Detection of bcr-abl Fusion Gene Expression in Chronic Myelogenous Leukemia At Sardjito Hospital: Case Reports

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Abstract

Leukemia is hematology malignancy caused by excess proliferation of hematopoetic or lymphoid cells. Leukemia cases in Indonesia were about 3,7 per 100.000 with mortality rate 83,6% and diagnosed based on FAB classification. The fact, WHO classification 2000, used worldwide based on cytogenesis and molecular biology profile, can define the clonal diseases more precisely and to choose the adequate therapy. CML case is about 15% of all adult leukemia cases and most of the cases are related with t(9; 2) (q11; q23) that result bcr-abl fusion gene.
The aim of this report is to show the rare case of CML that has been examined for bcr-abl fusion gene. Blood sample was obtained from CML patients diagnosed by hospital doctors based on FAB classification. Mononuclear cell was separated by Ficoll-hypaque gradient centrifugation, then RNA was isolated by Trizol and converted to cDNA by RT reaction. Beta-actin gene was used as internal control and bcr-abl gene was amplified by nested PCR.
We reported CML cases classified as atypical (case 1) and typical (case2) type based on FAB classification with post therapy for the second CML. At molecular level, bcr-abl fusion gene found at the second case with longer product than positive control.

Keywords: leukemia, CML, fusion gene, bcr-abl

Introduction

Leukemia is a blood disease resulting from the neoplastic proliferation of haemopoietic or lymphoid cells that can affect everyone, every age and sex (Bain, 2003). Leukemia prevalence varies at many countries, with range between 1- 4,8 per 100.000 populations. Prevalence of leukemia cases in Indonesia was around 3,7 per 100.000 with mortality rate 83,6% (WHO, 2001). Leukemia diagnosis in Indonesia is based on French-American-British (FAB) classification, even though World Health Organization (WHO) classification (2000) emphasized that cytogenetic and molecular biology detection are very useful to determine the disease clonality and to choose precise therapy.

Chronic Myelogenous Leukemia (CML) case is about 15% of all adult leukemia cases and account for 20% of leukemia death (Jandl, J.H., 1996). In FAB classification, CML is included in myeloproliferatif disorder, and clinically, it has 3 stages of disease: chronic, accelerated, and blast crisis phase. These three stages have different peripheral blood and bone marrow characteristic and different morphology pattern. The prognosis is getting worse for the extended stage (Bain, 2003; Jandl, 1996).

CML has known as the first type of leukemia that shown cytogenetic abnormality

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(9;22)(q34;q11) translocation, namely Philadelphia chromosome, and expressed bcr-abl fusion gene at molecular level (Bain, 2003; Jaffe, 2001). The expression of this fusion gene could be detected at mRNA level by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) technique (Weaver, 1999). In WHO classification (2000), CML is also grouping as myeloproliferatif disorder but with different subgroup. There are 2 subgroups based on cytogenetic and molecular characteristic: first, with Philadelphia chromosome and expressed bcr-abl fusion gene and second, without Philadelphia chromosome but also expressed bcr-abl fusion gene (Bain, 2003).

The fusion gene could influence disease progress in leukemia patient. CML patient with positive bcr-abl fusion gene have relapse possibility during 1-3 months after chemotherapy comparing with negative bcr-abl that could survive for several years after chemotherapy (Lowenberg, 1999; Drukker, 2001; Serrano et al., 2000)

Diagnosis and classification of leukemia by molecular technique based on genetic abnormality and clonal changing are never reported in Indonesia, especially at Sardjito Hospital Yogyakarta. This study was held to applied molecular technique in clinical setting by detect the expression of bcr-abl fusion genes at CML cases. We reported 2 CML cases with different clinical type and treatment, one was without chemotherapy case and the other was post therapy case that have been checked for the bcr-abl fusion gene by RT-PCR method.

Material and method

All reagents and primers in this study were provided by Hematology/Oncology Subdivision, International Centre for Medical Research (ICMR), Kobe University, Kobe, Jepang. The research was held at Clinical Pathology Instalation Sardjito Hospital, Tumor Biology Laboratory Histology Department Faculty of Medicine GMU Yogyakarta, Biochemistry Laboratory Inter University Centre GMU Yogyakarta and ICMR, Kobe School of Medicine, Kobe University, Kobe, Jepang from July 2003-Mei 2004.

