

## Fusion gene bcr-abl : from etiopathogenesis to the management of Chronic Myeloid Leukemia

Tri Agusti Sholikah\*<sup>1</sup>

<sup>1</sup>Histology Departement, Medical Faculty of Sebelas Maret University

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### ABSTRACT

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##### \*Corresponding author:

agusti3.dr@gmail.com

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Chronic Myeloid Leukemia (CML) is a myeloproliferative neoplasm. CML is relative frequent disorder. Most of CML patients have Philadelphia chromosome (Ph), which is derived from a reciprocal translocation between chromosome 9 and 22, t(9;22)(q34;q11), generating the BCR-ABL fusion gene. In general, there are 3 breakpoint cluster regions in BCR gene : mayor (M-bcr), minor (m-bcr) and micro ( $\mu$ -bcr). The BCR-ABL gene encodes proteins that vary in size depending on the breakpoint in the BCR gene. However, these proteins share a high tyrosine kinase activity. In the absence of activating stimuli, BCR-ABL tyrosine kinase able to transfer phosphate from ATP (autophosphorilation) to tyrosine residues on various substrates in the cell. It activates intracellular signaling pathways. These pathways cause increase proliferation or decrease apoptosis and differentiation of a hematopoietic stem cell; and defect in adherence of myeloid progenitors to marrow stroma resulting in CML. These discoveries determined that BCR-ABL fusion gene is critical event in etiopathogenesis of CML and a ideal target for therapy. Therapy of CML patients with BCR-ABL fusion gene-positif is by block autophosphorilation mechanism by Tyrosine Kinase Inhibitor (TKI), example imatinib. Molecular method to detect BCR-ABL transcript is necessary for monitoring response to TKI in CML pateints.

*Chronic Myeloid Leukemia (CML) merupakan keganasan myeloproliferatif yang relatif sering ditemukan. Sebagian besar pasien CML mempunyai kromosom Philadelphia, yaitu kromosom yang berasal dari hasil translokasi resiprokal antara kromosom 9 dan 22, t(9;22)(q34;q11) sehingga membentuk gen fusi BCR-ABL. Secara umum, terdapat 3 tipe breakpoint cluster region gen BCR-ABL yaitu mayor (M-bcr), minor (m-bcr) dan mikro ( $\mu$ -bcr). Gen fusi BCR-ABL mengkode protein dengan ukuran yang berbeda-beda tergantung tipe breakpointnya. Namun, semua protein tersebut mempunyai aktivitas tirosin kinase yang tinggi. Aktivitas tirosin kinase BCR-ABL dapat mengambil gugus fosfat dari ATP meskipun pada kondisi tidak ada stimulus (autofosforilasi). Gugus fosfat selanjutnya ditransfer ke residu tirosin berbagai substrat di dalam sel sehingga berbagai jalur sinyal intraseluler diaktifkan. Pengaktifan sinyal tersebut menyebabkan meningkatnya proliferasi, menurunnya apoptosis dan deferensiasi sel stem hematopoetik serta menurunnya perlekatan sel myeloid pada stroma sumsum tulang sehingga terjadi CML. Dengan diketahuinya mekanisme tersebut, dapat ditetapkan bahwa gen fusi BCR-ABL berperan penting dalam etiopatogenesis sekaligus dapat digunakan sebagai target yang ideal untuk terapi CML. Terapi tersebut adalah dengan cara menghambat autofosforilasi oleh suatu molekul yang disebut sebagai tyrosine kinase inhibitor (TKI), misalnya imatinib. Pemeriksaan molekuler untuk mendeteksi gen BCR-ABL diperlukan untuk*

*memonitor keberhasilan terapi menggunakan TKI tersebut.*

## INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by an increase of myeloid cells.<sup>1</sup> CML was first discovered in 1845 by Dr Rudolf Virchow and Dr John Hughes Bennett in France.<sup>2-4</sup>

