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A Discussion of Chronic Myeloid Leukemia

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Abstract

Chronic Myeloid Leukemia (CML) is a triphasic hematological malignancy characterized by the presence of the abnormal Philadelphia chromosome, linked to causing uncontrolled myeloid cell proliferation in the bone marrow and marked changes in the peripheral blood and bone marrow microenvironments. The discovery of the BCR-ABL1 fusion oncogene from the balanced translocation of chromosomes 9 and 22, creating the Philadelphia chromosome, and its encoded tyrosine kinase protein has been linked to causing uncontrolled cell proliferation, leading to a subsequent development of tyrosine kinase inhibitors (TKI). TKIs, along with traditional chemotherapy drugs and interferon treatment, have revolutionized CML treatment, enabling deep remissions and prolonging of the disease's chronic phase. This review will introduce CML and comprehensively explore the epidemiology, molecular basis, pathology, diagnostic approaches, and therapeutic strategies for CML, including their mechanisms of action and challenges, such as TKI resistance, with a focus on TKIs as the workhorses of CML therapy. By reviewing the current known literature, this paper aims to contribute to and guide the pursuit of new knowledge and research.

Keywords: Chronic Myeloid Leukemia (CML), relevant information of CML, treatment options, challenges

1. Myeloproliferative Neoplasms

Myeloproliferative neoplasms (MPN) are clonal disorders in hemopoiesis that arise in hematopoietic stem cells or early progenitor cells. Alongside cancers like polycythemia vera and primary myelofibrosis, they are characterized by affecting a particular lineage of mature myeloid cells. Also known as myeloproliferative disorders, these cancers tend to progress into leukemia, with the difference being the leukemic cells do not retain normal function, differentiation, and are not mature; leukemic cells are known as "blasts" to distinguish this fact. Across all MPNs, this overproduction causes abnormalities within the ratios of blood components, leading to diminished hemostatic and thrombotic performance (Barbui et al., 2013).

2. Chronic Myeloid Leukemia

Chronic myeloid leukemia is an MPN, manifesting through a gradual uncontrolled production of mature and maturing granulocytes — basophils, eosinophils, and neutrophils — building up over time, known as blasts. This is a distinguishing feature, being detectable through a blood count, but is not a conclusive diagnosis. CML can instead be characterized by the presence of the Philadelphia chromosome — the reciprocal translocation t(9;22)(q34;q11.2), where the ABL1 (Abelson murine leukemia) proto-oncogene tyrosine-protein kinase gene present on chromosome 9 is fused onto the BCR (breakpoint cluster region) gene on chromosome 22 (Kang et al., 2016). The product is a BCR-ABL1 fusion gene, encoding for a BCR-ABL1 protein hybrid, a dysregulated tyrosine kinase. This mutation is currently the determining diagnostic for CML and has been found to be a direct cause of leukemic behavior. Trials using mice expressing the mutant protein show that if ATP is not allowed to bind to BCR-ABL1, then cancer does not arise despite the expression (Zhang & Ren, 1998), whereas mutant-expressing mice crossed with the tetracycline-responsive element, tet-O, develop a similar form of human CML after induction of BCR-ABL1, with neutrophilia and leukocytosis (Koschmieder et al, 2005).

The progression of chronic myeloid leukemia has been ordered into three distinct stages: chronic phase (CP), accelerated phase (AP), and blast phase (BP). Chronic phase marks the beginning of leukemia, where total blasts equal to less than 10%. This is the stage with most response to tyrosine kinase inhibitors (TKI), the primary treatment of CML, and is asymptomatic to mildly symptomatic (Staging chronic myeloid leukemia, 2022). It has a median duration of 5-6 years.

Accelerated phase can be characterized by an unresponsiveness to TKI therapy, or exceeding thresholds in existing symptoms. A WBC count of over 10 x 10^9/L, thrombocytopenia under 10 x 10^10/L (or thrombocytosis over 10 x 10^11/L), 10 – 19% of blood/marrow blast presence, or blood basophils exceeding 20% (Eden & Coviello, 2023). Furthermore, any additional abnormalities in the Philadelphia chromosome, or new clonal differences, may also classify the leukemia into the accelerated phase (Eden & Coviello, 2023). Its median duration is 6-9 months.

