



# Chronic Myeloid Leukemia, from Pathophysiology to Treatment-Free Remission: A Narrative Literature Review

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**Abstract:** Chronic myeloid leukemia (CML) is one of the most common leukemias occurring in the adult population. The course of CML is divided into three phases: the chronic phase, the acceleration phase, and the blast phase. Pathophysiology of CML revolves around Philadelphia chromosome that constitutively activate tyrosine kinase through BCR-ABL1 oncoprotein. In the era of tyrosine kinase inhibitors (TKIs), CML patients now have a similar life expectancy to people without CML, and it is now very rare for CML patients to progress to the blast phase. Only a small proportion of CML patients have resistance to TKI, caused by BCR-ABL1 point mutations. CML patients with TKI resistance should be treated with second or third generation TKI, depending on the BCR-ABL1 mutation. Recently, many studies have shown that it is possible for CML patients who achieve a long-term deep molecular response to stop TKIs treatment and maintain remission. This review aimed to provide an overview of CML, including its pathophysiology, clinical manifestations, the role of stem cells, CML treatments, and treatment-free remission.

**Keywords:** chronic myeloid leukemia, remission, tyrosine kinase inhibitor

## Introduction

Chronic myeloid leukemia or chronic myelogenous leukemia (CML) is a hematopoietic stem cell (HSC) disorder, as are all leukemias. In CML, the disorder is characterized by translocation t(9;22)(q34;q11), resulting in the fusion of BCR and ABL1 genes into the pathogenic BCR-ABL1 oncogene, with many subsequent effects on downstream pathways.<sup>1,2</sup> The main pathway effect of this fused oncogene is to activate tyrosine kinase pathway constitutively, resulting in a proliferative advantage of the mutant HSCs compared to normal HSCs, and the gradual displacement of normal HSCs.<sup>3</sup>

CML symptoms range from asymptomatic to overt leukostasis, depending on the stage of CML; leukostasis often occurs in the blast phase. Meanwhile, hyperleukocytosis is the most common symptom, and is often present in all phases (chronic phase, accelerated phase, and blast phase).<sup>1</sup> The blast phase is similar to acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL). The diagnosis of CML often requires a basic hematology workup combined with identification of chromosomal abnormalities. Different phases of CML can be differentiated by clinical symptoms and blast percentage.

CML is one of the model diseases for targeted therapy. It is widely accepted that discovery of tyrosine kinase inhibitors (TKIs) has changed the treatment landscape of CML.<sup>4</sup> Prior to the introduction of TKIs, CML was universally fatal in the long term. However, since TKIs have become the first-line treatment for CML, the life expectancy of CML patients has become similar to that of people without CML.<sup>5,6</sup>

While TKIs have indeed improved the survival of CML patients, there are still unresolved issues associated with TKI treatment, such as adverse side effects and impact on quality of life. Furthermore, a very important issue associated with TKI treatment is the need for most patients to continuously take the TKI to prevent relapse. Thus, many patients are on

lifelong TKI treatment. However, several studies, such as STIM1 trial, have shown that patients with a deep molecular response (DMR) can achieve treatment-free remission (TFR) with no relapse after discontinuing their TKI.<sup>7</sup> This review article aimed to discuss all aspects of CML.

## Pathophysiology of CML

The presence of BCR-ABL1 fusion gene in hematopoietic stem cells has been shown to be sufficient for initiation of CML.<sup>8</sup> Thus, the pathophysiology of CML revolves around the BCR-ABL1 fusion gene. Approximately 90% to 95% of patients with CML have reciprocal translocation of t(9;22) (q34;q11.2).<sup>1,2</sup> This results in a shortened chromosome 22, termed Philadelphia chromosome, containing the BCR-ABL1 oncogene. However, It should be noted that BCR-ABL1 may also be present in other types of leukemia, such as acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).<sup>9</sup> The significance of BCR-ABL1 in ALL and AML is still under investigation.

