

Chronic Leukaemia

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Abstract: Chronic leukaemia manifests itself into two different types being Chronic Lymphocytic Leukaemia (CLL) and Chronic Myelogenous Leukaemia (AML) depending on the type of leukocyte being affected. CLL is the most common type of leukaemia in adults in contrast to CML which is the rarest type of leukaemia. This study shows the epidemiology and the etiology, such as chromosomal aberrations and gene mutations which include upregulation of Bcl2, mutation of p53, 13q deletion, 11q22-23 deletion, 12q trisomy and 17p deletion for CLL and the fusion of the BCR and ABL genes in CML. It also includes the clinical presentations consisting of both signs and symptoms as well as how these types of leukaemia are diagnosed and their pathophysiology which comprises a detailed description of the alterations in various cellular mechanisms. The treatment involving both chemotherapy and stem cell therapy, amongst others, has also been discussed.

Key words: Chronic lymphocytic, leukaemia, CML, epidemiology, chemotherapy

INTRODUCTION

Leukaemia is 'a progressive, malignant disease of the blood-forming organs, characterised by distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow'.

There are two common types of chronic leukaemia being the Chronic Lymphocytic Leukaemia (Chronic lymphoblastic leukaemia) and the Chronic Myelogenous Leukaemia.

The term chronic implies that the disease progresses slowly and the growth of relatively larger number of developed cells is permitted. Such cells can carry out some of the functions but this condition worsens progressively.

The types of leukaemia are also categorised according to the type of leukocyte being affected. In lymphocytic leukaemia, malignant lymphoid cells are observed while abnormalities in myeloid cells are found in myelogenous leukaemia.

CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

CLL is the most common leukaemia of adults in Western Countries (Schriever and Huhn, 2003). It accounts for 25% of all leukaemia cases and affects about 3 in every 100,000 persons in the United States (Yee and O'Brien, 2006). It originates from B-cells (B-CLL) in 95% of leukaemia cases and only a minority of patients are affected by leukaemia originating from T-Cells (T-CLL) (Schriever and Huhn, 2003). The T-Cell CLL has been

reclassified by the World Health Organisation as T-Cell Prolymphocytic Leukaemia (PLL) (Yee and O'Brien, 2006).

It is the only leukaemia not associated with exposure to ionising radiation, drugs or chemicals. But it was observed that the incidence of CLL in the relatives of the CLL patients is higher suggesting that CLL occurs in 5-10% of patients (Yee and O'Brien, 2006).

CLL patients presented night sweats, weight loss and fatigue as well as lymphadenopathy, splenomegaly and hepatomegaly. The peripheral blood lymphocyte count was found to be more than $5 \times 10^9 \text{ L}^{-1}$. The typical B-cell ALL immunophenotype is CD5+, CD19+, CD23+, FMC7-, negative expression of CD22, CD79b and surface membrane immunoglobulin (Yee and O'Brien, 2006).

During the process of normal B-cell maturation. Somatic hypermutation of the variable region of the Ig heavy chain gene (IgVh) in the lymphatic follicle is an important station. (Méhes, 2005). The presence of unmutated IgVh genes in CLL is considered to be an adverse prognostic factor (Yee and O'Brien, 2006). Therefore, the need to analyse the mutation state of the IgvH gene arises and this occurs via the presence of the CD38 and ZAP70 biomarkers (Méhes, 2005).

PATHOPHYSIOLOGY

Inhibition of the programmed cell death (apoptosis) and upregulation of the anti-apoptotic protein Bcl-2. Mutations of p53 were also observed in some CLL cases which result in poor clinical response to therapy with alkylating agents such as chlorambucil.

Genetic aberrations were also detected in 50-80% of CLL case (Méhes, 2005) and were listed according to their decreasing frequency being:

- 13q deletion (55%)
- 11q deletion (18%)
- 12q trisomy (16%)
- 17p deletion (7%) (Méhes, 2005; Schriever and Huhn, 2003)

13q deletion: The deletion can be either pinpointed on a single locus (q14) or can be accompanied with a loss of larger interstitial region of the long arm of the chromosome. Two hypothesis are involved in the manifestation of this deletion. The first one is about an unidentified tumour suppressor gene being included in the deletion. On the contrary, the second hypothesis states that the deleted 13q 14.3 region would include genes coding for micro RNA (miRNA) which are capable to regulate cellular activation by interacting with the genomic DNA. Therefore, this will impair disease progression (Méhes, 2005).