Blast phase is the final and most severe phase of CML. It is defined by a large concentration of blasts in the blood and marrow, with greater than or equal to 20% (Staging chronic myeloid leukemia, 2022). The median survival duration of this phase is 3-6 months, and recovery is not expected. Patients from CML primarily die from progression into AP and BP.

3. Epidemiology and Etiology

Chronic myeloid leukemia is about 15% of all leukemias (Leukemia – Chronic Myeloid – CML – Statistics, 2023). It is estimated that, in 2023, there will be 8090 new cases, 5190 men and 3740 women, as well as 1310 deaths (Key Statistics for Chronic Myeloid Leukemia, 2023). In past years, the yearly incidence has been 1.6 cases per 100,000 adults, with a median age of 65 years at diagnosis (Mandal, 2023). This cancer is rare in children, due to the initial speed of blast buildup, but most patients present in chronic phase as it is the longest duration (Leukemia – Chronic Myeloid – CML – Statistics, 2023).

There are no primary causes of CML. The formation of the Philadelphia chromosome may present as a random mutation, or have its chance augmented artificially. Currently, there are three recurring risk factors seen in most cases. Direct exposure to ionizing radiation, such as in nuclear weapons, directly increases the chance of genetic mutation or abnormality, leading to cancers and CML. In a study performed on the survivors of the 1945 Hiroshima bombing, CML was seen in17.6% – 36.5% of sample individuals with varying degrees of exposure and was the most prevalent leukemia type (Ichimaru et al., 1991). Furthermore, most patients of leukemia present at around 65 years and is uncommon in children, and the high median age at diagnosis suggests that old age is a risk factor to CML (Leukemia – Chronic Myeloid – CML – Statistics, 2023). Lastly, men are slightly more likely to acquire CML. The ratio of male to female cases is 1.4:1 (Mandal, 2023).

4. Molecular Basis

Using mice models, it has been proven that the ATP phosphorylation of BCR-ABL1's substrate causes myeloproliferative activity, and the prevention of ATP binding, in turn, does not lead to leukemogenesis (Zhang & Ren, 1998) (Koschmieder et al, 2005). The source of the mutant protein, the BCR-ABL1 fusion oncogene, is contained on the Philadelphia chromosome, making it the source of CML.

The Philadelphia (Ph) chromosome is a balanced translocation between chromosomes 9 and 22. Discovered in 1960 by Peter Nowell at the University of Pennsylvania (Koretzky, 2007), it was the first documented genetic relationship to malignancy. It results in the Abelson murine leukemia (ABL1) proto-oncogene, 9q34, being fused to the breakpoint cluster gene (BCR) on 22q11, forming the truncated Ph chromosome 22q- and a chromosome 9q+ (Haider and Answer, 2022). This translocation is demonstrated in Figure 1.