At the time of writing, the mechanism of Philadelphia chromosome formation and the time from the formation of the chromosome to the occurrence of clinical symptoms of CML are still not known with certainty. The Philadelphia chromosome was previously thought to have been formed due to the influence of radiation from the atomic bombs of Hiroshima and Nagasaki on CML patients in Japan.<sup>10</sup> Indeed, in vitro studies have shown that exposure of myeloid cell lines to high-dose radiation causes the expression of BCR-ABL1.<sup>10</sup> However, the majority of people are not exposed to chronic radiation. Some experts believe that the Ph chromosome can be formed as a result of spontaneous random translocation. Supporting this is the fact that the BCR-ABL1 gene has also been observed in a small proportion of healthy individuals.<sup>11,12</sup> This could also mean that not all BCR-ABL1 fusion genes develop into CML. Further studies are needed to elucidate the origins of the BCR-ABL1 gene; however, it will be very difficult to isolate the exact causes that contribute to the formation of the Philadelphia chromosome due to the relative rareness of the disease and issues with determining the onset of the disease, as it is initially asymptomatic.

As has been described by much of the scientific literature on this topic, the BCR-ABL1 hybrid gene located on the Ph chromosome synthesizes the oncoprotein 210 kD (p210), which plays a role in leukemogenesis of CML.<sup>13</sup> The p210<sup>BCR-ABL1</sup> protein can continuously activate the tyrosine kinase pathway, resulting in an increase in the signal transduction of unregulated downstream oncogenic pathways such as JAK/STAT, PI3K/AKT, RAF, MYC, and RAS/MEK.<sup>3,13</sup> All of these pathways are involved in cell growth, cell survival, and the inhibition of apoptosis. Thus, HSCs with BCR-ABL1 translocation have a proliferative advantage due to their enhanced response to growth factors. Furthermore, proliferation-inhibitory factors are less responsive. Thus, overall, the presence of BCR-ABL1 in hematopoietic stem cells will encourage their transformation into leukemia stem cells (LSCs) and replacement or displacement of normal hematopoietic stem cells.

In CML, there are other types of BCR-ABL1 fusion and breakpoints. For example, p190<sup>BCR-ABL1</sup> (e1a2), which encodes a hybrid 190 kDa protein, can be observed in CML, but the frequency is rare. Based on several studies, p190<sup>BCR-ABL1</sup> occurs in only 1–2% of CML patients.<sup>14,15</sup> Other BCR-ABL1 proteins include p230<sup>BCR-ABL1</sup>.

A point of interest is that not all CML patients have Philadelphia chromosome. In a minority of CML patients, the Philadelphia chromosome is lacking but identifiable BCR-ABL1 oncogenes are present. Observations have shown that the number of confirmed CML patients with no Philadelphia chromosome is up to 5–10%.<sup>16–18</sup> Interestingly, approximately 25–50% of these patients still have BCR-ABL1 gene rearrangement, as confirmed by molecular examinations.<sup>18,19</sup> In other words, the BCR-ABL1 genes in this subset of CML patients originate outside of the Philadelphia chromosome. All other patients have no confirmed BCR-ABL gene rearrangement.

According to a study by Wang et al, BCR-ABL-negative CML patients have a worse overall survival and have a higher risk of their leukemia transforming into acute myeloid leukemia.<sup>20</sup> Without the presence of BCR-ABL, most TKIs are not indicated as a main treatment, which may be one of the factors contributing to a worse prognosis in BCR-ABL-negative CML patients. Gene mutations commonly found in BCR-ABL-negative CML include SETBP1, ASXL1, NRAS/KRAS, SRSF2, CSF3R, U2AF1, and many others.<sup>21</sup> Some of these mutations may be used as potential targets for therapy in BCR-ABL-negative CML, but the evidence to support this is currently lacking.

## Pathophysiology of CML Progression

CML is grouped into three phases, namely the chronic phase, the accelerated phase, and the blast phase.<sup>1</sup> In the chronic phase, LSCs are confined to hematopoietic tissues. However, in the blast phase, the LSCs gain an invasive capability that enables them to infiltrate other organs, most notably the spleen, resulting in splenomegaly. Furthermore, immature blast cells now dominate in blast phase. The process of transformation from chronic CML to the accelerated phase or blast phase is thought to be caused by amplification of the BCR-ABL1 protein and increased activation of the downstream tyrosine kinase pathway, combined with the emergence of additional chromosomal abnormalities (ACAs).<sup>22,23</sup> Oxidative stress and impaired DNA repair have also been implicated.<sup>24–26</sup> In addition, mutations such as deletion of the IKZF1 gene and loss of CCAAT protein expression have been reported to cause the immaturity of granulocytes.<sup>27,28</sup> Blast-phase patients often have multiple gene mutations.<sup>29</sup> Common gene mutations due to genomic instability in blast-phase CML are summarized in Table 1.<sup>22,30,31</sup>