11q22-23 deletion: The ATM (Ataxia Telangiectasia) gene is included in this deletion. This gene is involved in the cellular apoptotic pathway through the activation of p53. The ATM gene loss results in reduced cell-death (Méhes, 2005).

12q trisomy: This trisomy has been linked to activated genes present at 12q which may be involved in disease progression. (Méhes, 2005).

17p deletion: This deletion is associated with the loss of certain genes which are important in cell cycle regulation since they are found at the 17p13 locus. One of such genes is the p53 tumor suppressor gene and as a result CLL cells may survive and accumulate further genetic defects (Méhes, 2005).

Apart from the previously mention chromosomal aberrations, further studies have shown that the level of activation of the human Telomerase Reverse Transcriptase (hTERT) gene was found to increase in B-CLL. hTERT is the gene which codes for the catalytic subunit of the telomerase enzyme. This was to blame for the clinical aggressiveness and poor prognosis in CLL (Tchirkov *et al.*, 2004).

TREATMENT

Chlorambucil is a bifunctional alkylating drug and is the standard treatment against CLL. It presents remission rates up to 40% with only 3% complete remission with the latter having a short duration.

Fludarabine is a purine-analogue and it inhibits DNA synthesis by interfering with ribonucleotide reductase and DNA polymerase. It is active against both dividing and resting cells. It is the most active single agent in CLL treatment and is indicated for B-CLL patients. However, it is also used as a first line of treatment with 80% remission rates and up to 60% complete remission rates. If given to relapsed patients after treatment with this drug, remission rates increase to 83%. The adverse effects connected to Fludarabine are myelosuppression, lymphocytopenia and specific damage of CD4+ T cells (Schriever and Huhn, 2003).

Another purine analogue involved in CLL treatment is Cladribine having an efficacy similar to fludarabine. It presents complete remission rates of 67%. The adverse effects are similar to fludarabine.

Rituximab is a chimeric monoclonal antibody that binds to the surface antigen CD20 which expression is restricted to B-cell malignancies (Schriever and Huhn, 2003).

Alemtuzumab is another chimeric monoclonal antibody which binds to the CD52 antigen. CD52 is strongly expressed on malignant B-cells including CLL cells and lymphoblastic leukemia cells. The drug promotes transmembrane signaling and induces apoptosis through a caspase-3-dependent pathway. Infections are its major adverse effects (Schriever and Huhn, 2003).

High dose therapy followed by autologous stem cell transplantation ie transplantation of stem cell obtained from host. This always leads to reinfusion of contaminating leukemic cells. Stem cell purging must be performed by B cell negative selection, CD34+ B-cell selection or combination of both methods. Ineffective purging leads to relapse. Transplant related mortality varies between 4 and 19%. Approximately 80% of patients achieve complete remission and 40-50% are alive after 4 years. But in most studies, 50% of patients have relapsed at 4 years.

High dose chemotherapy followed by allogeneic stem cell transplantation that is stem cells obtained from a donor. Higher mortality rates have been observed that varied between 25 and 50% of all patients with relapse rates escalating between 10-25% of patients (Schriever and Huhn, 2003).

CHRONIC MYELOGENOUS LEUKAEMIA

Chronic myelogenous leukaemia is a rare disease represents 14% of all leukaemias and 20% of adult leukaemias worldwide. The incidence is 1.6 cases per 100,000 adults per year and the male to female ratio is 1.4:1 in the Unites States (Cardama *et al.*, 2006). CML incidence increases exponentially with age. It was

statistically proven that the median age at diagnosis is 65-67 years (Cardama and Cortes, 2006; Lee, 2000) On the other hand, CML is exceedingly rare in children (Lee, 2000).

The disease is not preventable and neither inherited since there is no known hereditary, familial, geographic, ethnic, or economic association with CML. It can be concluded, that neither chemical exposure nor genetic predisposition is thought to induce CML (Cardama and Cortes, 2006). Ionizing radiation in high doses is the only known risk factor (Lee, 2000). CML can also be due to a reciprocal chromosomal translocation which involves the ABL1 proto-oncogene on chromosome 9 and the BCR gene (i.e. the breakpoint cluster region) on chromosome 22 to form the Philadelphia chromosome (Cardama and Cortes, 2006).

