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Literature Review

Molecular Pathogenesis and Treatment Strategies of Chronic Myeloid Leukemia (CML)

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Abstract

Chronic Myeloid Leukemia (CML) is a myeloproliferative disease diagnosed in bone marrow, arising from a chromosomal translocation between chromosomes 9 and 22, resulting in the formation of fusion oncogene *BCR–ABL*. The product of this fusion oncogene is a new oncoprotein bcr–abl which possesses abnormal tyrosine kinase activity. In response to this, abnormal signaling pathway activation occurs, leading to cell transformation. *BCR–ABL* oncogene could be targeted by tyrosine kinase inhibitors (TKIs) to delay or inhibit the disease progression. Imatinib is the first drug designed against CML but resistance to this has led to the development of the second- and third generations of inhibitors that are active against many types of *BCR–ABL* gene mutations. However, somehow, due to disease progression, TKIs do not remain as effective. There are three well-characterized phases of CML: The chronic phase (CP), the accelerated phase, and the terminal stage which is the blast crisis (BC) stage. In the CP of CML, mature granulocytes and myeloid precursors become aggregated majorly in the bone marrow and peripheral blood. The accelerated phase is marked by increased disease severity and an increase in progenitor/precursor cell number. In the BC stage, undifferentiated blast cells grow in number. Many patients with CML are diagnosed during the CP of disease, so the survival rate of CML is high. However, 20% of CML patients proceed to advanced stages that result in drug resistance, intolerance, and mortality. So, for proper CML treatment, drugs are needed to target multiple *BCR–ABL* mutations, delay or stop disease progression, and overcome resistance caused by *BCR–ABL* independent mechanisms, especially during advanced phases of CML. Moreover, drugs could be developed to eradicate the stem cells of CML. These targets could be achieved by understanding mechanisms of disease progression, disease relapse, and drug resistance by utilizing high throughput molecular genetics, cell biology and immunology techniques.

Keywords: CML, Philadelphia chromosome, *BCR–ABL* gene, blast crisis, leukemia

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1. Introduction

CML is one type of cancer originating in the bone marrow and manifested in blood cells. During CML, a hematopoietic stem cell tumor, immature granulocytes accumulate in the body. Since these are immature cells, they do not function properly. Due to the excessive progression of hematopoietic cells and their progenitors, the balance between regeneration and differentiation is disrupted, causing leukemia. Out of all the leukemias, 15% of patients have CML, which shows that 2 out of 100,000 individuals are diagnosed with CML yearly. Moreover, 5–10% of those patients are exposed to radiation [1].

At the cytogenetic level, CML happens due to the chromosomal translocation between chromosome no. 9 and 22 t (9;22) that leads to the formation of the Philadelphia chromosome [2]. At the molecular level, this translocation occurs due to the formation of the *BCR–ABL* fusion oncogene. BCR (Breakpoint Cluster Region) gene lies on chromosome 9 while ABL protooncogene resides on chromosome 22, so after translocation, fusion oncogene *BCR–ABL* is produced, which is the hallmark of chronic myeloid leukemia (CML) [3]. *BCR–ABL* oncogene produces bcr–abl protein that possesses deregulated tyrosine kinase activity leading toward activating signaling pathways in cells, ultimately causing leukemia. About 95–99% CML patients have this Philadelphia (Ph) chromosome and *BCR–ABL* gene [4].

2. CML Disease Stages

There have been three well-characterized phases of CML. Depending upon individual patient characteristics, treatment strategies, and efficiency of drugs, CML progresses from the initial to accelerated and blast crisis (BC) stage [5]. The initial phase is the chronic phase (CP), in which mature myeloid cells increase in number, resulting in an elevated white blood cell count. This phase is treatable and may extend up to 25 years while receiving proper treatment. If not properly treated, the CP may proceed to an accelerated stage marked by an increase in immature myeloid cells in peripheral blood and bone marrow. Besides, some new cytogenetic changes could also occur during this phase. The next step in the progression is BC, which is indicated by immature blast cells increasing the production of normal hematopoietic cells. This stage is highly resistant to treatment; death could occur from infection or bleeding complications in addition to the decrease in the number of normal granulocytes and platelets [6]. Multiple genetic changes may be responsible for different phases of the disease. In addition to some

biological, molecular, and chromosomal factors, different splice variants of the *BCR–ABL* gene may be responsible for disease progression, pathogenicity, and even treatment strategies [7].