## Additional Chromosomal Abnormalities in CML

LSCs are genetically unstable, so they have a tendency to present with other genomic or cytogenetic abnormalities.<sup>32,33</sup> Additional cytogenetic abnormalities (ACAs) have been shown to increase in the later phases of CML. For example, in the chronic phase or at diagnosis, only approximately 5–10% of CML patients have ACAs, but this increases to up to 80% in the blast phase.<sup>34,35</sup> Cytogenetic changes commonly observed in the blast phase include +8, +19, double Philadelphia chromosome, and isochromosome 17q.<sup>36,37</sup>

The presence of ACAs in the early stages appears to be associated with disease progression in CML. A study by Clark et al showed poor progression-free survival in CML patients with ACAs.<sup>37</sup> Additionally, a study by Chandran et al also found a poor prognostic effect of ACAs in CML.<sup>35</sup> The latest CML guidelines from the European LeukemiaNet state that the appearance of ACAs is one of the early indicators of disease progression, and the high-risk type of ACAs are associated with TKI treatment resistance.<sup>38</sup> A study by Meggyesi et al showed that 30 out of 65 imatinib-resistant patients (46%) had ACAs.<sup>39</sup> Monitoring of ACAs during TKI treatment is important as ACAs are associated with treatment resistance.<sup>40</sup>

## CML Stem Cells

Evidence of the HSC origin of CML comes from the observation that the translocation of BCR-ABL1 is observed in myeloid, erythroid, and megakaryocytic cells. However, the effects of this translocation on erythroid and megakaryocytic cells are still unclear. The suppression of erythroid and megakaryocytic cells in CML is very likely due to the high proliferation of myeloid cells causing disruption. The malignant HSCs, termed leukemia stem cells (LSCs), provide a reservoir of self-replenishing leukemia cells.<sup>2</sup> Thus, to completely eradicate CML, LSCs ideally must be targeted and removed to prevent relapse.

**Table 1** Common Gene Mutations in Blast-Phase CML

Gene Mutations	Gene Functions
p53 loss of function	Growth arrest, DNA repair, and apoptosis
CDKN2A loss of function	Tumor suppressors
GATA-2 gain of function	Gene expression regulation through transcription factor
RUNX1 mutations	Hematopoietic stem cell maturation
IKZF1 mutations	Regulator of lymphoid differentiation
ASXL1 mutations	Gene transcription regulator
WT1 mutations	Tumor suppressor

LSCs in chronic-phase CML can be identified via positive CD34 markers and negative CD38 markers.<sup>2,41–43</sup> However, using only these surface antigen markers provides a heterogeneous population, and these markers are not unique to CML LSCs.<sup>41</sup> For example, the population of normal HSCs can also be identified using positive CD34 markers and negative CD38 markers as well. CD33 marker is also expressed by both normal stem cells and LSCs. However, there is a greater density of CD33 in LSCs.<sup>44</sup> Indeed, LSC identification is also a challenge in other cancers.<sup>45</sup>

In an article by Hoshmand et al, it is stated that markers such as CD25, CD26, and interleukin-1-receptor accessory protein (IL1RAP) have been reported to be expressed specifically on CML stem cells.<sup>41</sup> CD25 is encoded by the IL2RA gene and the CD25 marker has been reported to be expressed by more than 90% of CD34<sup>+</sup>/CD38<sup>-</sup> LSCs.<sup>46,47</sup> Meanwhile, neither CD26 nor IL-1RAP have been shown to be expressed in LSCs in most other malignancies.<sup>48</sup> However, it should be noted that both CD26 and IL-1RAP are expressed in normal mast cells, which may complicate identification.<sup>49,50</sup> There are some possible combinations of cell surface markers for reliable identification of CML LSCs such as Lin-negative, CD34-positive, CD38-negative/low, CD45RA-negative, KIT-negative, and CD26-positive but this requires further confirmations.<sup>41,51</sup>

LSCs have a persistent presence, even with tyrosine kinase inhibitor treatment.<sup>52</sup> Thus, patients in remission are not “cured” and may technically relapse once TKI treatment is stopped. This suggests that there may be BCR-ABL1-independent pathways that maintain the self-renewal of CML LSCs. A study by Zhao et al showed that the maintenance of cancer stem cells requires activation of Hedgehog signaling.<sup>53</sup> Additionally, a study by Chen et al demonstrated that the Alox5 gene is important for LSC survival.<sup>54</sup> Other pathways or processes that support CML LSCs persistence include JAK1-STAT3 signaling, PI3K/AKT/FOXO signaling, Wnt signaling, and impaired DNA damage responses.<sup>55–59</sup> LSCs also have increased expression of IL-1 receptors, which, if activated, reduce the growth of LSCs.<sup>60</sup> Other cytokines that provide support to LSCs include TNF-alpha and IL-6.<sup>61</sup> Thus, it is not unexpected that a significant proportion of patients in remission relapse after stopping treatment.