CLINICAL PRESENTATION AND DIAGNOSIS

CML is described in 3 phases of disease progression. These are:

- Chronic Phase (CP)
- Accelerated Phase (AP)
- Blast Phase (BP) (Cardama and Cortes, 2006)

Patients suffering from chronic phase CML account to 90% of all CML patients. They have effective immune systems and generally feel well for a relatively long period of time. When symptoms and signs are present, they are usually mild and related to expansion of the CML cells. Thus the common symptoms include fatigue, weight loss, bone ache and splenomegaly. This phase is usually described as a benign premalignant state, as the disease is easily managed with outpatient therapy during the early stage. Still after 3 to 8 years it becomes an acute leukaemia that is rapidly fatal. The progression of the disease after 2 years of diagnosis results in 5-10 patients developing blast crisis. The progression rate then increases by 20-25% per year (Lee, 2000).

When CML enters an advanced stage, patients present also an inability to control leukocyte counts with previously stable doses of medication, fevers, night sweats and splenic infarcts from excessive splenomegaly. The platelet count may either be high or low. There is also an increase in basophil, eosinophil, promyelocyte and blast count. This hastened phase suggests a more aggressive cytogenetic abnormality apart from the Philadelphia chromosome. Additional abnormalities include trisomy 8, trisomy 19, a duplicate Philadelphia chromosome and isochromosome. The blast crisis mentioned previously is characterized by having a 20-30%

blast and promyelocyte count in marrow or peripheral blood samples. Several definitions are still being studied for the accelerated phase and blast crisis as there are still some discrepancies in the blood count values obtained (Cardama and Cortes, 2006).

PATHOPHYSIOLOGY

The fusion of the ABL1 and BCR genes results in the activation of the c-ABL protooncogene to its oncogenic active form. The breakpoints at the 9q34 within the ABL1 gene represent an area spanning more than 300 kilobase pairs (upstream of exon b1 and downstream of exon 1a). On the other hand, breakpoints within the BCR gene are restricted to 3 main breakpoint cluster regions.

In most patients with CML, the break occurs within a 5.8-kb area spanning the BCR exons e12-e16 (or the b1-b5). This is referred to as the M-bcr-major breakpoint cluster region. Due to alternative splicing, a fusion transcript with either b2a2 or b3a2 junctions can result and will give rise to a 210-kd hybrid protein (p210BCR-ABL).

Figure 1 depicts the location of the M-BCR in the BCR gene on chromosome 22 and the location of breakpoints within the ABL gene on chromosome 9.

The p210BCR-ABL protein possesses various important functional domains which are shown in Fig. 2.

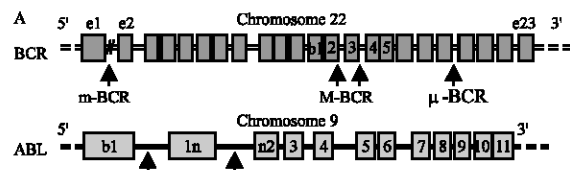


Fig. 1: Locations of the breakpoints in the BCR and ABL genes (Inokuchi, 2006)

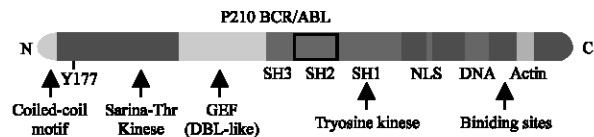


Fig. 2: The functional domains on the p210 BRR/ABL include the oligomerisation domain (coiled-coil motif), the tyrosine 177 (Grb-2 binding site), the phosphoserine/threonine-rich SH2-binding domain and the rho-GEF (DBL-like) domain on the BCR portion and the regulatory src homology regions SH3 and SH2, the SH1 (tyrosine kinase domain), the Nuclear Localization Signal (NLS) and the DNA- and actin-binding domains in the ABL portion (Inokuchi, 2006)

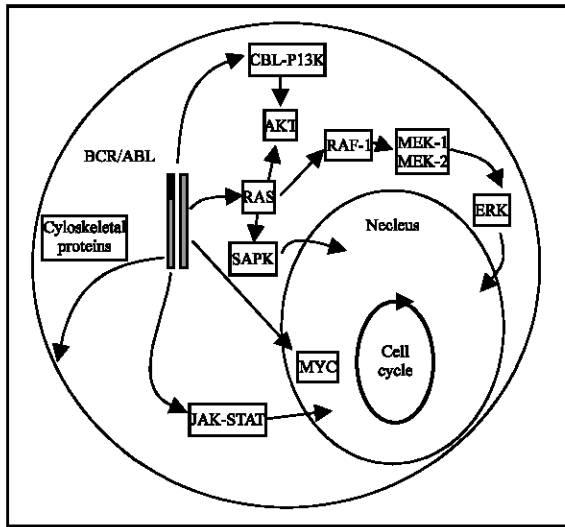


Fig. 3: The activation of signal transduction pathways by BCR/ABL (Cardama and Cortes, 2006)