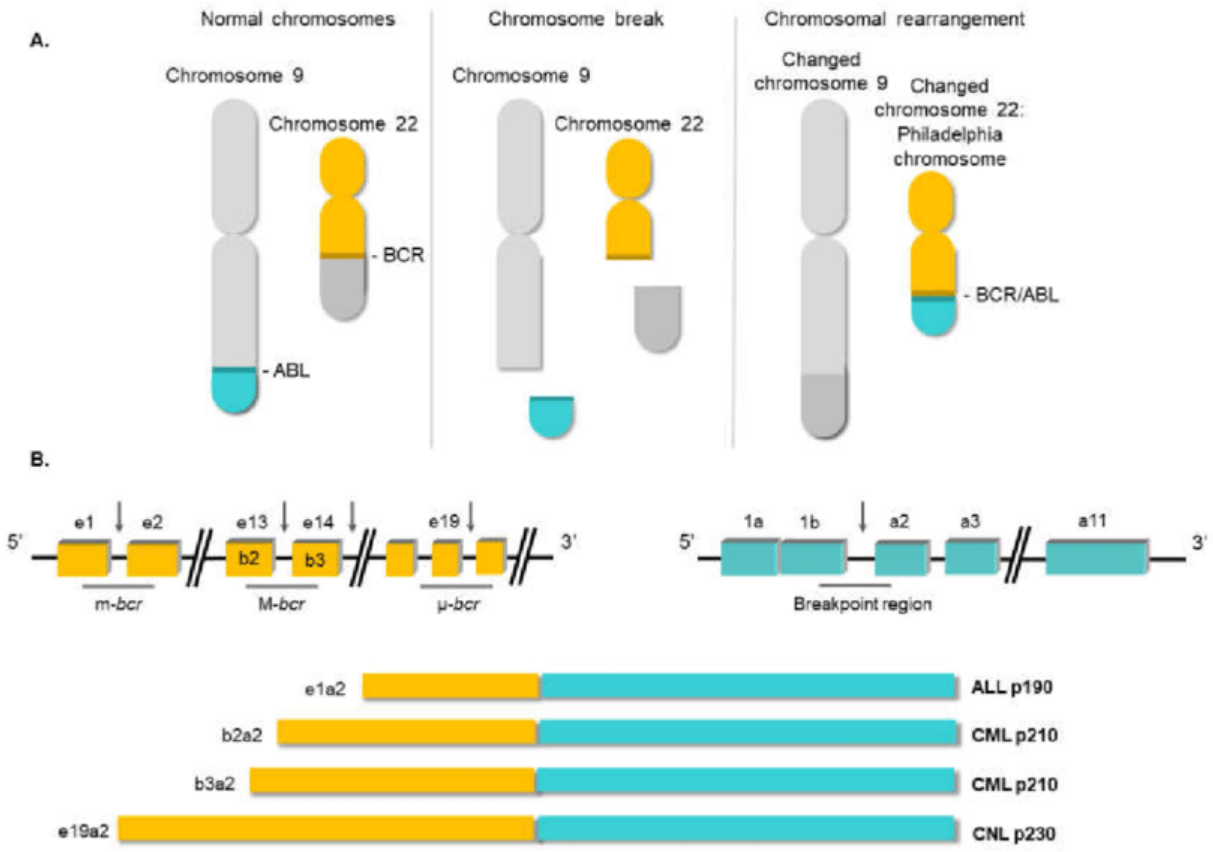

Figure 1. Structure of the BCR/ABL1 oncogene

(A) Schematic representation of the t(9;22) (q34;q11) translocation triggering the Philadelphia chromosome.

(B) Breakpoint locations between BCR and ABL1 genes. Different fusion protein combinations yield different outcomes (Vuelta et al., 2021).

BCR-ABL1 has 3 isoforms encoded by the fusion gene when translocation occurs at different exons: p190, p210, and p230; they have been found to contribute to acute lymphoblastic leukemia, chronic myeloid leukemia, and chronic neutrophilic leukemia (Li et al., 1999). Furthermore, it is known that fusion at exons 13 and 14 of the BCR gene both produce the p210 isoform (Deb et al., 2014), where 55% possess the exon 14 fusion and 40% on exon 13 (Kang et al., 2016), and 5% of cases are seen where both isoforms (e13 and e14) are detected (Okamoto et al., 1997). Ph is thus an acquired mutation and is not passed through the germline.

4.1 Signaling Pathways in CML

Initial development of CML-CP appears to be a direct result of p190 BCR-ABL1 due to Ph. Leukemogenesis can be contributed to the protein's tyrosine kinase (TK) activity, resisting cell death and differentiation (Kang et al., 2016). BCR-ABL1 interferes in multiple cell signal pathways to promote cell proliferation.

4.1.1 Activation of Janus Kinase 1-3 (JAK)

BCR-ABL1 promotes the JAK2/STAT pathway, leading to cell growth and oncogenic transformations (Valent, 2014). It is also found that JAK2 can mutually enhance BCR-ABL1 by phosphorylation at Y177 (Samanta et al., 2011).

4.1.2 PI3K-AKT-mTOR

BCR-ABL1 has been seen to evade cell cycle arrest through the PI3K-AKT-mTOR pathway (Keeshan et al., 2003), as well as induce expression of K-phase kinase-associated protein 2 (Skp2), leading to proliferation (Andreu et al., 2005). Metabolic changes also occur due to BCR-ABL1, increasing glucose metabolism (glycolysis) and affecting PI3K-AKT-mTOR electron transport chains (Kim et al., 2005).