Based on its history, the treatment of CML had changed a few times.<sup>5</sup> In the 1800s, the main treatment for CML was using Fowler fluid. This fluid was developed by Dr. Thomas Fowler in mid 1700s. Potassium arsenite was the active ingredient of Fowler fluid. In the 1900s, the treatment of CML switched into radiotherapy; chemotherapy like busulphan, hidroxyurea, and interferon  $\alpha$  (IFN  $\alpha$ ); as well as bone marrow transplant.<sup>3</sup> Busulphan and hidroxyurea could control the amount of white blood cells for a few years. However, busulphan could cause fibrosis and hidroxyurea has a lot of side effects like nausea, stomatitis and rash. Both of this medicine could not slow down the progression of the disease. IFN  $\alpha$  could preserve stable state of CML patients longer than busulphan and hidroxyurea. However, this medicine is quiet expensive. In its administration, injection is done 3 times a week, and it has side effects like depression and extreme exhaustion. These chemotherapy medicine are only paliative treatment.<sup>6</sup> Bone marrow transplantation is the curative treatment, but its mortality and morbidity is quiet high. In 1960-1990, BCR-ABL fusion gene was known to have important etiopathogenesis role in CML and could be use as treatment target.<sup>3</sup>

## CHRONIC MYELOID LEUKEMIA

Chronic myeloid leukemia (CML) is the most common haematology neoplasm.<sup>7</sup> In Asia, the incidence of CML is 0,4-1 per 100.000 population per year. This number is lower than Western country which is 1-1,5 per 100.000 population per year. In Asia, the average age of patients first diagnosed with CML is approximately 45 years old. While in the Western country it is 20 years older, which is 65 years old. CML is found

more in male patients compare to female.<sup>8</sup> The occurrence of CML is not affected by hereditary factor.<sup>9</sup>

The symptoms of CML, just like other neoplasms, include : fatigue, lethargy, profuse sweating at night, decreased body weight, fever, abdominal pain, easy satiety, and bone-joint pain.<sup>1,9,10</sup> On CBC (Complete blood count), a very high increase of white blood cell (leukosytosis) will be found, even until  $100 \times 10^9/L$  or  $1000 \times 10^9/L$ . Decrease of red blood cells (erythropenia) and trombocyte (trombocytopenia) are often found, or increase of trombocyte (trombocytosis) might also be found. On blood smear, immature myeloid cells, such as myeloblast, would be found. Bone marrow biopsy would show hypercellularity.<sup>9,10</sup>

Based on its pathogenesis, CML is divided into three phases, which are chronic phase, acceleration phase, and blastic phase or crisis phase. More than 90% CML patients are diagnosed in chronic phase. During this phase, patients usually have no complains or asymptomatic. Thus, patients are often diagnosed accidentally, usually when they undergo blood laboratorium testing for other purposes.<sup>1</sup> Chronic phase is characterized by leukocytosis and the amount of myeloblast cells 1-15%. If patients are not treated properly during chronic phase, then the disease would progress into acceleration phase.<sup>11</sup>

Acceleration phase usually last for 6 to 9 months.<sup>11</sup> In this phase, patients are usually starting to show symptoms like profuse sweating at night, unexplainable fever, and bone-joint pain.<sup>11,12</sup> Hepatomegaly and splenomegaly might also be found.<sup>11</sup> The diagnosis of CML in acceleration phase could be made if there are one of these criteria : (1) blast cell is 15-29%, (2) peripheral blood basophil is  $> 20\%$ , (3) persistent thrombocytopenia ( $< 100.000/\mu l$ ) that is not related to therapy, (4) persistent thrombocytosis ( $> 1.000.000/\mu l$ ) even after adequate treatment, (5) increase of white blood cell count or enlarging spleen even after adequate treatment, (6) additional chromosome abnormality.<sup>11-13</sup>

Blastic phase or blastic crisis is a fatal acute

phase.<sup>3</sup> This phase last for approximately 3-6 months. It is characterized by severe anemia, haemorrhage, severe infection, and lymphadenopathy.<sup>11</sup> The diagnosis of blastic phase could be made if there are one of these criteria : (1) blast cells is >30%, (2) extramedullary blast cells proliferation, (3) the prescence of blast cell focus in bone marrow biopsy.<sup>11,12</sup>