The mechanism of how CML progresses is still not completely understood [8]. Moreover, biomarkers for the early prediction of disease progression are not available. So, there is a need for the discovery of biomarkers common for the advancement of CML disease so that patients at risk of the disease progression are diagnosed early, and these patients are appropriately managed to avoid or delay the progression of the disease.

3. Pathogenesis of CML

In 1960, Peter Nowell and David Hungerford predicted an anomaly in the chromosome of individuals suffering from CML. That anomaly was the formation of an acrocentric chromosome within the patients, which was then thought of as a deletion in the chromosome. This anomaly was supposed to be the first anomaly associated with specific malignancy. As time passed and the banding pattern of chromosome improved, it was deduced that the anomaly was a shortening of chromosome 22 [9]. Then in 1973, it was described by Dr. Janet Rowley that the anomaly arose when the reciprocal translocation occurred between chromosomes 9 and 22, and the resultant shortened chromosome was called the Philadelphia chromosome. The *ABL* gene lies on the long arm of chromosome 9. In contrast, the *BCR* gene is found on chromosome 22, so after translocation, the *BCR–ABL* gene seems to be located on derivative chromosome 22, which was then called the Philadelphia chromosome [10].

4. CML Etiology

Fusion genes are characteristic of a particular type of leukemia and are referred to as the cause in studies where distinct cell lines were exposed to radiation. *BCR–ABL* gene production is the primary cause that gradually leads toward CML. However, it is unknown how this rearrangement forms. *BCR–ABL* fusion genes were seen through polymerase chain reaction (PCR) in 25–30% of individuals and 5% of children in their bone marrow cells. Through this research, it became evident that the processes that regulate immune responses or other events perform a vital function in the progress of CML. However, CML has no evidence of a genetic or hereditary basis. The *BCR–ABL* gene can be found only in hematopoietic cells or monozygotic twins. However, there

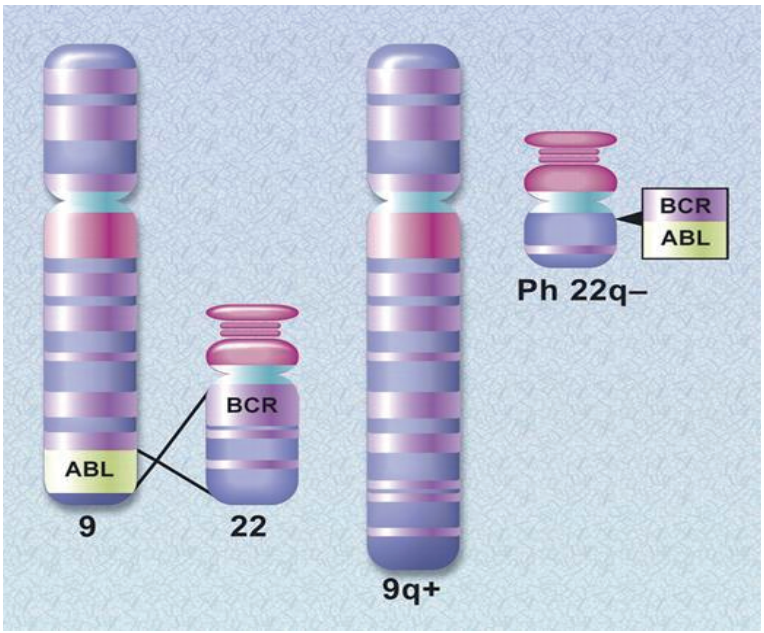

Figure 1: Representation of formation of Philadelphia chromosome after translocation [11].

are limited chances of CML to transfer in families [12]. CML is found to be enhanced in a ratio where there is the exposure of radiation or in the vicinity of the atomic bomb [13].

5. Clinical Manifestations of CML Patients

The most common symptoms of CML are fever, fatigue, enlarged spleen and liver, weight loss, and body aches. For improved quality of life, there should be timely interventions and treatment of patients. About 58–95% of CML patients show splenomegaly initially in disease, 22–65% of patients have fever, hepatomegaly is seen in 20–100% of CML patients, while fatigue is seen in 18–100% of patients [14].