4.1.3 RAS Pathway

BCR-ABL1 uses the RAS signal pathway to transduce nuclear transcription factors via phosphorylation of F177 (Steelman et al., 2004; Mandanas et al., 1993). This can be proved with mice models, where RAS inhibition

slows development of MPDs similar to human CML (Baum & Ren, 2008).

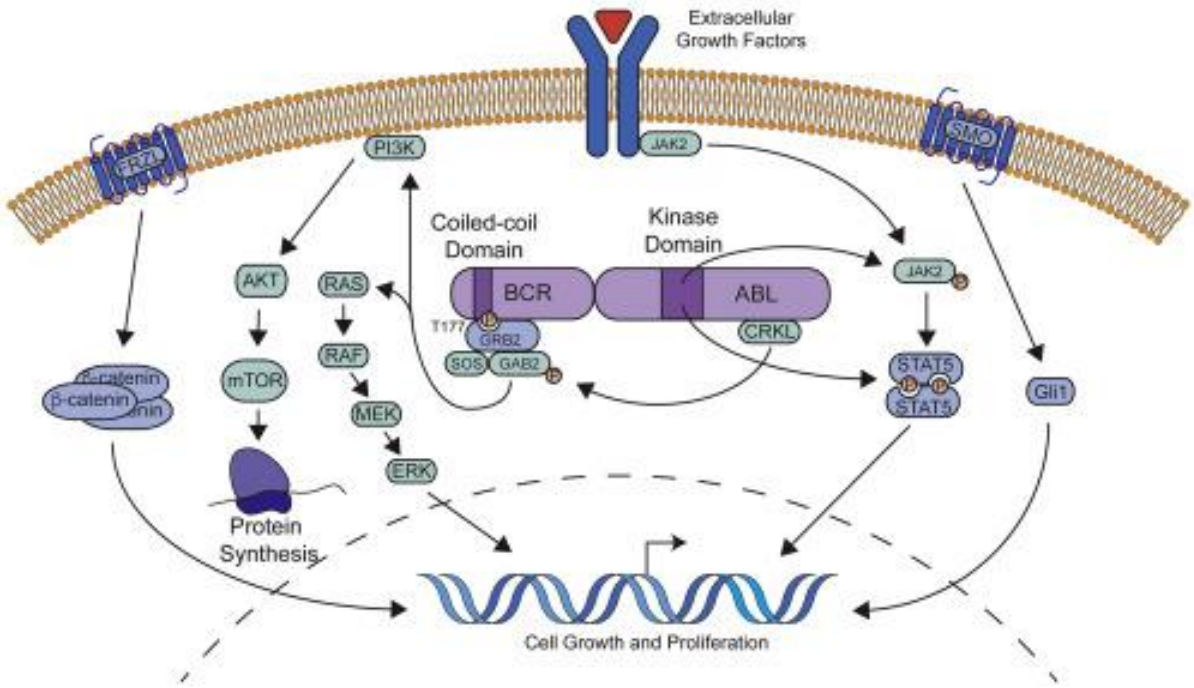

Figure 2. Molecular pathways downstream of BCR-ABL1 (Braun et al., 2020)

5. Clinical Manifestations

While CML is often asymptomatic in about half of all patients, some common symptoms of the chronic phase include:

- Splenomegaly: The enlargement of the spleen, resulting in left upper quadrant pain, tenderness, and fullness without eating much food (Eden & Coviello, 2023).
- Bone soreness and pain: Pain is caused by leukemic blasts exit the marrow and move to bone surface or joint (American Cancer Society, 2018). Cases have been reported where CML manifests as gouty arthritis (Pavithran & Thomas, 2001).
- Anemia: The overproduction of blasts restricts the number of RBCs in the bloodstream, leading to anemia (American Cancer Society, 2018). Weakness, fatigue, and nausea manifest as a result.
- Bleeding episodes: Blast presence also impacts platelets, causing dysfunction and leading to thrombosis, priapism, or poor hemostasis (Eden & Coviello, 2023).

As CML progresses, existing symptoms worsen. Classic symptoms of cancer also arise, such as weight loss. Additionally, CML patients are increasingly immunocompromised over the course of the cancer due to improper WBC functionality.