The sample was mononuclear cell isolated from inward patients Sardjito Hospital that diagnosed as CML based on FAB classification and gave the informed consent. Mononuclear cell was isolated by Ficoll-Hypaque gradient centrifugation from 5-10 mL heparized peripheral blood then kept at -80°C as frozen pellet until the RNA isolation. Total RNA was isolated from the mononuclear cell using Trizol reagent (Life Technologies), according to the manufacture recommendation. Briefly, approximately 10^7 cell of frozen pellet were thawing and mix with 1 ml of Trizol then let for 10 min at room temperature. After centrifugation at 12,000 rpm for 10 min in a minicentrifuge, the supernatant was treated with 0.2mL chloroform then precipitated with 0.5ml isopropanol. The pellet was washed with 70% cold ethanol and dissolved in 100 µl of sterile water. The purity and concentration of the RNA preparation were spectrofotometrically measured. The cDNA was made from RNA by a reverse transcryptase reaction with random primers and β-actin gene was used as internal control. Primer for β-actin gene were b-actin-1 (with sequence 5′→3′ GGA GAA GCT GTG CTA CGT CGC CC) and β-actin-2 (with sequence 5′→3′ TAC ATG GTG GTG CCG CCA GAC AG). The tube containing amplification mixture subjected to 30 cycles on a thermal cycler (Takara thermocycler, Japan) with the following programs: 1x5 min precycle at 94°C for 5 min, for the next cycle denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, and the last extension at 72°C for 9 min.

Bcr-abl fusion gene was amplified by nested PCR with following primers: ABL-1 (with sequence 5′→3′: GGC CCA TGG TAC CAG GAG TG), ABL-2 (with sequence 5′→3′: GTT TCT CCA GAC TGT TGA CTG), BCR-1 primer (with sequence 5′→3′: GCT TCT CCC TGA CAT CCG TG), and BCR-2 (with sequence 5′→3′: GGA GCT GCA GAT

GCT GAC CAA C). The amplification programs were: 2x1 min precycle at 94°C, 35x1 min at 94°C, 1 min at 65°C, and 1 min at 72°C followed by a final extension incubation at 72°C for 10 min. The target fragment was 371 bp.

The presence of PCR product was determined by electrophoresis of 7 mL of the reaction product in 2 percent Agarose gel, with tris acetate-electrophoresis buffer TAE (0.04 mol Tris, 0.001 mol EDTA, pH 7,8) and a 100 bp DNA ladder (Gibco) as molecular marker. The electrophoresis was visualized by UV light and documented by Polaroid film.

Result and discussion

Case 1

a. Patient characteristic and history

Leukemia cell was isolated from a 69 years old woman that admitted to hospital cause of weakness and dyspneu. The weakness has been suffered since 2 months ago with fullness and distention of the abdomen, then many lumps appeared at her neck, axilla and inguinal. When admitted to hospital, physical examination revealed anemia, hepatosplenomegaly and multiple lymphadenopaty at the neck, axilla and inguinal and also sternal tenderness.

b. Laboratory examination

At admission, blood test showed leukocytosis (WBC 196 x109/L), anemia (Hb 5.1 g%), and trombocytopenia (PLT 50 x109/L) with complete leukocyte spectrum at differential telling (from blast cell to neutrofil, with promyelocyte predominance (33,6%) at peripheral blood and bone marrow smear). Peripheral blood morphology showed elevated blast count (9%) and hypercellularity at bone marrow examination with higher blast count (26%) (Figure 1). There is no dysplastic morphology at all cell lineages in bone marrow (erythrocyte, leukocyte or megakaryocytic lineage). Bone marrow cytochemistry staining pointed to myeloid type with diffuse positive result for Periodic Acid Schiff (PAS) staining at granulocyte series above myeloblast series. Positive result was also found in Sudan B Black (SBB) staining (Figure 2).