The most common symptom found in the CP of CML is leukocytosis. Approximately 50–70% of patients with CML have a leukocyte ratio of as much as 100×109/L, whereas, in 10–20% of patients, there have been variations in their leukocyte count. Moreover, 30–50% of patients are affected by thrombocytosis, which can elevate to 1000×109/L in rare cases. If a patient's WBC (White Blood Cell) count exceeds 10×109/L even after taking imatinib, it indicates a loss of complete hematological response. The aggregation of platelets in some patients has also been seen as well [15].

6. Treatment for CML

Treatment for CML has been revolutionized by advances in molecular biology, cytogenetics, and microbiology during the last two decades. Earlier, CML was treated by conventional therapies, including Busulphin (BUS), hydroxyurea (HU), and Interferon alpha (IFN-α), sometimes combined with a low dose of cytarabine. In 50–75% of patients, hydroxyurea produced a complete hematological response, but no significant effect on Ph+(Philadelphia-positive) leukemia cells or cytogenetic response has been seen. However, resistance to hydroxyurea or interferon alpha could occur. Allogenic hematopoietic stem cell transplantation (allo-SCT) is considered the essential treatment for CML. Still, it has some associated risks so that it can be provided to 40% of patients. Most of these conventional therapies have significant limitations, so new drugs are designed to target CML cells [16].

6.1. BCR–ABL As a Target for Therapy

The target most used in CML is *BCR–ABL* tyrosine kinase. Because *BCR–ABL* is present in 95% of CML patients, it is considered a significant target in treating CML [17]. *BCR–ABL* is an accelerated tyrosine kinase that has multiple effects on a wide range of signaling pathways [18]. *BCR–ABL* got translated in hematopoietic stem cells to form an oncoprotein, that is, $p210^{BCR/ABL}$,while the normal gene, that is, p145c-ABL, is found in cytoplasm and has the normal activity of tyrosine kinase. This activity of tyrosine kinase plays an important role in transformation process of cell. Moreover, its residence in cytoplasm causes the aggregation of phosphorylated substrates in different multi-protein complexes that cause the production of antiapoptotic and different mitogenic signals. The over activity of tyrosine kinase enzyme delays apoptosis, cell cycle alteration could occur, and cell division regulation is disrupted that leads to leukemia [19].

Multiple signaling pathways like Ras/Raf/mitogen-activated protein kinase [MAPK], phosphatidylinositol 3 kinase, STAT5/Janus kinase, and Myc are regulated by *BCR–ABL* oncogene. The activity of this tyrosine kinase results in uncontrolled proliferation of cells and reduced apoptosis rate that cause the expansion of pluripotent stem cells in bone marrow. The Wnt/ ß-catenin pathway is also involved in the production of BC cells since its activation has been observed in samples of patients with CML. Several transcription factors seem to play a role in CML progression like δEF1, MZF1, and Jun B which is downregulated during progression. The other transcription factors like δEF1

and MZF1 play role in hematopoietic stem cell differentiation and CD34,cMyb expression is being modulated by these transcription factors [20].

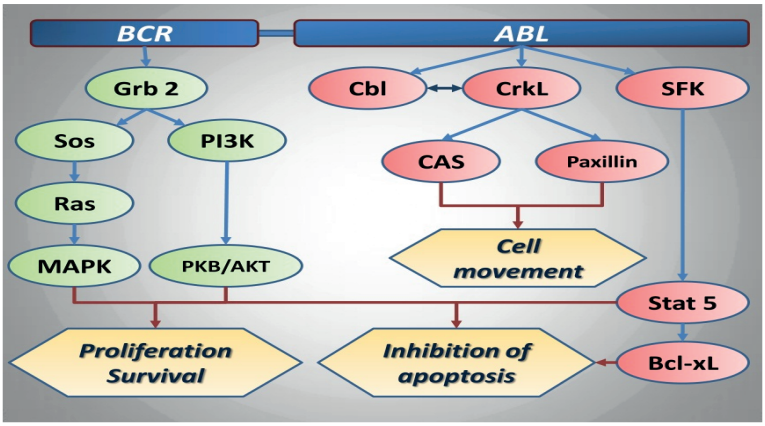

Figure 2: Mechanisms involving the interaction of *BCR–ABL* with different receptors [21].