6. Pathology

6.1 Peripheral Blood

Most commonly, the peripheral blood shows a high degree of leukocytosis with granulocytes. There exist many cells from the neutrophil maturation line (Eden & Coviello, 2023). During the chronic phase, blasts usually account for less than 2% of all white blood cells (Eden & Coviello, 2023). Basophilia is also commonly associated with CML and is seen as a poor prognostic sign (Goh & Anderson, 1979).

Additionally, a hallmark of CML is the abnormal neutrophil cytochemistry as a result of leukemic activity. Leucocyte alkaline phosphatase (LAP) is found in secretory vesicles of neutrophils and is an indicator of post-mitotic granulocytes (Gianni et al., 1996). Its lack in CML is a sign of improper differentiation and immaturity and is used for blood smear testing (Dotti et al., 2005).

6.2 Bone Marrow

The uncontrolled proliferation of HSCs has consequences on the structure and microenvironment of the bone

marrow. Erythroid islands are decreased significantly, and erythroid cells are diminished compared to myeloid cells (Eden & Coviello, 2023), leading to disturbances in RBC production. Megakaryocytes, the precursors to platelets, are found to be atypically small, or "dwarfs" (Hidalgo-Lopez et al., 2018; Eden & Coviello, 2023). Furthermore, there are varying levels of fibrosis between cases, from reticulin to collagen, along with cases where no fibrosis is present (Thiele et al., 2009). A presence of sea-blue histiocytes and pseudo-Gaucher cells can be seen in the marrow (Büsche et al., 1997; Kelsey & Geary, 1988).

6.3 Methods of Diagnosis

An affirmative diagnosis of chronic myeloid leukemia is based upon detection of Ph or the BCR-ABL1 mRNA/protein. After extraction of a blood or marrow sample, the current prolific methods of diagnosis are as follows: (1) Conventional karyotyping and cytogenetic analysis: The most widely used method of diagnosis, occurring in 95% of all cases (Khajehmarjany et al., 2015). Creates a karyotype, allowing one to observe translocation and Ph. (2) Florescence ion situ hybridization (FISH): A rapid method based on florescent probes attaching to certain base pair sequences in DNA. By labeling each chromosome with known sequences, one is able to view cell chromosomes more efficiently than traditional methods (Khajehmarjany et al., 2015). This method also shows translocations between chromosomes due to the nature of the florescent probes, allowing for easy identification of Ph. (3) Real-time PCR (RT-PCR): High efficiency method of amplifying the BCL-ABL1 mRNA for detection, without needing to visualize the Ph chromosome, like in karyotyping (Branford & Hughes, 2006). An alternative is seen in plain multiplex PCR, where the gene itself may be amplified.

While obtaining a blood cell count is highly indicative of leukemic activity, it is still unable to distinguish between different leukemias and MPDs, and thus detection of Ph or BCR-ABL1 is essential in diagnosing CML.

Currently, new methods of diagnosis, such as digital PCR (dPCR) and next-generation sequencing (NGS), are being developed. Their goal is to increase sensitivity and accuracy of detection, being able to amplify trace amounts of genetic material (Soverini et al., 2020). Potential points of investigation include simple detection methods with greater availability to patients in rural or undeveloped regions.

7. Treatment Options

When approaching therapeutic strategies of CML, it is most effective to relieve present symptoms, caused by hyperleukocytosis, splenomegaly, and thrombocytosis, and prolong the chronic phase, rather than attempting to cure the leukemia outright. This is due to the only known curative treatment being bone marrow transplantation, a rare and potentially toxic option where 30% of recipients die from treatment-related complications (Fausel, 2007). Instead, there are many other readily available and safe methods that have greatly decreased the lethality of CML by treating the chronic phase.

7.1 Chemotherapy

7.1.1 Busulfan

A chemotherapy drug, it is an alkylating agent that inhibits RNA transcription, ceasing much of the cell's protein activity. It further damages the cancer cell DNA structure by creating cross-links in the molecular structure through reacting guanine with carbonium ions from the drug (Patel & Tadi, 2022). This stops the uncoiling required for division and impedes proliferation. It also poses challenges common to all alkylating chemotherapeutics, such as intestinal mucosal damage, blood cell deficiencies, and impairment of rapidly dividing cell groups, such as the gametes (Patel & Tadi, 2022).