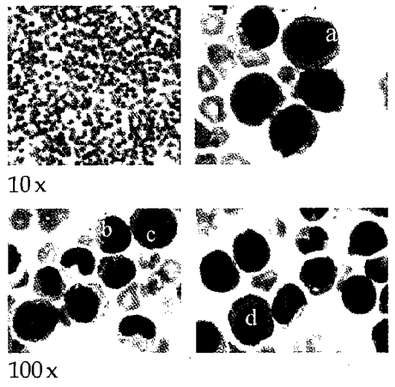

Figure 1. Bone marrow cellularity and leukocyte cell morphology in case 1 (May-Grunwald Giemsa staining, 10x and 100x objective magnification). Note:a=myeloblast,b=myelocyte, c=metamyelocyte, d=promyelocyte

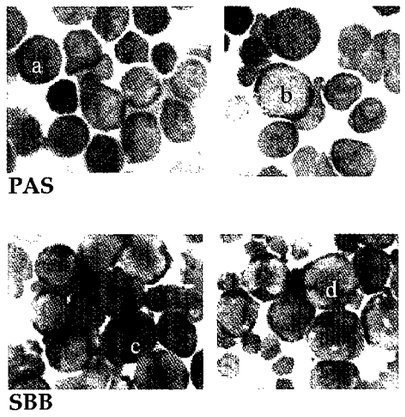

Figure 2. Bone marrow cytochemistry staining of case 1 (PAS and SBB staining, 100x objective magnification). PAS= Periodic Acid Schiff, SBB= Sudan B Black. Note: a=myelocyte, PAS (+) diffuse; b=myeloblast, PAS (+) diffuse; c=metamyelocyte, SBB(+); d=myeloblast, SBB(+)

During hospitalization, the patient got cephalosporin therapy and hydroxyurea but after 2 days, she developed gastric stress ulcer's sign as melena and also showed renal failure with increasing BUN and serum creatinine (BUN 32.8 mmol/L, creatinine

2.01 mmol/L). Gastric ulcer can be overcome by anti-ulcer therapy but the patient developed hyperuricemia (uric acid 14,4 mmol/L). Anemia was overcome by 8 kolf of Packed Red Cell (PRC) transfusion. No remission induction treatment was given for leukemia therapy. After 9 days treatment, the families asked to take care her at home but 2 days later she died at home.

c. Molecular examination

There was no bcr-abl fusion gene expression that found by RT-PCR with primers for b3 exon of bcl gene and a2 exon for abl gene (Figure 3).

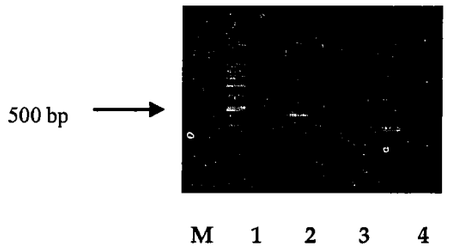

Figure 3. Elektrophoresis of bcr-abl fusion gene in 2% agarose gel (M=marker, 1=case 1, 2=case 2, 3= negative control, 4= positive control, 371 bp)

Based on FAB classification, the 1st case diagnosed as leukemia because of the existence of trias leukemia such as bisitopenia with leukocytosis in routine blood test, and evidence of leukemia cell infiltration to the organs and lymphoid tissues like hepatosplenomegaly and multiple lymphadenopaty. Diagnosis of CML characterized by very high leukocyte count (>100 x 10^9/L) with blast cell less than 10% and all leukocyte spectra were found in peripheral blood morphology. Similar pattern was found in bone marrow examination but there was no basophylia, eosinophylia or monocytosis.

The absence of bcr-abl fusion gene in this case (Figure 3) might be caused by 1) **different gene breakpoint.** Technically, the primers used for bcr-abl fusion gene amplification were annealed at M-BCR breakpoint between b3 exon of bcr gene and a2 exon of abl gene (b3a2). In fact, bcr-abl fusion gene has several different breakpoint such as M-BCR, m-bcr and m-bcr that gave different fusion proteins (Vardiman et al, 2001, Bain, 2003). As a result, this different breakpoint or exon/intron different breakpoint can not revealed by primers used in this study; 2) **there is no bcr-abl fusion gene** in this case. Several researcher explained that bcr and abl gene rearrangement resulted bcr-abl fusion gene was found in 90-95% of CML cases in the world, so only 5-10% of CML case was negative for bcr-abl fusion gene (Bain, 2003, Vardiman, 2001). There are 3 possibilities of myeloproliferatif disorder without bcr-abl fusion gene: atypical CML, CNL (Chronic Neutrophylic Leukemia) and CMML (Chronic Myelomonocytic Leukemia) (Kurzock, et al, 2001).