Therapies to target CML LSCs has not been used clinically. One may argue that since long-term sustained molecular responses from TKI produce a significant proportion of patients remaining in molecular response when stopping TKI, therapies to target CML LSCs may not be needed. However, there will always be a relatively high proportion of patients that will not be able to achieve TFR status and thus, therapies targeting CML LSCs may be useful for this population. We the authors speculate that using combination therapies of TKI with one or several drugs targeting LSCs pathways may be the new norm in the future. EZH2 inhibitor and JAK inhibitor combine with TKI seem to be effective in eliminating LSCs and should be further researched.<sup>62,63</sup>

## Clinical Manifestations and Diagnosis

As stated above, the majority of CML patients are asymptomatic, especially in the early stages.<sup>1</sup> Patients are often suspected to have CML following a medical check-up revealing leukocytosis and basophilia. In the chronic phase, when the majority of CML patients are diagnosed, the symptoms, if there are any, are fatigue, weight loss, cold sweats, and abdominal pain or feeling full quickly due to splenomegaly. Spleen size should be measured, as it is a component of CML prognosis scores such as the Sokal score and the ELTS score. Other symptoms that can occur, albeit rarely, are low-grade fever, bleeding, thrombosis, gouty arthritis, priapism, and gastric ulcers. The baseline diagnostic workup recommended by the ELN 2020, ESMO 2017, and NCCN 2021 guidelines is presented in [Table 2](#).<sup>38,64,65</sup>

Upon routine hematological examination, an increase in the number of leukocytes and a shift in the differential count to the left, and myeloid series cells in various stages of differentiations, are often found.<sup>66</sup> Leukocyte counts are often above  $25 \times 10^9/L$  with an increase in basophil count.<sup>67</sup> In the chronic phase, the blast percentage is often low or negative. Platelets are also elevated in the majority of patients.

Bone marrow examination reveals a hypercellular marrow with more than 75% cellularity, and a granulocyte-to-erythroid ratio of 10:1 to 30:1.<sup>67</sup> The increased cellularity is due to elevated myelopoiesis.<sup>64</sup> An increase in cellularity is also observed in other leukemia. In CML, basophilia in bone marrow is often observed.

An increase in megakaryocytopoietic activity with hypolobated nuclei and micromegakaryocytes is often observed in the bone marrow.<sup>68</sup> Examinations of bone marrows by Mughal et al showed the presence of megakaryocytic clustering in 60.6% of patients and found that megakaryocytic clustering was correlated with a high Sokal score.<sup>69</sup> Elevated reticulin fibrosis in the bone marrow has been observed in approximately 30% of CML patients and can be associated with worse symptoms; however, this has not been

**Table 2** Baseline Diagnostic Workup Recommended by the ELN 2020, ESMO 2017, and NCCN 2021 Guidelines

ELN 2020	ESMO 2017	NCCN 2021
<ol style="list-style-type: none"> <li>1. Physical examination with particular reference to spleen and liver size</li> <li>2. Complete blood cell count with microscopic differential</li> <li>3. Bone marrow aspirate for cytologic examination and cytogenetics; core biopsy if dry tap</li> <li>4. Fluorescence in situ hybridization (FISH) only in the case of Ph negativity</li> <li>5. Qualitative reverse-transcription polymerase chain reaction (PCR) for the detection of BCR-ABL1 transcripts and identification of the transcript type</li> <li>6. Electrocardiogram</li> <li>7. Standard biochemical profile with hepatitis B serology</li> </ol>	<ol style="list-style-type: none"> <li>1. Blood counts and differential</li> <li>2. Bone marrow cytology</li> <li>3. Bone marrow karyotype</li> <li>4. Qualitative RT-PCR</li> <li>5. Mutational analysis in the accelerated phase or blast phase</li> </ol>	<ol style="list-style-type: none"> <li>1. History and physical exam, including palpation of spleen</li> <li>2. Complete blood count with differential</li> <li>3. Chemistry profile</li> <li>4. Hepatitis B panel</li> <li>5. Bone marrow aspirate and biopsy for morphologic and cytogenetic evaluation</li> <li>6. Quantitative reverse-transcription polymerase chain reaction (RT-PCR)</li> </ol>