By means of mechanisms involving the interaction of Bcr–Abl with the growth factor receptor-binding protein (Grb-2)/Gab2 complex, the RAS pathway is made to become constitutively active. As a result, the activity of the Sos guanosine diphosphate/guanosine triphosphate (GDP/GTP) exchange factor is increased, which encourages the buildup of the active Ras that is GTP-bound. Indirect interactions between Bcr–Abl and the p85 regulatory component of PI3K are also made possible by several docking proteins, including GRB-2/Gab2 and c-cbl. The *BCR/ABL* transformation is significantly influenced by an Akt-dependent cascade that is activated by the PI3K pathway. This cascade controls the subcellular location or activity of various targets, including BAD, MDM2, IB-kinase, and transcription factors from the Forkhead family. Signal transducer and activator of transcription 5(STAT5) is a further signaling pathway that *BCR/ABL* activates [22].

So, drugs with *BCR–ABL* kinase-inhibiting activity would be considered an effective treatment for CML.

The first generation of inhibitors: CML could be treated with Imatinib, resulting in enhanced response, better outcomes, and higher survival rates. Through the molecular analysis of imatinib, it has been shown that it is sandwiched between two lobes, the N-lobe and C-lobe of the ABL kinase domain, and it penetrates the central region of the kinase. Imatinib has its efficiency due to its ability to persist in the inactive form of the kinase domain via the induced fit model. As a kinase molecule is dynamic, it switches between its active and passive states, while imatinib causes the molecule to remain inactive as it has potency for this conformation. Imatinib has shown better activity toward the CP of CML, like >95% hematologic response and >73% cytogenetic

remission. However, the response is not that well in the later stages of CML, with a ratio of only 65% of patients showing hematologic response [23].

Besides many benefits, some patients get resistance against this therapy and become in tolerated. Due to the inappropriate or toxic response, approximately one-third of CML patients treated with imatinib discontinue their treatment. There are multiple reasons for an ineffective response from imatinib. Imatinib resistance could develop by the occurrence of point mutations in the domain of ABL kinase that leads to the changes in the structure of this domain, and ultimately *BCR–ABL* gene is over-expressed. Moreover, resistance against imatinib could be developed by the loss of the target at which kinase act or if chimeric genes got multiplied or evolution through cloning could occur. Mechanisms other than the *BCR–ABL* target could also be a reason for imatinib resistance, such as weak absorption through intestines, reaction of drugs, and poor administration of drugs [24].

The second generation of inhibitors: For patients that become resistant to imatinib, there is a second generation of inhibitors that include nilotinib (Tasigna®) and dasatinib (Sprycel®) that showed enhanced activity in many patients. Still, there is a reduced effect in patients with T315I *BCR–ABL* mutation. Therefore, it is essential to identify significant components involved in CML's pathogenesis to find new approaches to overcome resistance against tyrosine kinase inhibitors (TKIs) [25].

The third generation of inhibitors: Except T3151 mutation, all mutations are treated by the second generation of inhibitors, including dasitinib. T3151 is recognized as the most challenging mutation with no cure available to date. During preclinical research, AP24534 (ponatinib) was identified as a potent inhibitor of T3151 and all other mutations exhibited by the *BCR–ABL* gene. Now, phase II trials of ponatinib are going on, which is showing effective results even against T3151. Ponatinib exhibits a Carbon–Carbon triple bond linker which is the cause of making contact hydrophobically with different side chains of T3151. It also helps correct the binding domains' orientation toward their pocket and acts as inflexible connectors. Other contact points are being incorporated to increase its inhibiting capacity, improve the binding affinity, and make it less render to point mutations. Bosutinib (SKI-606), a third-generation inhibitor, is active against the *BCR–ABL* gene in the less nanomolar concentration. It is in phase III clinical trials and is active against patients showing resistance to imatinib or other inhibitors in phase II clinical trials. It can work in nanomolar concentration and inhibits ABL and SRC kinase to auto-phosphorylate, which results in cell growth inhibition and apoptosis. Because of its double action, this inhibitor is effective in CML disease and other tumors or

malignancies. It has a better advantage because it inhibits only the faulty proteins in tumorous cells, and appropriate proteins are not disrupted like in earlier drugs [26].