7.1.2 Hydroxyurea

An antineoplastic analog of urea which prevents cell proliferation by inhibiting ribonucleotide diphosphatase reductase, impeding DNA synthesis and mitosis. The cell cycle is stopped at the G1 phase to S as a result (Jinna & Khandhar, 2022). By causing myelosuppression, hydroxyurea faces the same issues as busulfan, where anemia, leukopenia, and thrombocytopenia may develop. Furthermore, continuous hydroxyurea therapy risks the development of multiple skin cancers and thus treatment must be carefully monitored and controlled (Best & Petitt, 1998). It must also be noted that hydroxyurea only induces a hematologic response, not a cytogenic response (Chem et al., 2021).

7.1.3 Interferon Alfa (IFN-alfa) + Cytarabine (Ara-C)

IFN-alfa is an anti-leukemic interferon with partial understanding of its mechanisms of action. It is known to drive activation of multiple genes encoding apoptotic, antiviral, immunomodulatory, and transcription factor proteins (Der et al., 1998), such as TRAIL, caspase 4, 8, MHCs, and interleukins 6 and 15, as well as regulating the cell cycle through modulating cyclins and cyclin-dependent kinases. It is found that IFN-alfa thus promotes cell differentiation and apoptosis, preventing proliferation and leukemogenesis. It is also believed that IFN-alfa activates the immune response against leukemic cells, which may also pose issues and interfere with treatments (Talpaz et al., 2012). Additionally, IFN-alfa is toxic and may cause flu-like symptoms, culminating in immune

disorders, heart dysfunction / arrhythmia, or nerve damage during misguided treatment (Talpaz et al., 2012). On the other hand, Ara-C is a pyrimidine analog that incorporates into the DNA, restricting the replication process during the S phase and targets rapidly dividing cells, similar to other chemotherapies. This also indicates that it poses the same limitations as the aforementioned drugs. When taken together, the two drugs are one of the most popular and effective treatments to prolong chronic phase CML, second only to TKI therapy in recent years.

7.2 Tyrosine Kinase Inhibitors

As a tyrosine kinase, the BCR-ABL1 is inherently susceptible to tyrosine kinase inhibitor (TKI) treatment. With the goal of preventing phosphorylation of the tyrosine substrate, TKIs can prevent the effects of the mutated TK by deactivating its effects. Split into 5 types, CML treatment often employs type 1 and 2 inhibitors. Type 1 competitively inhibits ATP binding at the binding site of active TKs, and type 2 does the same for inactive TKs (Thomson et al., 2023).

Imatinib mesylate is the first TKI approved for use by the FDA, in 2001, and is currently the most popular primary treatment when CML is diagnosed in the chronic phase (Iqbal & Iqbal, 2014). Imatinib possesses the same mechanism of action as other TKIs, aiming to block BCR-ABL1's activation by binding to the ABL kinase domain, shown in Figure 3. Furthermore, it is highly targeted towards this target domain and its derivatives, allowing for applications in inhibiting c-KIT and other kinases, while having little effect on other kinases (Deininger & Druker, 2003). Following the advent of imatinib as a forerunner of TKI therapy, many other inhibitors have entered widespread use as secondary treatments after imatinib or used in combination, such as nilotinib, dasatinib, bosutinib, and ponatinib (Pophali, 2016). These drugs work in similar ways to the original, imatinib, in that they are targeted therapies against the ABL domain.