Based on diagnosis criteria of those 3 possibilities, CNL and CMML possibilities can be excluded because in CNL diagnosis criteria, the case should have stab and mature neutrophyl more than 80%, immature granulosit less than 10%, myeloblast less than 1% of the peripheral leukocyte differential telling (Imbert et al., 2001). Diagnosis criteria for CMML are persistent monocytosis more than 1x10^9/L, blast cell less than 20% of peripheral and bone marrow leukocyte count (Vardiman et al., 2001)

This case, based on WHO classification, was diagnosed as atypical CML because of leucocytosis in peripheral blood with increasing mature and immature neutrophyl percentage, dysgranulopoesis, the absence of bcr-abl fusion gene, neutrophyl precursor more than 10%, no basophylia or monocytosis. The accelerated phase supported by the infiltration signs in lymph node that frequently found in accelerated phase or blast crisis CML (Kjeldsberg, et al, 1995), and increasing blast cell percentage but less than 30% in bone marrow and less than 10% in peripheral blood (Bain, 2003, Vardiman, 2001). Atypical CML have worse prognosis than typical type (Kjeldsberg, et al., 1995; Bain, 2003) especially with accelerated phase as shown in this case.

This case is a very rare case, especially in Indonesia and Yogyakarta because until now there is no report or publication about atypical CML accelerated phase with negative bcr-abl fusion gene expression.

Case 2

a. Patient characteristic and history

A 41 years old man complained about bleeding in his mouth mucosae, under his arm skin and a lump on his thigh. He checked himself to Sardjito Hospital and admitted for 3 days. There were no organomegaly or lymphadenopaty signs at admission but blood tests revealed anemia (Hb 9 g%), leukocytosis (AL 140x109/L), and trombocytopenia (9x109/L). Peripheral and bone marrow cell morphology showed CML chronic phase pattern based on leukocytosis with complete cell spectrum dominated by promyelocyte (20%), basophylia pattern, eosinophylia and bone marrow hypercellularity (erythroid: myeloid ratio = 1:25). Elevated erythroid series and nucleated erythrocyte cell (4%) was also found with normal megakaryocyte morphology. Philadelphia chromosome was found at cytogenetic test.

Patient was treated with Hydrea for 1,5 months then the dose was tapering off and he was scheduled for bone marrow transplantation in Singapore. But, the lack of donor causes the therapy changing to imatinib mesylate for 7 months. Patient can reach complete hematology remission (CHR) until present. Imatinib mesylate therapy was still continued although there was a slight side effect as athralgia. The patient's white blood count increased in every reducing dose. The peripheral blood examination was performed routinely every week at Sardjito Hospital to monitor the patient condition. The results and morphology was in normal range (Figure 4) with WBC range between 9–11 x109/L. When involved in this study, patient was still in therapy and in good condition.

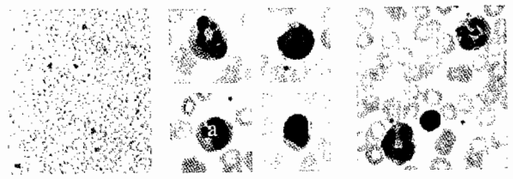

Figure 4. Peripheral blood morphology of case 2 after imatinib mesylate therapy (Wright staining, 10x and 100x objective magnification). Note: a= segmented neutrophyl, b=lymphocyte, c=stab neutrophyl

b. Molecular examination

Fusion gene examination revealed positive result for bcr-abl fusion gene with the amplification product size about 400-500 bp, longer than the positive control that has length 371 bp (Figure 3).

In the second case, classical CML diagnosis was supported by leukocytosis with increasing percentage all myeloid cell spectra in peripheral and bone marrow, the existence of basophylia, eosinophylia and Philadelphia chromosome.

Hydroxyurea is a chemical agent that can inhibit DNA synthesis in cell by inhibiting ribonucleotide reductase enzyme then disturbing deoxyribonucleotide formation and dNTP pool in cell (Adams and Lindsay, 1967; Bianci, et al., 1986). Hydroxyurea therapy is a recommended therapy for CML and other myeloproliferative disorder (Cortelazo, et al., 1995; Silver, et al., 1999) and known to give better short term survival than other therapy such as busulfan (Silver, et al., 1999), bone marrow transplantation (Gale, et al., 1998), or combination therapy with interferon. (The Benelux CML Study Group, 1998). But, for long term survival, bone marrow transplantation is more effective especially in youth group (Gale, et al., 1998; Silver, et al., 1999).

In this patient, hydroxyurea is chosen as early therapy to reduce the leukemia cell proliferation rate, as preparation for bone marrow transplantation and gave good response as shown by complete

hematological remission (CHR). But, the lack of bone marrow transplantation donor, the therapy is switched to imatinib mesylate.