## ETIOLOGY

In 1960, Peter Nowell and his apprentice, David Hungerford, found an abnormal

chromosome in the leukocyte of CML patients. Later, it was found that the chromosome was chromosome 22 and was called Philadelphia (Ph) chromosome because it was first found in Philadelphia.<sup>14</sup> In 1973, Dr Janet Rowley described that Philadelphia chromosome originated from reciprocal translocation between chromosome 9 and 22, t(9;22)(q34;q11).<sup>3</sup> This translocation, merge the 3' part of ABL gene inside chromosome 9 with the 5' part of BCR gene inside chromosome 22, forming fussion BCR-ABL gene (Figure 1).<sup>3,9</sup>

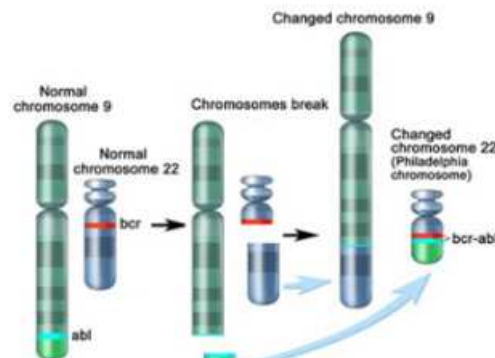


Figure 1. Philadelphia Chromosome.<sup>15</sup>

Further research showed that BCR-ABL fusion gene was found in more than 95% CML patients.<sup>13</sup> This gene is considered as the cause of CML. It is supported by a few other research, such as the culture of CD34 cells that expressed BCR/ABL and mouse cells that carry BCR-ABL induced retroviral then developed CML.<sup>16</sup>

The cause of chromosomal translocation that produce BCR-ABL fusion gene is unknown, but ionization radiation, in radiotherapy for cancer patients and atomic bomb explosion, is considered a risk factor of CML. This is because the incidence of CML is increased in the population exposed to these radiation.<sup>9,17</sup> This could be seen in the event of atomic bomb explosion in Japan.<sup>12,17,18</sup> Ten years after this event, the incidence of CML was increased 50 times in the population exposed to the explosion compare to population unexposed to the explosion, and children less than 15 years old suffered from CML

faster than adults > 30years old.<sup>18</sup> The risk of CML is also increased with aging. The incidence of CML is not affected by cigarette smoking, diet, or infection.<sup>9</sup>

## BREAKPOINT TYPE

BCR-ABL fusion gene has various transcription types, depend on the breakpoint position of BCR gene that merge with ABL gene.<sup>19</sup> Generally, BCR-ABL fusion gene is classified into 3 breakpoint cluster region of BCR gene, which are major (M-bcr), minor (m-bcr), and micro ( $\mu$ -bcr). Major breakpoint has 2 subtypes, which are b2a2 (e13a2) and b3a2 (e14a2). Type b2a2 (e13a2) is formed if BCR gene broke at exon 13 (b2) and its fragment translocate with ABL gene at exon 2 (a2). Breakpoint b3a2 (e14a2) is a combination of BCR gene fragment in which the breakpoint located at 14 exon (also called b3) with the fragment of ABL gene that broke

at exon 2 (a2). Minor breakpoint would form e1a2 type which is a combination of BCR gene fragment that broke at exon 1 with the ABL gene fragment that broke at exon 2 (a2). Micro breakpoint would form e19a2 type (c3a2). It is formed when the BCR gene fragment that broke at

exon 19 translocate with ABL gene fragment that broke at exon 2 (a2) (Figure 2).<sup>19,20</sup> In addition to that, there are a few other less common types, such as e1a3, e13a3 (b2a3), e12a1(b1a1), e8a2, e6a2.<sup>19,21,22</sup>

