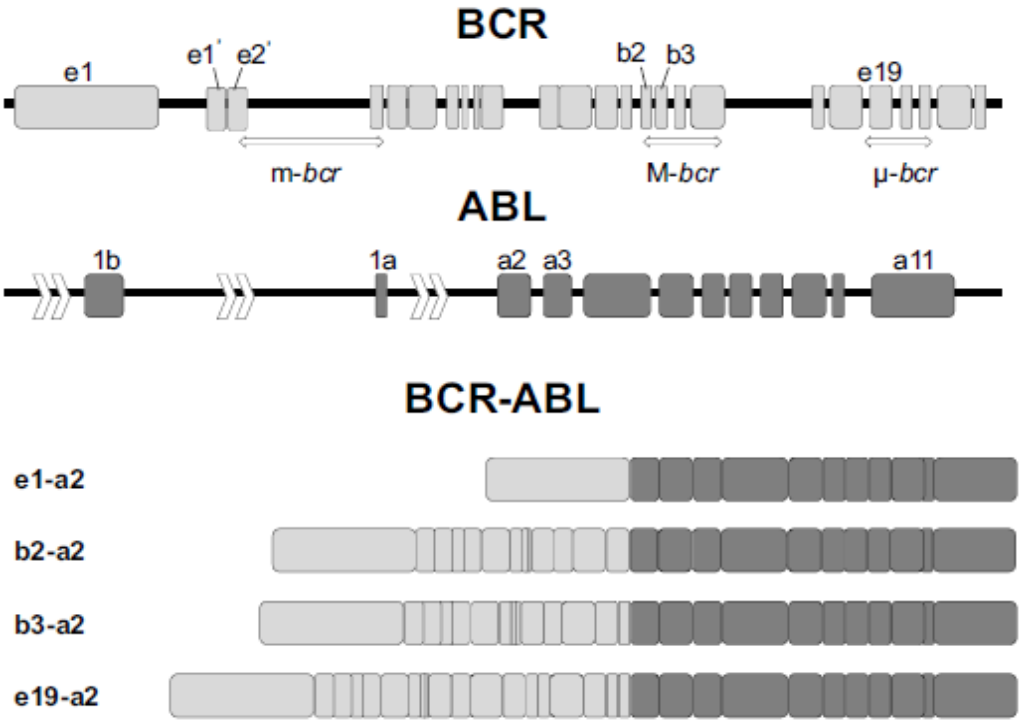

Figure (1): A schematic diagram showing different breakpoint cluster regions in BCR-ABL1 ***(Cea et al., 2013).***

Disruption of intracellular network signaling by BCR-ABL

The leukemogenesis caused by the BCR-ABL1 oncoprotein is a multi-step process proceeding from initial to tumor maintaining events and finally results in a complex tumor supporting network. This process is accomplished through kinase dependent and kinase independent signaling pathways (Wnt/beta-catenin, Hedgehog, Alox5, PTEN and FoxO pathways) ***(Chen et al., 2010).***