Imatinib mesylate is a 2-phenylaminopirimidine derivate, with brand name Gleevec (STI571), has specific inhibition effect to fusion protein that encoded by bcr-abl gene (Savage and Antman, 2002; Holtz, et al., 2002). Imatinib mesylate has been proven as leukemia cell proliferation inhibitor for progenitor cells (Holtz, et al., 2002), for newly diagnosed CML patient (Kantarijan, et al., 2003), for chronic phase, accelerated or blast crisis during 2nd phase clinical trial (Braziel, et al., 2002; Talpaz, et al., 2002) and for CML with secondary chromosomal abnormality (Mohamed, et al., 2003) but with different percentage successfulness of hematological remission, cytogenetic and survival or relapse rate also intolerance side effect (Druker, et al., 2001).

The problem found in this patient is persistence of bcr-abl fusion gene after hydroxyurea and bcr-abl inhibitor therapy at RT-PCR checking, although hematologically the patient reached complete hematological remission. This bcr-abl persistence indicated that leukemia clone in this patient can not be eradicated by bcr-abl inhibitor and resulted resistance to imatinib mesylate.

Several investigators have reported imatinib mesylate resistance and the mechanism are predicted as multifactorial. The following resistance mechanism has been reported: 1) bcr-abl gene amplification, 2) point mutation in ATP-binding pocket in ABL kinase domain like mutation at position 315 that change tyrosine to leucine, at position 1127 that change glycine to lysine (Hofmann, et al., 2002) and several other mutation (Barthe, 2001; Branford, et al., 2002) that change the conformation of bcr-abl and prevent imatinib binding to bcr-abl protein, 3) increasing of AGP (alpha-glycoprotein) plasma concentration that binded with imatinib and blocked imatinib capability to inhibit kinase activity of BCR/ABL (Marcuci, 2003).

Several new chemical agents include in piridopirimidin (PD180970 and PD166326) are investigating and developing to replace imatinib therapy in resistance cases (Rosee, et al., 2002; Huron, et al., 2003).

In the case, longer bcr-abl product (around 400-500bp; positive control 371 bp) has been detected (Figure 3). It means there were several base additions to patient bcr-abl gene. The addition might be caused by gene amplification with very short distance or only the part of gene meanwhile point mutation does not cause length changing but might change coded protein conformation especially if the point mutation change the ABL-binding pocket sequence. To determine the exact changing in this bcr-abl product, a further investigation and sequencing is needed.

The patient prognosis can not be determined but routine monitoring and Gleevec therapy are continuing.

Conclusion

We reported CML cases classified as atypical (case 1) and typical (case2) type based on FAB classification with post therapy for the second CML. At molecular level, bcr-abl fusion gene found at the second case with longer product than positive control.

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References

Adams, R.L., and Lindsay, J.G. (1967) Hydroxyurea Reversal of Inhibition and Use as a Cell-synchronizing Agent. *The Journal of Biological Chemistry*. 242:1314-1317.

Bain, B.J. (2003) *Leukemia Diagnosis: A Guide to the FAB Classification*. Third Edition Gower Medical Publishing

Barthe, C., Cony-Makhoul, P., Melo, J.V., Rosley, J., Mahon.F-X. (2001) Roots of Cinical Resistance to STI-571 Cancer Therapy. *Science*. 293:2163a

Bianchi, V., Pontis, E., Reichard, P. (1986) Changes in Deoxyribonucleoside Triphosphate Pools Induced by Hydroxyurea and Their Relation to DNA Synthesis. *The Journal of Biological Chemistry*. 261:16037-16042.

Branford, S., Rudzki, Z., Walsh, S., Grigg, A., Arthur, C., Taylor, K., Hermann, R., Lynch, K.P., and Hughes, T.P. (2002) High Frequency of Point Mutations Clustered Within The Adenosine-Triphosphate-binding region of BCR/ABL in Patients With Chronic Myeloid Leukemia or Ph-positive Acute Lymphoblastic Leukemia Who Develop Imatinib (STI571) Resistance. *Blood*, 99:3472-3475.

Braziel, R.M., Launder, T.M., Druker, B.J., Olson, S.B., Magenis, R.E., Mauro, M.J., Sawyer, C.L., Paquette, R.L., and O'Dwyer, M.E. (2002) Hematopathologic and Cytogenetic Findings in Imatinib Mesylate-treated Chronic Myelogenous Leukemia Patients:14 Months' Experience. *Blood* 100:435-441.