**Notes:** For additional information, readers should refer to CML guidelines by ELN 2020, ESMO 2017, and NCCN 2021. Adapted from *Ann Oncol*, 1(28), Hochhaus A, Saussele S, Rosti G, et al. Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. iv41–51, Copyright 2017, with permission from Elsevier<sup>64</sup> and with adaptation from these other studies.<sup>38,65</sup>

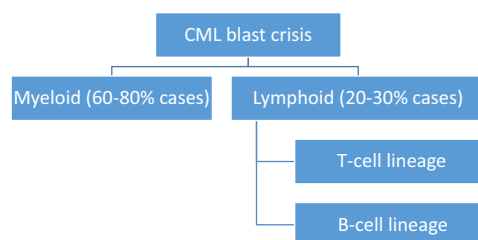
incorporated into the current CML prognostic stratification, similarly to megakaryocytic clustering.<sup>64,70</sup> The blast proportion in the chronic phase should be <15%, and a number above this indicates progression to the accelerated phase or blast phase.<sup>64</sup> Immunohistochemistry through CD34 staining may be used to help blast identification.<sup>71</sup>

Cytogenetic analysis and qualitative PCR are recommended for CML diagnosis.<sup>38,64</sup> In cytogenetic analysis, the Philadelphia chromosome may not be identified in some patients due to abnormal locations of the BCR-ABL1 gene. Hence, a FISH examination should be conducted to identify the location.<sup>38</sup> PCR examination of BCR-ABL transcript is the most reliable method of diagnosing CML. However, false-negative qualitative and quantitative PCR results may occur due to atypical BCR-ABL1 transcripts.<sup>64</sup>

The accelerated CML phase occurs after one to three years of the chronic CML phase.<sup>1</sup> This phase can be suspected when there is an enlargement of the spleen that had shrunk previously following therapy, anemia that becomes more severe, or the development of petechiae or ecchymosis. The presence of a fever is usually a sign of infection. The majority of chronic CML patients will enter the accelerated phase before the blast phase.

The blast phase is characterized by symptoms of extramedullary blast infiltration in organs other than the liver and spleen, such as the lymph nodes, central nervous system, and lungs. Indeed, both the WHO and ELN guidelines use this symptom as one of the criteria for blast-phase diagnosis.<sup>38,72</sup> A high blast percentage further differentiates this phase from the accelerated phase. According to the ELN 2020 CML guidelines, the blast percentage needed for diagnosis is  $\geq 30\%$  in the peripheral blood or bone marrow.<sup>38</sup> This is in contrast with the WHO guideline, which state that a blast percentage of  $\geq 20\%$  is needed.<sup>72</sup> Overall, it is recommended to check for ACAs and gene mutations in the blast phase or when TKI treatment does not produce an adequate response. Furthermore, ACAs and gene mutations are useful for determining treatment.

The blast phase of CML resembles acute leukemia. The blast type is myeloid in up to 80% of cases, or lymphoid in up to 30% of cases (Figure 1).<sup>73</sup> In lymphoid blast crisis, B-cell lineage is much more common than T-cell lineage.<sup>74,75</sup>

**Figure 1** Type of blast phase in CML.

Lymphoid blast crisis presents a unique diagnostic and therapeutic challenges. Readers are suggested to refer to an article by Yohannan et al for further information.<sup>23</sup>

## Therapies for CML

### Chemotherapies

Busulfan was one of the earliest chemotherapies used for CML. In the past, busulfan was administered until leukocyte counts reduced to below  $30 \times 10^9$  /L. As is the case for all chemotherapies, busulfan is associated with serious adverse effects. Those adverse effects include aplasia, pulmonary fibrosis, and severe infection.

Therapy with short-duration hydroxyurea can be given to CML patients with high leukocyte or high platelet counts with the aim of reducing leukocyte and/or platelet levels prior to the administration of a TKI.<sup>76</sup> The recommended duration of administration of hydroxyurea is not stated in the ELN's CML guideline. Another alternative to the use of hydroxyurea is apheresis (in certain conditions, such as hyperleukocytosis with leukostasis), but this is rarely used because the decrease in leukocytes following apheresis is only temporary. Nowadays, chemotherapies for CML have mostly been abandoned in favor of more effective therapies such as TKIs.