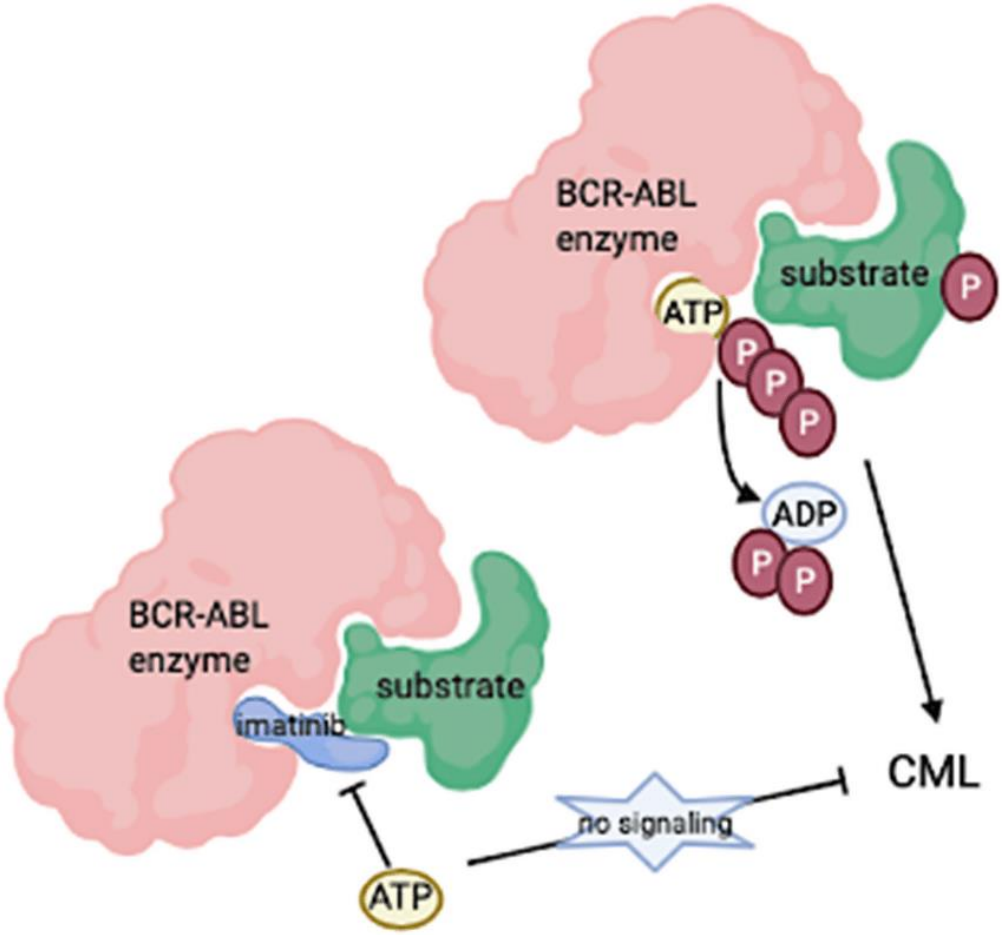

Figure 3. Mechanism of action of Imatinib, binding to the ATP binding site and preventing phosphorylation (Karasu et al., 2021)

A 2003 study performed by O'Brien et al compared the effectiveness of imatinib mesylate against the interferon-alfa and cytarabine combination therapy for 1106 newly diagnosed chronic phase CML patients

(O'Brien et al., 2003). Each drug was randomly given between the patients so that one-half was on each therapy. During the treatment, if lack of response, intolerance, loss of response, or worsening WBC count was detected, crossover to the other therapy track would ensue. By tracking hematological and cytogenic responses of each drug, it was shown that imatinib had greater than 50% higher percentage of recipients with a major cytogenic response than IFN-alfa + Ara-C, with 85.2% against 22.1%. Imatinib also had significantly higher hematological responses in 2 years, with 95.3% against 55.5%, and was 1.5 months faster, on average, than the combination therapy in median interval to a complete hematological response (O'Brien et al., 2003). Furthermore, 89.2% of patients in the combination therapy reached the requirements for crossover, while only 14.3% on imatinib were changed (O'Brien et al., 2003). Thus, it can be concluded that while IFN-alfa and cytarabine combination therapy is still possible for CML treatment, the advent of imatinib and new TKIs have shown their dominance in achieving significantly superior results.

7.2.1 Limitations of Tyrosine Kinase Inhibitors

As with all treatments, TKIs are imperfect. While targeted and highly specific, their application is limited by side effects, including but not limited to fatigue, abdominal pain, diarrhea or constipation, thrombosis, strokes, hypokalemia, thyroid dysfunction, anemia, thrombocytopenia, and/or neutropenia (Thomson et al., 2023).