Cortelazzo, S., Finazzi, G., Ruggeri, M., Vestri, O., Galij, M., Rodeghiero, F., and Barbui, T. (1995) Hydroxyurea For Patients With Essential Thrombocythemia and a High Risk of Thrombosis. *N Engl J Med* 332:1132-6.

Druker, B.J., Sawyer, C.L., Capdeville, R., Ford, J.M., Baccarani, M., Goldman, J.M. (2001) Chronic Myelogenous Leukemia *Hematology 2001*:86-112.

Druker, B.J., Talpaz, M., Resta, D.J., Bin Peng, Buchdunger, E., Ford, J.M., Lydon, N.B., Kantarijan, H., Capdeville, R., Ohno-Jones, S., and Sawyers, C.L. (2001) Efficacy and Safety of a Specific Inhibitor of the BCR-ABL Tyrosine Kinase in Chronic Myeloid Leukemia. *N Engl J Med*, 344:1031-1037.

Gale, R.P., Hehlmann, R., Zhang, M-J., Hasford, J., Goldman, J.M., Heimpel, H., Hochhaus, A., Klein, J.P., Kolb, H-J., McGlave, P.B., Passweg, J.R., Rowlings, P.A., Sobocinski, K.A., Horowitz, M.M., and German CML Study Group. (1998) Survival With Bone Marrow Transplantation Versus Hydroxyurea or Interferon for Chronic Myelogenous Leukemia. *Blood*, 91:1810-1819

Hofmann, W-K., Jones, L.C., Lemp, N.A., de Vos, S., Gschaldmeier, H., Hoelzer, D., Ottman, O.G., Koeffler, H.P. (2002) Ph^+ Acute Lymphoblastic Leukemia Resistant to the Tyrosine Kinase Inhibitor STI571 Has a Unique BCR-ABL Gene Mutation. *Blood*, 99:1860-1862.

Holtz, M.S., Slovak, M.L., Zhang, F., Sawyers, C.L., Forman, S.J., and Bhatia, R. (2002) Imatinib Mesylate (STI571) Inhibits Growth of Primitive Malignant Progenitor in Chronic Myelogenous Leukemia Through Reversal of Abnormally Increased Proliferation. *Blood*, 99:3792-3800.

Huron, D.R., Gorre, M.E., Kraker, A.J., Sawyer, C.L., Rosen, N., and Moasser, M.M. (2003) A Novel Pyridopyrimidine Inhibitor of Abl Kinase Is a Picomolar Inhibitor of Bcr-abl-driven K562 Cells and Is Effective against ST571-resistant Bcr-abl Mutants[1]. *Clinical Cancer Research*, 9:1267-1273.

Imbert, M., Vardiman, J.W., Bain, B., Brunning, R.D., Pierre, R., Flandrin, G. (2001) Chronic Neutrophilic Leukemia dalam Jaffe, E.S., Harris, N.L., Stein, H., and Vardiman J.W. *World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Haematopoetic and Lymphoid Tissues*, IARC Press, Lyon

Jaffe, E.S., Harris, N.L., Stein, H., and Vardiman J.W. (2001) *World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Haematopoetic and Lymphoid Tissues*, IARC Press, Lyon

Jandl, J.H. (1996) *Blood:Textbook of Hematology*. Second edition. Little Brown.

Kantarijan, H.M., Cortes, J.E., O'Brien, S., Giles, F., Garcia-Manero, G., Faderl, S., Thomas, D., Jeha, S., Rios, M.B., Letvak, L., Bochinski, K., Arlinghaus, R., and Talpaz M., (2003) Imatinib Mesylate Therapy in Newly Diagnosed Patients with Philadelphia Chromosome-positive Chronic Myelogenous Leukemia: High Incidence of Early Complete and Major Cytogenetic Responses. *Blood*, 101:97-100.

Kjeldsberg, C., Foucar, K., McKenna, R. (1995) *Practical Diagnosis of Hematologic Disorders*. Second edition. ASCP Press.

Kurzrock, R., Bueso-Ramos, C.E., Kantarijan, H., Freireich, E., Tucker, S.L., Siciliano, M., Pilat, S., and Talpaz M. (2001) BCR Rearrangement-Negative Chronic Myelogenous Leukemia Revisited. *Journal of Clinical Oncology*, 19:2915-2926.