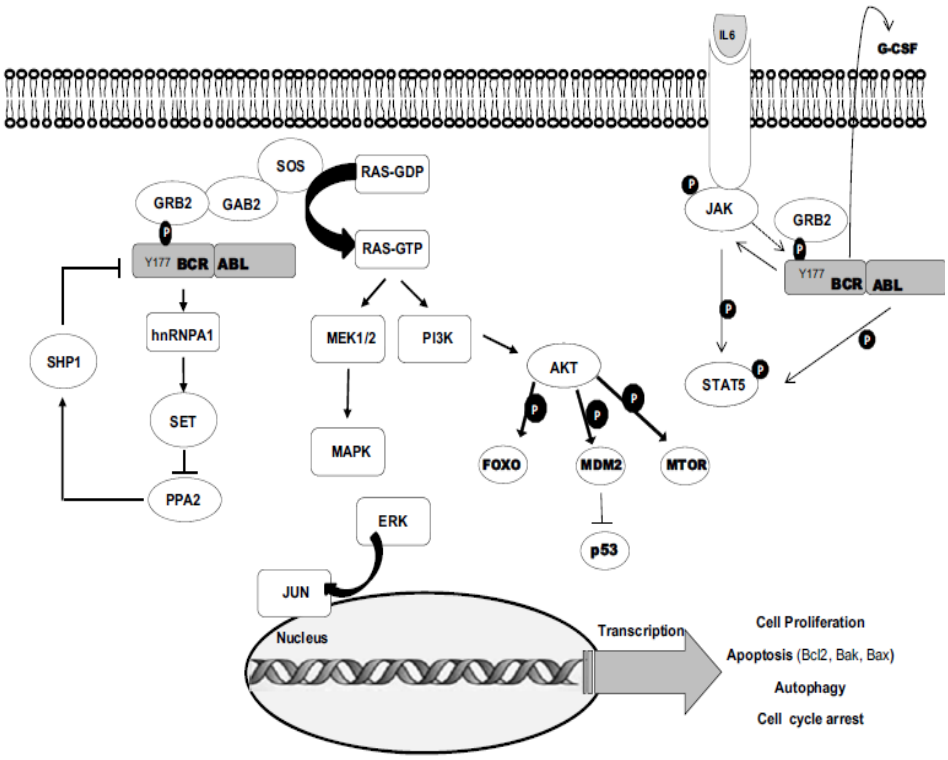

Figure (2): Schematic diagram of Intracellular Network signaling in BCR-ABL1 transformed leukemia cells ***(Cea et al., 2013).***

Unlike ABL which shuttles between nucleus and cytoplasm, the oncoprotein BCR-ABL1 is mainly retained within the cytoplasm resulting in interactions with many cellular substrates involved in oncogenic pathways ***(Cea et al., 2013).***

The constitutive tyrosine kinase activity increases the number of phosphotyrosine residues on BCR-ABL1 and, therefore, the binding sites for the SH2 domains of other proteins, that in concert with the genomic instability promoted by BCR-ABL1 itself, drive disease progression ***(Cilloni and Saglio, 2012).***

Thus, all BCR-ABL1 regions (SH-2, Kinase and Factin bind) are equally required for its own transformation ability ***(Cea et al., 2013).***

The abnormal interaction between this oncoprotein and other cytoplasmic molecules causes the perturbation of a variety of signaling cascades. An example is the mitogenic and transforming activities of CML cells that derive from BCR-ABL1's ability to disrupt the RAS-mitogen activated protein (MAP) kinase signaling cascade ***(Sacha et al., 2012).***

The oncoprotein contains in the BCR-NH terminal sequence a tyrosine-phosphorylated site which interacts directly with SH2 domain of the cytoplasmic adapter protein GRB-2 ***(Cea et al., 2013).***

Recent studies demonstrate the crucial role of Tyr177 phosphorylation in BCR-ABL1mediated leukemogenesis, consequently its mutation by disrupting GRB2 binding, affects BCR-ABL1induced Ras activation ***(Rumpold and Webersinke, 2011).***

Moreover, the interaction of BCR-ABL1 with cytoplasmic adapter proteins creates several multiprotein signaling complexes. BCR-ABL1/GRB2 by recruiting Son of Sevenless (SOS) stimulates the activation of the scaffold adapter GRB2associated binding protein 2 (GAB2) and the

conversion of inactive GDP bound form Ras to its active GTP-bound state ***(Cilloni and Saglio, 2012).***

The resulting GRB2/GAB2/SOS complex causes constitutive activation of RAS downstream pathway with abnormal cell proliferation ***(Cea et al., 2013).***

Another cellular pathway liable of uncontrolled proliferation and frequently deregulated in CML cells is the phosphoinositide 3-kinase (PI3K)/AKT signaling ***(Rumpold and Webersinke, 2011).***

Many molecules downstream of AKT have been shown to be important players in mediating BCR-ABL1 leukemogenic effect, including the pro-apoptotic protein BAD, the negative regulator of p53 MDM2 (upregulated by BCRABL), and the FOXO subclass of forkhead factors (FOXO1, FOXO3a, and FOXO4). All these molecules are directly inhibited by BCR-ABL1 by inducing their cytoplasmic retention ***(Cea et al., 2013).***

Also, the mammalian target of rapamycin (mTOR), participates to leukemogenic effect of BCR-ABL1, given that the suppression of cells growth as well as other cellular processes, such as autophagy, is observed after treatment with the specific mTOR inhibitor, rapamycin ***(Chen et al., 2010).***

Transcription factors such as STAT family members (1 and 5) are also involved in the oncogenic network controlled by BCR-ABL signaling ***(Chen et al., 2010).***

Usually the nuclear translocation of STATs occurs exclusively after cytokine binding to receptors and is mediated by activation of the receptor-associated JAK kinases. CML cells exhibit STATs activation in a JAK-independent manner through a direct association of STAT SH2 domains with phosphorylated tyrosines on BCR-ABL1 ***(Hoelbl et al., 2010)***