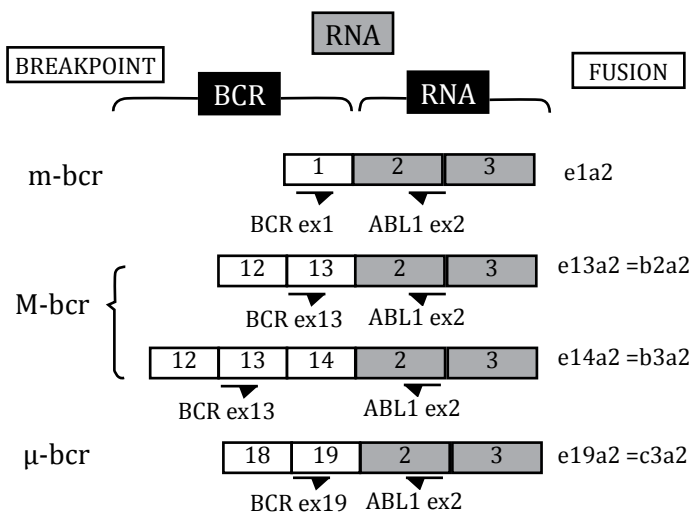


Figure 2. Transcription type structures of BCR-ABL.<sup>23</sup>

Three main types of breakpoint (major, minor and micro) code different sizes of protein, but all three have high tyrosin kinase activity. Major breakpoint type code protein 210-kDa (p210), minor type code protein 190-kDa (p190), and micro breakpoint type code protein 230-kDa (p230). A lot of research in various countries showed that 95% CML patients who tested positive for BCR-ABL gene, has major breakpoint type (M-bcr). For instance, a research in Korea reported that 98,18% of all CML patients who became the subjects of the research, had major breakpoint type, while other type was only found less than 1%.<sup>19</sup>

Types of breakpoint is related to the clinical manifestation of CML patients.<sup>24</sup> Patients with major breakpoint type is characterized with neoplastic expansion in myeloid and megacaryocytic, as well as differentiation failure and moderate degree of maturation on granulocyte progenitor. Patients who carry minor type BCR-ABL gene showed monocytosis. Micro breakpoint type is characterized by high number of neutrophils, low immature myeloid cell count,

and minimal splenomegaly. Patients with this type of CML showed a more mild symptoms compare to other types.<sup>20</sup>

### MOLECULAR PATHOGENESIS

Some domains of BCR-ABL gene that contribute to the occurrence of CML have been identified. ABL protein domains include SH3 domain, Src2-homology (SH2) domain, tyrosin kinase domain, DNA-binding domain, and actin-binding domain (Figure 2). BCR protein domains include oligomerization domain and phosphoserin/treonin rich SH2 binding domain.<sup>25</sup> Tyrosin kinase ABL domain contributes in the activity of kinase which worked by transferring phosphate group of ATP to tyrosin residue of various protein substrates, thus activating various intracellular pathway. This activity is strictly regulated intra cells. If there are no stimuli, then the activity of ABL gene kinase would not occur.<sup>16,26</sup> This is due the presence of autoinhibition mechanism by myristoyl group located at Ncap ABL region. Myristoyl are able to pull the C lobe of kinase domain, thus binding the SH2 and SH3 to kinase

domain. Therefore, deletion or mutation of Ncap, SH2 domain, and or SH3 domain would increase kinase activity.<sup>16,26</sup>

In BCR-ABL fusion gene, ABL Ncap region did not join BCR gene (Figure 3), thus autoinhibition mechanism did not occur. The merging of BCR

with ABL would cause autophosphorilation in BCR-ABL kinase protein, causing an increase of kinase protein activity.<sup>26</sup> This event would affect various protein and adaptor molecule inside cells to bind into BCR-ABL protein and activating various intracellular signaling pathway<sup>3</sup>.