Lowenberg, B., Downing, J.R., Burnett, A. (1999) Acute Myeloid Leukemia, *N Engl J Med*, 341:1051-1062.

Marcucci, G. Perrotti, D., and Caligiuri M.A. (2003) Understanding the Molecular Basis of Imatinib Mesylate Therapy in Chronic Myelogenous Leukemia and the Related Mechanisms of Resistance[1]. *Clinical Cancer Research*, 9:1248-1252.

Mohamed, A.N. Pemberton, P. Zonder, J. and Schiffer C.A. (2003) The Effect of Imatinib Mesylate on Patients with Philadelphia Chromosome-positive Chronic Myeloid Leukemia with Secondary Chromosomal Aberrations. *Clinical Cancer Research* 9:1333-1337.

Rosee, P.L., Corbin, A.S., Stoffregen, E.P., Deininger, M.W., and Druker B.J. (2002) Activity of the Bcr-Abl Kinase Inhibitor PD180970 against Cancer Relevant Bcr-Abl Isoforms That Cause Resistance to Imatinib Mesylate (Gleevec, ST571)[1]. *Cancer Research* 62:7149-7153.

Savage, D.G., and Antman, K.H. (2002) Imatinib Mesylate – A New Oral Targeted Therapy. *N Engl J Med*, 346:683-693.

Silver, R.T., Woolf, S.H., Hehlmann, R., Appelbaum, F.R., Anderson, J., Bennet, C., Goldman, J.M., Guilhot, F., Kantarijan, H.M., Lichtin, A.E., Talpaz, M., Tura, S. (1999) An Evidence-Based of the Effect of Busulfan, Hydroxyurea, Interferon, and Allogeneic Bone Marrow Transplantation in Treating the Chronic Phase of Chronic Myeloid Leukemia: Developed for the American Society of Hematology. *Blood*, 94:1517-1536.

Serrano, J., Jose Roman, Joaquin Sanchez, Antonio Jimenez, Juan, A. Castillejo, Concepcion Herrera, Maria Gracia Gonzalez, Luisa Reina, Maria del Carmen Rodriguez, Miguel A. Alvarez, Juan Maldonado, and Antonio Torres. (2000) Molecular analysis of lineage-specific chimerism and minimal residual disease by RT-PCR of p210BCR-ABL and p190BCR-ABL after allogeneic bone marrow transplantation for chronic myeloid leukemia: increasing mixed myeloid chimerism and p190BCR-ABL detection precede cytogenetic relapse. *Blood*, 95:2659-2665.

Talpaz, M., Silver, R.T., Druker, B.J., Goldman, J.M., Gambacorti-Passerini, C., Guilhot, F., Schiffer, C.A., Fischer, T., Deininger, M.W.N., Lennard, A.L., Hochhaus, A., Ottmann, O.G., Gratwohl, A., Baccarani, M., Stone, R., Tura, S., Mahon, F-X., Fernandez-Reese, S., Gathmann, I., Capdeville, R., Kantarijan, H.M., and Sawyer C.L. (2002) Imatinib Induces Durable Hematologic and Cytogenetic Responses in Patients with Accelerated Phase Chronic Myeloid Leukemia: Results of a Phase 2 Study. *Blood* 99:1928-1937.

I J Biotech., June (2004), 681-720

The Benelux CML Study Group (1998) Randomized Study on Hydroxyurea Alone Versus Hydroxyurea Combined With Low-Dose Interferon-a2b for Chronic Myeloid Leukemia. Blood, 91:2713-2721.

Vardiman, J.W., Imbert, M., Pierre, R., Brunning, R.D., Thiele, J., Flandrin, G. (2001) Chronic Myelogenous Leukemia dalam Jaffe, E.S., Harris, N.L., Stein, H., and Vardiman J.W. *World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Haematopoetic and Lymphoid Tissues*, IARC Press, Lyon

Vardiman, J.W., Imbert, M., Pierre, R., Brunning, R.D., Bain, B., Flandrin, G., Bennett, J.M. (2001) Chronic Myelomonocytic Leukemia dalam Jaffe, E.S., Harris, N.L., Stein, H., and Vardiman, J.W., *World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Haematopoetic and Lymphoid Tissues*. IARC Press. Lyon.

Weaver, R.F. (1999) *Molecular Biology*. WCB McGraw-Hill, 80-82.

World Health Organization. (2001) GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence World Wide. IARC Press. Lyon.