### Interferon Alpha

Prior to the TKI era, the use of IFN $\alpha$  was dominant as a first-line therapy. Since the emergence of TKIs, IFN $\alpha$  has not been used as a first-line therapy because TKIs provide better survival and a better molecular response. However, several recent studies have shown that the combination of pegylated-IFN $\alpha$  (PEG-IFN $\alpha$ ) with imatinib provides a faster and more potent molecular response.<sup>77,78</sup> Currently, several clinical studies of the combination of PEG-IFN $\alpha$  with nilotinib or other TKIs are underway.

### Stem Cell Therapy

The use of stem cell transplantation as an initial therapy has now been replaced by TKI therapy. Allogeneic stem cell transplantation may be considered for chronic CML patients who fail to respond to treatment with at least two TKIs. According to the European Society for Medical Oncology (ESMO) guidelines, chronic-stage CML patients who have a T315I mutation are also recommended for stem cell transplantation after a ponatinib trial.<sup>38</sup> Patients who are at high risk for transformation to the blast phase are candidates for stem cell transplantation because the outcomes of stem cell transplantation in the blast phase are poor.<sup>79</sup>

### Tyrosine Kinase Inhibitors

The first-line therapy for CML is a tyrosine kinase inhibitor (TKI), except in pregnant women. TKI therapy is a targeted therapy that inhibits the tyrosine kinase pathway in CML, which has been proven in clinical trials to provide better outcomes than previous CML treatments such as hydroxyurea, interferon alpha, and busulfan.

The first trial of a TKI in CML was imatinib compared with interferon and low-dose cytarabine in the newly diagnosed chronic-phase chronic myeloid leukemia (IRIS) study, which showed that 76.2% percent of CML patients given imatinib achieved a complete cytogenetic response (CCyR) after a median follow up of 19 months.<sup>80</sup> Meanwhile, in the cytarabine +IFN- $\alpha$  group, the CCyR only reached 14.5%. This striking difference indicated that imatinib provided significantly better CCyR outcomes in the CRF patients. In addition, imatinib provided better survival rates than previous therapies. Therefore, after the publication of this study, TKIs became the first-line therapy used for CML, replacing IFN- $\alpha$  and chemotherapy.

Imatinib works via competitive inhibition of the ATP-binding site in the kinase domain, which subsequently blocks the ability of BCR-ABL to phosphorylate tyrosine residues.<sup>81</sup> Side effects of TKI treatment include hypertension, thrombosis, and pleural effusion.<sup>82,83</sup> Currently, the mechanisms that cause these side effects are unknown and are the subject of ongoing investigation.

With continuous treatment, approximately 90% of CML patients treated with imatinib have a similar survival to with people without CML. However, the remaining 10% of patients display resistance to imatinib, shown by failure to achieve a molecular response; these patients can often be treated with a second-generation TKI such as dasatinib or nilotinib.<sup>38</sup> It should be noted that even with TKI treatments, there is a continuous presence of LSCs, as TKI usually does not eliminate

quiescent stem cells.<sup>52</sup> A strategy that is currently being developed to eliminate LSCs is the combination of a TKI with thiazolidinediones. Thiazolidinediones, in a study by Prost et al, were shown to impair hematopoiesis through PPAR- $\gamma$ -STAT5 signaling activation, which may be of benefit in destroying quiescent stem cells.<sup>84</sup>

The second-generation TKIs consist of dasatinib, nilotinib, bosutinib, and radotinib. They tend to have a higher potency in inhibiting BCR-ABL1 mutants.<sup>85</sup> The DASISION trial showed that dasatinib 100 mg once daily was superior to imatinib 400 mg once daily in the outcomes of a MMR.<sup>86</sup> The outcome of MR4.5 was also higher from dasatinib than imatinib. The recent DASCERN study also showed a better MMR outcome from dasatinib.<sup>87</sup> Similarly, other second-generation TKIs (nilotinib, bosutinib, and radotinib) have higher MMR responses than imatinib.<sup>88–90</sup>