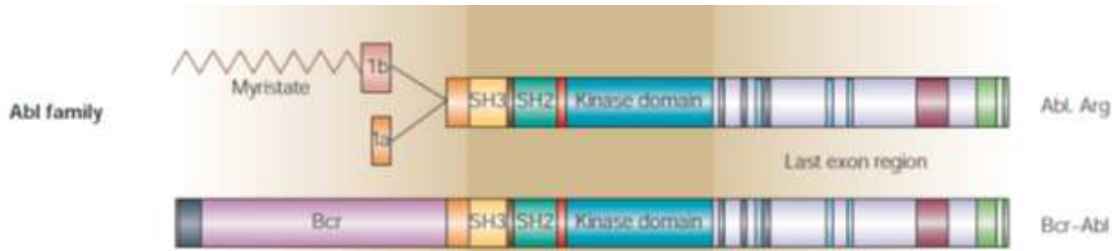


Figure 3. ABL1 domain and BCR-ABL fusion gene.<sup>27</sup>

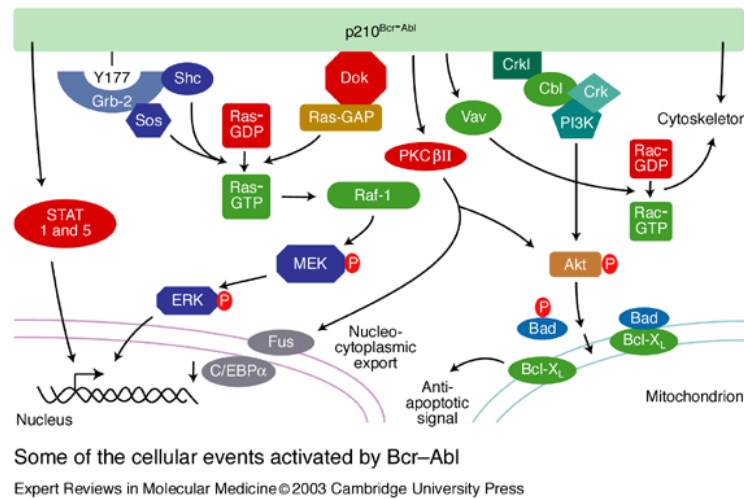


Figure 4. Some of the cellular events activated by BCR-ABL.<sup>28</sup>

Intracellular signaling pathway activated by BCR-ABL include RAS, PI3K, and STAT pathway.<sup>25,28</sup> Ras pathway would activate when BCR-ABL interact with adaptor protein like Grb2, Shc, Sos and Dok. Activated Ras would bind GTP. Ras-GTP would then activate Raf-1 and Raf-1 would activate MAP kinase (MEK). MEK activates extracellular-signal-regulated kinase (ERK) which then initiate gene transcription that contributes in proliferation (Figure 4).<sup>3,17,28</sup>

The interaction of BCR-ABL protein with Crkl adaptor protein would cause the activation of PI3K (phosphoinositide 3-kinase) pathway.

Activated PI3K would phosphorylate Bad, causing Bad to detach from BCL-XL. Free BCL-XL prevents the release of c-cytochrom from mitochondria and preventing apoptosis (Figure 4).<sup>11,17</sup>

Phosphorilation of Signal Transducer and Activator Transcription (STAT) 1 and 5 by BCR-ABL causing these protein to activate. This STATs activation would activate gene transcription that contributes in cell growth, for instance CBL-XL, cyclin D1 and D2 (Figure 4).<sup>17</sup>

In addition to the activation of these three pathway, BCR-ABL could also interact with kinase

C  $\beta$  II (PKC $\beta$ II) protein. The activation of this protein would inhibit the degradation of Fus protein. Fus contributes in decreasing the CCAAT/enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ) expression which is a transcription factor for myeloid cell differentiation.<sup>17</sup> Therefore, this activation would inhibit myeloid cells differentiation.<sup>11,17,28</sup> In addition to that, the presence of Hes1 protein could suppress CEBP $\alpha$  transcription factor.<sup>29</sup> Previous research from Nakahara et al. (2010) reported that CML patients in blastic crisis phase have high Hes 1 expression, different from the other phases. So that protein Hes1 upregulation become one of the causes of blastic crisis in CML.

Protein that took part in cytoskeleton organization, like actin, paxillin, talin, vinculin and Focal Adhesion Kinase (FAK) also could interact with BCR-ABL (Figure 4). This would alter adhesion and the function of cytoskeleton, thus causing the detachment of immature blood cells from bone marrow into blood stream<sup>17</sup>.