Beyond the molecular activation of intracellular pathways, it has been suggested that the bone marrow niche may influence leukemic cells survival and proliferation. In fact, culturing leukemic cells on bone marrow stroma induces resistance to imatinib through increased levels of STAT3 and its downstream targets Bcl-XL, Mcl-1, and survivin ***(Cea et al., 2013).***

The adoptive secretion of stromal cell derived factors such as interleukin6(IL-6) and granulocyte macrophage colony stimulating factor (GM-CSF), produced by bone marrow stromal cells (BMSCs) lead to activation of JAK2 and furnish extrinsic protection of CML cells from TKIs ***(Traer et al., 2012).***

Therefore, CML cells promote a soil for their own growth by inducing BMSCs to upregulate growth factors, which not only stimulate BM angiogenesis, but also promote CML proliferation and metabolism, in part independently of BCR-ABL signaling ***(Nair et al., 2012).***

Cytogenetics and chromosomal aberration in CML

The Ph chromosome is the result of a reciprocal translocation between the long arm fragment (9q34) of chromosome 9 containing the ABL protooncogene a human homologue of v-abl oncogene carried by the Abelson murine leukemia virus (A-MuLV) (discovered by Abelson and Rabstein 1970) and a part of the long arm of chromosome 22, which breaks in a specific breakpoint cluster region (BCR). As a result, the shortened chromosome 22q-(Ph) is formed with the BCR/ABL fusion gene in the region of a junction on chromosome 22, and the ABL/BCR gene on the elongated chromosome 9q+ ***(Balabanov et al., 2014).***

The rearrangement of the BCR and ABL1 gene can also be visualized by fluorescence in situ hybridization (FISH) in interphase nuclei and on metaphase chromosomes using dual color dual fusion probes ***(Bennour et al., 2016).***

The standard translocation t (9; 22) (q34; q11) is found in about 85–90 % of all CML patients ***(Marzocchi et al., 2011).***

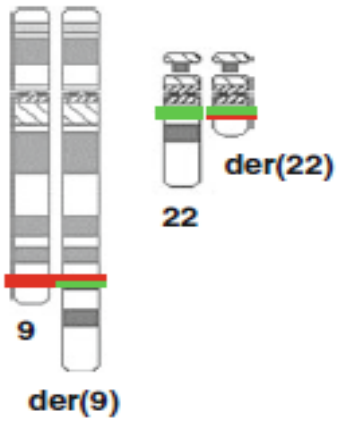

Figure (3): The Ph chromosome ***(Balabanov et al., 2014).***

- ***Ph negative karyotype in CML***

About 5–10% of CML patients lack cytogenetic evidence of the Ph chromosome. These Cryptic BCR-ABL rearrangements can be found in cases with a normal karyotype and in cases with complex karyotype in which the t (9; 22) is not detected by conventional cytogenetic analyses. Such rearrangements can be revealed by FISH ***(Bennour et al., 2011).***

The most frequent location of the BCR-ABL fusion gene in complex chromosomal rearrangements is 22q11.2 and in some cases the fusion gene is located at 9q34, but in rare variant cases BCR-ABL is translocated on sites other than 22q11 ***(Bennour et al., 2012).***

We proposed 2 major mechanisms of how a cryptic BCR/ABL rearrangement can arise. The simplest explanation is

that there is a nonreciprocal insertion of chromosome material between chromosomes 9 and 22, resulting in a gene fusion. The insertion can occur within either chromosome, although the fusion gene is situated at its usual locus (22q11) more frequently than on the derivative chromosome 9 (der9) ***(Marzocchi et al., 2011).***

The more complex explanation involves two sequential translocations. First, there is an initial standard translocation t (9; 22) (q34; q11). This is followed by a reverse translocation involving different breakpoints, which reconstitutes the partner chromosomes. The BCR-ABL fusion gene can be situated at the usual locus (22q11) or at the reciprocal locus on 9q34, depending on whether the second breakpoints are distal or proximal to the fusion genes, respectively ***(Bennour et al., 2011).***

The prognosis of Ph-negative, BCR-ABL positive patients is not different from those that are Ph positive, and therefore, treatment remains the same ***(Bennour et al., 2016).***

- ***Variant Ph rearrangement in CML***

In 5–10% of CML cases, variant rearrangements involving 9q34, 22q11.2, and one or more additional genomic regions generate the BCR-ABL chimeric gene ***(Bennour et al., 2016).***

Some researchers have invoked a one-step mechanism, where in chromosome breakage occurs on three different chromosomes simultaneously in a three, four, or five-way