Ponatinib is a third-generation TKI which has been shown to be more efficacious than other TKIs (Approximately 500 times more potent than imatinib) and is a TKI that has activity against T31UI BCR-ABL1 point mutation.<sup>38,91,92</sup> Recommended dose for ponatinib is 45 mg daily but this can be lowered into 30mg daily or 15 mg daily if side effects of thrombotic events, vascular occlusions, and heart failure are of major concern.<sup>1,38,93</sup>

## TKI Resistance

CML patients with TKI resistance should be treated with a second-line TKI and be examined for BCR-ABL1 mutations as the most common cause of TKI resistance is due to point mutations in the kinase domain of BCR-ABL1.<sup>94</sup> These point mutations cause a decrease binding affinity of TKI.<sup>95</sup> Recommended method to detect BCR-ABL1 resistance mutations is next generation sequencing (NGS).<sup>38</sup>

The type of TKI given depends on the BCR-ABL1 resistance mutations found. For example, patients with a T315I mutation are recommended to be treated with ponatinib only as patients harboring this mutation have resistance to other TKIs.<sup>38,96</sup> In contrast, patients with a Y253H, E255V/K, F359V/I/C mutations can be treated with dasatinib, bosutinib, or ponatinib.<sup>38</sup> ELN 2020 CML guideline has a list of recommended tyrosine kinase inhibitors for BCR-ABL1 resistance mutations.<sup>38</sup> In general, second-generation TKIs are recommended when there is BCR-ABL1 point mutations. The selection of second-generation or third-generation TKIs should be based on a balance between efficacy and side effects.<sup>1</sup>

## Treatment Response

The goal of therapy in CML is to achieve a hematological response, a cytogenetic response, and a long-term major molecular response. A complete hematological response (CHR) is defined as a normal leukocyte count, a normal platelet level, a normal differential result, and the disappearance of CML symptoms.<sup>97</sup> A partial hematological response is defined as a decrease in leukocyte count of less than 50% of the leukocyte level before treatment, or normalization of the leukocyte level with persistent splenomegaly or the presence of blasts in the peripheral blood. Meanwhile, a complete cytogenetic response (CCR) is defined as the absence of metaphase Ph+ in the bone marrow cells.<sup>97</sup>

Currently, the major molecular response is more often used to monitor therapy, especially for TKI therapy.<sup>97</sup> Treatments such as chemotherapies and interferon alpha rarely achieve a major molecular response. Molecular responses should be quantified on an international scale using the ratio of BCR-ABL1 transcripts to ABL1 transcripts.<sup>98</sup> A molecular response of BCR-ABL1 1% is equivalent to that of CCR. The molecular response of BCR-ABL1 0.1% is defined as a major molecular response (MMR) or MR<sup>3</sup>. The molecular response of BCR-ABL1 0.01% is defined as MR<sup>4</sup> (Table 3). There is also the term deep molecular response (DMR), defined as MR<sup>4</sup> and above.<sup>99</sup> CD26, perhaps as a marker of LSCs, can be used as an alternative to detect response, especially for BCR-ABL-independent CML cells; however, it is also difficult to detect small numbers of CD26-positive LSCs.<sup>100</sup> Thus, currently, the monitoring of CML patients revolves around the molecular response.

## Prognosis

There are currently four prognostic systems for CML: (1) Sokal, (2) Euro, (3) EUTOS, and (4) ELTS.<sup>101,102</sup> However, it should be noted by clinicians that the Sokal score was developed in the chemotherapy era. Meanwhile, the Euro score was developed in the interferon alpha (IFN $\alpha$ ) era. Thus, the eras in which these scoring systems were developed may reduce the validity of the Sokal and Euro scores, because almost all CML patients today are treated with a TKI. Indeed, it is now recommended that the EUTOS and ELTS scores be used instead.

**Table 3** BCR-ABL1 International Scale and Molecular Response

BCR-ABL1 International Scale	Log Reduction	Molecular Response	Categories
10	1	MCyR	Non-Deep Molecular Response
1	2	CCyR	
0.1	3	MMR	
0.01	4	MR <sup>4</sup>	Deep Molecular Response
0.0032	4.5	MR <sup>4.5</sup>	
0.0001	5	MR <sup>5</sup>	
0.00001	6	MR <sup>6</sup>	

**Abbreviations:** McyR, major cytogenetic response; CCyR, complete cytogenetic response; MMR, major molecular response.

The European treatment and outcome study (EUTOS) scoring system predicts CCR at 18 months after TKI therapy. In contrast to other scoring systems, the EUTOS long-term survival (ELTS) score predicts the mortality risk of CML patients given a TKI therapy using the same variables as the Sokal scoring system. Currently, the ELN recommends the use of the ELTS scoring system compared to the other scoring systems.<sup>38</sup>

## Treatment-Free Remission

Previously, the aim of CML therapy was to delay progression to the blast phase, minimize symptoms, and increase overall survival. These aims are mostly achieved in most patients today. Hence, new outcomes for CML patients, such as treatment-free remission with TKI discontinuation, are now being pursued, especially since there are concerns due to the long-term toxicity of TKI therapies. Another important reason is the cost associated with TKI therapy. Currently, research on CML is focused on achieving DMR to achieve treatment-free remission (TFR).