Activation of various intracellular signaling pathway would increase proliferation, decrease apoptosis, differentiation and attachment of

myeloid cells in the stroma of bone marrows, hence causing CML.<sup>3,11</sup> All these activation are dependant on the activity of tirosin kinase BCR-ABL, therefore BCR-ABL could be use as an ideal target for treatment.<sup>3</sup>

### TREATMENT WITH TKI

Tirosin kinase BCR-ABL works by taking the phosphate groups from ATP (autophosphorilation) that would be transfered to tirosin residue of various cell substrates and causing the activation of intracellular signaling pathway.<sup>3,26</sup> Therefore, tretment in which BCR-ABL is the target, wouldd contribute in inhibiting the activity of tirosin kinase, so-called Tyrosine Kinase Inhibitor (TKI). An instance of TKI is imatinib. This medicine works by inhibiting the binding of ATP on BCR-ABL tirosin kinase, therefore also inhibits phosphorilation mechanism (Figure 5). TKI is a curative treatment option that has less risk compare to other treatment options (bone marrow transplant), that is why TKI is currently preferred.<sup>3</sup>

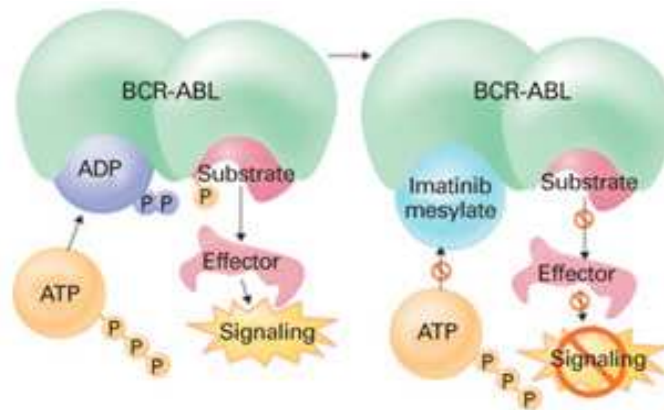


Figure 5. Action mechanism of imatinib.<sup>30</sup>

In vitro, imatinib suppressed the proliferation of cells that expressed BCR-ABL gene selectively. On colony forming-assay which uses CML patients cells, showed that imatinib could decrease the positive colony of BCR-ABL 92-98% and inhibits the formation of normal colony minimally. On animal model experiment, imatinib could inhibit BCR-ABL positive cells, depending on selective dosage. Clinical trial that uses IFN $\alpha$  intolerant or resistant chronic phase

CML patients, blastic crisis phase patients, and ALL Ph<sup>+</sup> patients, showed that imatinib could be well-tolerated. The most common side effects are nausea, periorbital oedem, and muscle cramp.<sup>3</sup> Imatinib is metabolized by the liver with half life 13 to 16 hours, so that it could be given only once daily.<sup>3,31</sup>

Most chronic phase CML patients, respond well to imatinib treatment, however some patients showed resistance causing relaps or

development to acceleration phase or even blastic crisis phase.<sup>3</sup> This resistance is caused by drug efflux mechanism, so that imatinib could not reach its target or mutation on BCR-ABL kinase domain occur and causing the target to be insensitive.<sup>3,32</sup> To resolve this resistance, new medicines which are second generation tyrosin kinase inhibitor, are developed, dasatinib and nilotinib.<sup>3,33</sup> In vitro, dasatinib has 350 times more potential compare to imatinib, while nilotinib 50 times. Research showed that patients who received dasatinib 100 mg or nilotinib 300 mg, has faster treatment response compare to patients who received imatinib 400 mg.<sup>34</sup>