Rebastinib is a third generation of inhibitor called as switch pocket inhibitor. Large number of CML patients cause relapse od disease due to the T3151 mutation that exhibit resistance against all kind of TKIs. So, there was urgent need to develop drug against this mutation. Rebastinib act as a potent inhibitor against ABL portion of gene and it adheres to the switch control site that play roles in the configuration of kinase domain. This drug has shown beneficial effects against T3151 mutation as well as imatinib refractory patients. While, it has shown non-satisfactory results for CML patients, it has proven effective for some other type of leukemias like breast cancer, pancreatic endocrine tumors, and glioblastoma [27]. Aurora kinases is another class of protein kinase that play important roles during mitotic stages. It has been demonstrated that some of the aurora kinase members specially aurora A become over expressed in many tumors and malignancies. So, certain compounds are designed to selectively inhibit aurora A and other types of aurora kinases [28]. Now, Alisertib (MLN8237), AT9283, and Danusertib (PHA-739358) show better results against CML and ALL patients that possess T3151 mutation. This may cause their use as off target agents in addition to other FDA-approved drugs [29].

7. Resistance to Drugs

Bonnet and Dick first time proposed the idea of leukemic stem cells (LSCs) which was based on the idea that only small number of leukemic cells is able to renew themselves and have the ability to propagate themselves [30]. Now, LSCs presence is somehow linked with resistance to drugs, initiation, and CML relapse. CML treatment has been revolutionized by the use of TKIs that increases the chance of survival by 90%, however, 25% of the patients become immune to these inhibitors [31]. This happens because of the occurrence of leukemic clones that express ABL1 mutations. Although the perseverance of LSCs is not only dependent on *BCR–ABL*1 kinase mutations [32]. So, it has been demonstrated through biological evidence that in CML, the therapy must not be only dependent on kinase activation, but other mechanisms of resistance should also be taken into consideration. Hence, there would be less chances of revival of disease [33].

Therefore, nowadays, mechanisms have been applied to target LSCs, and specific drugs are being designed to control the LSC number. These mechanisms include (1) Target-specific surface markers on LSCs surface; (2) Signaling pathways like Wnt or

Hedgehog (Hh) being modulated to alter the dormancy of LSC with the help of specific transcriptional factors (i.e., FoxO, BCL6, or PML); (3) Interaction between different hematopoietic niche; (4) Drug efflux pumps being inhibited like ABC transporters; and (5) Epigenetic regulation between LSCs and normal cells being targeted [34].

Research is being done in developing strategies to particularly target the LSCs. Many new types of drugs are being made for this purpose like DNA methylation inhibitors (DNMTi, i.e., 5aza-2-deoxycitidine or decitabine, hydralazine, and valproate) and HADC inhibitors (i.e., phenylbutyrate, romidepsin, entinostat, and vorinostat). Virnostat and decitabine effects have been studied in phase I study of clinical trial. Moreover, combined treatment strategies of dasatinib and decitabine are being studied in phase I or II clinical trials. In addition, Panobinostat efficiency is being determined in association with imatinib to induce apoptosis in BC CML cells and lowering the levels of *BCR–ABL1* transcripts. Likewise, microRNA antisense oligonucleotides and microRNA mimics are also to be applied in the treatment of CML for best possible results [35].

So, for accurate CML treatment there is need for developing drugs that can target multiple *BCR–ABL* mutations, delay or stop disease progression, and overcome resistance caused by *BCR–ABL* independent mechanisms specially during advanced phases of CML. Moreover, drugs could be developed to eradicate stem cells of CML. These targets could be achieved by understanding mechanisms of disease progression, disease relapse, and drug resistance by utilizing high through put techniques of molecular genetics, cell biology, and immunology.

8. Conclusion

CML is a hematopoietic stem cell clonal disorder with an incidence rate of 1–2 cases per 100,000 per year. The Philadelphia chromosome is the characteristic of CML disease formed due to the reciprocal translocation of chromosomes 9 and 22. The primary cause of CML is uncontrolled and enhanced tyrosine kinase activity in the *BCR–ABL* gene. To control CML progression, it is necessary to control tyrosine kinase functions, so drugs that show inhibitory effects against this tyrosine kinase enzyme are recommended. Three-generation inhibitors are available for treating CML, including imatinib, dasatinib, ponatinib, and bosutinib. But still, work is going on to predict the biomarkers of disease progression so that disease can be cured at even later stages.

9. Future Recommendations

Although drugs are available for CML patients, some patients become resistant to these drugs due to their disease severity, so significant work must be done on CML to target its novel biomarkers involved in CML progression. Moreover, patients can be cured at the early stages of the disease, however, BC stage patients have no treatment available, so much more work is needed. In addition, there is a need to detect the biomarkers of disease progression so that CML progression can be delayed or inhibited.

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Ethical Considerations

Not applicable.

Competing Interests

None.

Availability of Data and Material

Not applicable.

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