Furthermore, the possibility of imatinib inhibiting kinases other than the targeted leukemic cells pose a threat to organ health, potentially causing cardiac failure and liver damage (Mughal & Schrieber, 2010). With mild to moderate toxicity, dosage is an important factor to consider when conducting imatinib therapy, thus potentially impacting the drug's effectiveness. The question of linking higher dosage to greater efficacy is highly debated, with some studies showing faster responses when doubling the imatinib dose (from 400 mg to 800mg daily) is administered with similar frequency of side effects (Kantarjian et al., 2004; Hehlmann et al., 2011), whereas other studies do not report significant response differences in the two values (Baccarani et al., 2009). This area is still currently unclear and requires more study to determine if greater results can be achieved by raising the dosage, while still maintaining safety. It must still be noted that imatinib has decades of safety data associated with its proper use since its introduction, and that newer, next-generation TKIs may have greater safety and efficacy.

Currently, the greatest setback of TKI therapy is the potential of mutation leading to loss of response. It has been observed that the leukemic cell may render a 400 mg daily dosage of inhibitor ineffective through brute quantity, amplifying the fusion gene or overexpressing the protein (Sacha, 2014). The leukemic cells may downregulate influx transporters or upregulate efflux transporters, reducing the amount of TKI within the cell (Amir & Javed, 2021) It is also possible that the protein itself has become resistant to the therapy. If the inhibitor is not able to bind to its target site, the ABL-kinase domain, due to changes in the conformational structure, then it is unable to prevent phosphorylation of the substrate and thus allows normal functioning BCR-ABL1 within the cell. A protein mutation accounts for 40-90% of resistant cases of CML to TKI therapy (Amir & Javed, 2021). Mutations may cause resistance to certain drugs while still being susceptible to others. These are the major mutations to the BCR-ABL1 protein that may cause resistance (O'Hare et al., 2007; Redalli et al., 2009; Branford et al., 2009; Jabbour et al., 2011; Soverini et al., 2011; O'Brien et al., 2011; Cortes et al., 2013):

- G250E/F311I/H396R: Resistance to imatinib, susceptible to dasatinib, niolotinib, and bosutinib.
- V299L/T315A/F317K/V/I/C: Resistance to dasatinib and bosutinib, susceptible to nilotinib and imatinib.
- Y253H/F/E255K/V/E355G/V379I/F359V/C/I: Resistance to imatinib and nilotinib, susceptible to dasatinib.
- T315I: Resistance to imatinib, dasatinib, nilotinib, bosutinib, susceptible to ponatinib.
- T315M: Resistance to all TKIs.

With the possibility of these mutations, it is thus unrealistic to treat CML with a single TKI. While imatinib is the most popular therapy, a plethora of mutations can cause it to be ineffective, and without proper monitoring of the patient's status, it is possible for one to advance to accelerated phase even while undergoing TKI therapy. When a patient develops resistance, secondary and tertiary treatments are thus necessary, potentially utilizing other TKIs, traditional chemotherapies, or the IFN-alfa combination therapy. If the resistance is a change in BCR-ABL1 quantity, then raising the dose of the current treatment may also prove successful. However, the nature of resistance must be further studied, with identification of the effects of multiple mutation combinations.

8. Conclusion

Chronic myeloid leukemia is a myeloproliferative disorder where an uncontrolled proliferation of blast granulocytes takes place in the bone marrow and blood, directly caused by the effects of the BCR-ABL1 fusion oncogene. Its balanced translocation results in the formation of a tyrosine kinase protein, which disrupts multiple

cell pathways and incites leukemogenesis. Thus, tyrosine kinase inhibitors, which prevent phosphorylation and activation of the kinase, were developed, and put into widespread use with the advent of imatinib. Greatly more effective and targeted than regular chemotherapeutics, TKIs are still imperfect, with multiple side effects and the possibility of developing resistance. Going forward with inhibitor research, the field of combining TKIs and chemotherapies may prove to be fruitful, being able to achieve results despite resistance. The development of next-generation TKIs has the potential to improve upon these shortcomings, by bypassing certain mutations and increasing safety. Lastly, time of diagnosis is crucial to administering the treatment early. New knowledge in the fields of dPCR and NGS may prove beneficial to detecting CML with greater accuracy and sensitivity. Thus, the ever-evolving landscape of research and therapeutic advancements holds great promise in improving outcomes for the management of chronic myeloid leukemia.

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