### TKI TREATMENT MONITORING

The therapeutic response of CML patients to TKI is done by evaluating their clinical conditions in addition to another 3 types of monitoring which are hematology test, cytogenetic test, and molecular test.<sup>35</sup> Hematology test is done by evaluating the number of blood cells (blood count) and blood smear to determine the type of white blood cells differentiation. If the number of patients blood cells are normal (leukocyte  $<10 \times 10^9$  /L, trombocyte  $<450 \times 10^9$ /L) and no immature granulocyte cells are found, then patients are considered as complete hematologic response (CHR).<sup>31</sup> In CHR, CML could not be detected through hematology test, but could still be detected through cytogenetic and molecular test. Cytogenetic test is done to determine

the number of cells carrying Philadelphia chromosome. Cytogenetic test would be done with conventional cytogenetics which is karyotyping, or a more advance cytogenetic which is Fluorescence In Situ Hybridization (FISH).<sup>11</sup> If the number of cells carrying Philadelphia (Ph<sup>+</sup>) chromosome is between 66-95%, then patients are considered as minimal cytogenetic response. If the number of cells Ph<sup>+</sup> is between 36-65%, then patients are considered as minor cytogenetic response, and if the number is between 1-35% then patients are considered as partial cytogenetic response (PCyR). If no Ph<sup>+</sup> cells are found, then patients are considered as complete cytogenetic response (CCyR). PCyR and CCyR are called major cytogenetic response (MCyR). In CCyR, leukemia cells could not be detected through cytogenetic test but could be detected through molecular test. Molecular test is done to detect the number of BCR-ABL transcript. If the number of BCR-ABL transcript  $<0,1\%$  then patients are considered as major molecular response (MMR) and if the number of BCR-ABL transcripts could no longer be detected then patients are considered as complete molecular response (CMR) (Figure 6).<sup>11,31</sup> Molecular test could be done by quantitative real-time PCR (RQ-PCR).<sup>36</sup> It is important to gather information about treatment success and patients prognosis so that optimal therapeutic course could be determined.<sup>11,13</sup>

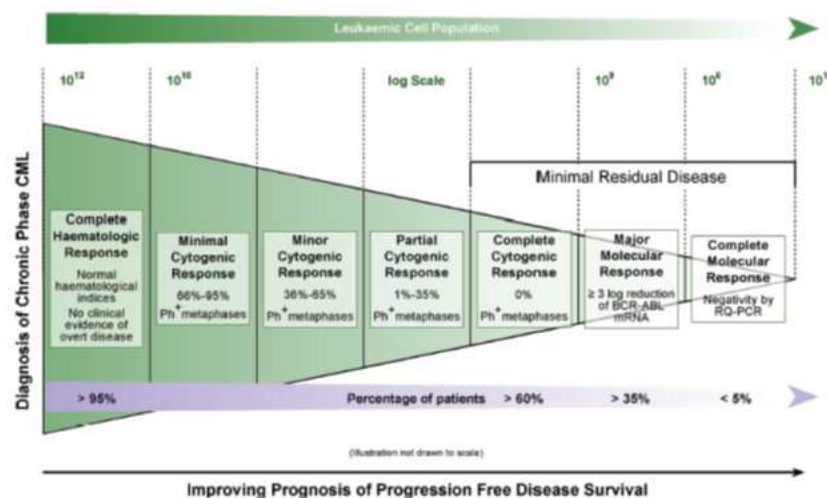


Figure 6. Therapeutic Response of CML patients.<sup>11</sup>

## CONCLUSION

Most CML cases are caused by the presence of BCR-ABL fusion gene. This gene increases the activity of various hematopoietic stem cells intracellular pathway, thus causing an increase of myeloid cell proliferation, and in the other hand, the apoptosis and differentiation process into mature cells are decreased. The impact is an increase of number of myeloid cells in the bone marrow and blood circulation, thus causing CML. Currently, the treatment of CML patients who has positive BCR-ABL gene, are mainly using TKI, which is a molecule that could inhibits the activity of BCR-ABL tyrosin kinase. This treatment is a curative treatment that has minimal risks. Hence, a test to evaluate BCR-ABL fusion gene expression in CML patients need to be done not only as diagnostic tool but also to determine treatment options. In addition to that, molecular test to detect the number of BCR-ABL transcript is also important to monitor CML patients response to TKI treatment.

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