Several pathways are activated in p210BCR-ABL expressing cells. Phosphorylation at the Y177 site of BCR is very important in p210BCR-ABL leukemogenesis. This residue makes up a high-affinity docking site for the SH2 domain of Grb2 (growth factor receptor-bound protein 2). Subsequently, Grb2 engages SOS (a guanine-nucleotide exchanger of RAS) and a scaffold adapter Grb2-Associated Binding protein 2 (GAB2) through its SH3 domain. GAB2 is phosphorylated and this leads to the recruitment of Phosphatidylinositol 3-Kinase (PI3K) and SHP2 (also known as PTPN11). The SOS activates RAS (Cardama and Cortes, 2006).

Grb2 binding and the Bcr-Abl induced RAS activation are interrupted due to a mutation of Y177 to phenylalanine (Y177F). The Y177F mutation ceases the transformation of primary bone marrow cultures. The BCR-ABL complex also phosphorylates Src kinases i.e. Lyn, Hck and Fgr. Following phosphorylation, the Hck activates STAT5 (Signal Transducer and Activator). Disease progression has been correlated with over expression of the previously mentioned Hck/Lyn (Cardama and Cortes, 2006).

It was concluded that BCR-ABL promotes the haematopoietic cell transformation. This occurs mainly through the RAS, PI3K and STAT 5 activation. All these steps cause upregulation of the expression of cyclins especially cyclin D1 which causes the cell to progress from the G1 to the S phase. When BCR-ABL1-positive K562 cells were induced to express the dominant negative forms of RAS, PI3K, or STAT 5, apoptosis was observed (Cardama and Cortes, 2006).

Studies proved that Bcr-Abl also enhances SET expression (nucleus/cytoplasm-localized phosphoprotein

that potentially inhibits the tumor suppressor protein phosphatase 2A-PP2A). This occurs mainly during progression to the Blast Phase. PP2A regulates cell proliferation, survival and differentiation (Cardama and Cortes, 2006). Figure 3 illustrates the main signal transduction pathways activated by BCR/ABL.

TREATMENT

Tyrosine kinase inhibitors are essential in treating CML. Until a few years ago, interferon alfa and SCT (Stem Cell Transplantation) were the only therapeutic options. Currently, SCT is still being used as an option especially for patients who do not respond appropriately to Tyrosine Kinase Inhibitors (TKIs). However, the use of molecularly targeted TKIs in recent years led by imatinib mesylate has brought a change in the treatment of CML (Cardama and Cortes, 2006).

Using interferon: The use of recombinant human interferon alfa has shown relative antitumor and immunomodulatory activity in the treatment of CML. Several results have been reported such as Major Cytogenetic Responses (MCGRs) (<35% Ph chromosome-positive cells) in up to 40% of patients and Complete Cytogenetic Responses (CCGRs) (0% Ph chromosome-positive cells) in up to 25% of the population studied. When cytarabine was used in combination with this therapy this resulted in a significant higher probability of a CCGR in about 35% of the patients. This proves the fact of increased survival rates thus proving the use of CCGR the interferon alfa therapy (Cardama and Cortes, 2006).

Stem Cell Transplantation (SCT): This is still and remains an important treatment option. SCT performed within 12, 24 months and within first 36 months, from the diagnosis has been suggested to possibly have similar outcomes. Recently it was discovered that interferon alfa therapy had no adverse effect and that exposure to imatinib does not affect the outcome either. A strategy was recently proposed to overcome the lack of compatible donors-this is the use of Matched Unrelated Donors (MUDs). Still this is still being studied. The result of MUD SCT may be improved by careful molecular typing, especially for HLA-A, HLA-B and HLA-DRB1. Still, the use of stringent typing criteria is closely linked to a lower probability of finding a compatible donor. Additional disadvantages and risks associated with SCT include graft-vs-host disease, veno-occlusive disease, life-threatening infections, risk of secondary malignancy, decreased overall quality of life and the risk of late relapse. The long-term survival rate after MUD-SCT for young patients in the early CP stage of disease is 57% when compared with 67% in patients who receive grafts

from HLA-matched siblings. The long-term survival in patients has been improved by recent molecular matching incorporation. This has decreased the rate of graft-vs-host disease. Reduced-intensity conditioning regimens have been used to make SCT available to an increased number of patients who otherwise would be ineligible for standard SCT. This makes use of the T cells found in the graft to eliminate the CML cells (graft-vs-leukaemia effect). This increases tolerability and decreases morbidity and mortality of the patient (Cardama and Cortes, 2006).

Imatinib mesylate: Imatinib mesylate (Gleevec) can be described as an orally bioavailable 2-phenylamino-pyrimidine with targeted inhibitory activity against the active tyrosine kinase of the Bcr-Abl chimeric fusion protein. Imatinib also inhibits other kinases, such as c-Kit, platelet-derived growth factor α and β and Abl-Related Gene (ARG). Nowadays Imatinib has become the standard therapy for CML because it has an effective and efficient activity and mild toxicity profile. Imatinib therapy was found to have an increased effect when compared to interferon alfa plus low dose cytarabine (when taking into account the hematologic, cytogenetic responses, tolerability and transformation to CML-AP). The results from the International Randomized Study of Interferon and STI571 have established imatinib 400 mg d⁻¹, as the standard therapy for CML. Still a disadvantage with Imatinib therapy is that most patients who have discontinued it experience a molecular and cytogenetic relapse. Some patients with CML also exhibit either primary or secondary resistance to imatinib (Cardama and Cortes, 2006).

Treatment strategies being developed for the future Bcr-abl inhibitors: This is one of the strategies currently being developed to overcome imatinib resistance. New Abl TKIs have been developed with an increased potency and less stringent binding requirements and also with inhibitory activity against other kinases. New agents that block Bcr-Abl in a non-ATP competitive manner are being proposed as an alternative for patients who develop imatinib-insensitive Bcr-Abl kinase mutations.

Nilotinib (AMN107): This is a phenylamino-pyrimidine derivative with a 20-30 fold increased potency when compared with imatinib against Bcr-Abl.

Dasatinib (BMS-354825): It was found that inhibition of Bcr-Abl alone may not be sufficient to destroy all of the CML cells, particularly the leukemic imatinib in sensitive quiescent stem cells. Dasatinib is an ATP

competitive, dual-specific Src- and Abl-kinase inhibitor with a 100-300 fold higher potency against Bcr-Abl compared with imatinib.

Non-ATP-competitive Bcr-Abl inhibitors: Imatinib, nilotinib and dasatinib are ATP-competitive inhibitors. Therefore, these molecules can be affected by the T315I mutation, which was described as the gatekeeper of the kinase domain. Due to this, non-ATP- competitive compounds are also being used. They compete with natural substrates such as Crkl but not with ATP (in the case of ON012380) (Cardama and Cortes, 2006). Additional therapy include:

- Farnesyl transferase inhibitors (such as lonafarnib - these have demonstrated activity both as single agent and in combination with imatinib.
- Homoharringtonine - A *Cephalotaxus* alkaloid which is found to be the best therapeutic option in patients in whom interferon alfa-based therapies failed.
- Hypomethylating agents and histone deacetylase inhibitors.

Use of vaccines to elicit specific immune responses directed toward CML-restricted tumor antigens since immune-mediated events play an important role in the suppression of the CML clone (Cardama and Cortes, 2006).

REFERENCES

- Cardama, A.Q. and J.E. Cortes, 2006. Chronic myeloid leukemia: Diagnosis and treatment. *Mayo Clinic Proc.*, 81: 973-988.
- Inokuchi, K., 2006. Chronic myelogenous leukemia: From molecular biology to clinical aspects and novel targeted therapies. *J. Nippon Med. School*, 73: 178-192.
- Lee, S.J., 2000. Chronic myelogenous leukaemia', *British J. Haematol.*, 111: 993-1009.
- Méhes, G., 2005, Chromosome abnormalities with prognostic impact in B-cell chronic lymphocytic leukemia. *Pathol. Oncol. Res.*, 11: 205-210.
- Schriever, F. and D. Huhn, 2003. New directions in the diagnosis and treatment of chronic lymphocytic leukaemia. *Drugs*, 63: 953-969.
- Tchirkov, A., C. Chaletix, C. Magnac, Y. Vasconcelos, F. Davi and A. Michel *et al.*, 2004. hTERT expression and prognosis in B-chronic lymphocytic leukemia. *Anna. Oncol.*, 15: 1476-1480.
- Yee, K.W.L. and S.M. O'Brien, 2006. Chronic lymphocytic leukemia: Diagnosis and treatment. *Mayo Clinic Proc.*, 81: 1105-1129.