In general, CML patients require lifelong therapy to prevent recurrence, even when the patient has achieved an MMR status for several years; however, this trend is now changing. The antiproliferative effects of TKIs on LSCs have been shown to be strong; however, the effect on apoptosis is weaker.<sup>103</sup> The reason for this is that quiescent LSCs are resistant to apoptosis due to TKIs. A further problem is associated with the BCR-ABL-independent pathway, which maintains the survival of CML stem cells. A study by Jeanpierre et al showed that quiescent stem cells activated BMPR1B, Stat3, and BMP4-niche signals to maintain their survival.<sup>104</sup> These signals may not be targeted by TKIs, especially early-generation TKIs.

Recently, it has become known that CML patients who successfully achieve a long-lasting DMR can achieve complete recovery. Such patients have a lower risk of CML relapse when TKI therapy is discontinued. However, only a small proportion of CML patients can achieve a DMR status with their current therapy, due to the presence of CML stem cells left over after therapy. Several trials have shown that a significant proportion of CML patients (approximately 60%) with undetectable minimal residual disease still have a relapse, shown by the identification of BCFR-ABL1-positive cells within the first 2 years after imatinib discontinuation.<sup>7,105,106</sup>

Based on the results of the stop imatinib (STIM) clinical trial, with a median follow-up of 77 months, 39% of CML patients who achieved MR<sup>4</sup> status for two years were able to maintain their remission status without TKI therapy.<sup>105</sup> A meta-analysis by Campiotti et al (2017) of 15 cohort studies of patients with undetectable BCR-ABL1 transcript criteria showed that the mean molecular recurrence rate was 51% (95% CI 44–58%; I<sup>2</sup>: 55%).<sup>107</sup> The percentage of TFR in patients who achieved an MMR alone without a DMR was lower than that in patients who achieved a DMR. This result can be interpreted as showing that a significant proportion of patients may achieve TFR.

From all of the available studies, it can be inferred that only CML patients who achieve DMR are candidates for treatment-free remission (Table 4). The factors associated with successful TFR have not yet been established. However, a study by Irani et al demonstrated that CML patients who achieved TFR had higher levels of natural killer (NK) cells.<sup>108</sup> This finding was similar to the results of a sub-analysis of EURO-SKI.<sup>109</sup> It should be noted that even in patients who

**Table 4** Summary of TFR Studies

Trials	Year of Publication	Country	Sample Size (n)	TKI Used	Minimum TKI Treatment Duration	DMR Definition Used	Minimum DMR Duration	Results of TFR Rate After Stopping Treatment
5-year update of the ENESTfreedom trial <sup>110</sup>	2021	Multiple countries	190	Nilotinib	2 years	MR <sup>4,5</sup>	1 year	42.6% at 5 years
ISAV <sup>111</sup>	2020	Italy	107	Imatinib	2 years	UMRD	18 months	48% at 36 months
DADI (2020) <sup>112</sup>	2020	Japan	58	Dasatinib	3 years	DMR	2 years	55% at 6 months
Fava et al <sup>113</sup>	2019	Italy	293	Imatinib, Nilotinib, dasatinib, bosutinib	7 years	MR <sup>4</sup>	Median of 46 months (IQR 31;74)	62% (95% CI: 56;68) at 34 months; 68% (95% CI: 62;74) for imatinib, 73% (95% CI: 64;83) for second generation TKI
Pagnano KB <sup>114</sup>	2019	Brazil	48	Imatinib	3 years	MR <sup>4</sup>	2 years	61% (95% CI 47–75) at 20 months
DASFREE <sup>115</sup>	2019	USA	84	Dasatinib	2 years	MR <sup>4,5</sup>	1 year	46% at 2 years
NILSt <sup>116</sup>	2019	Japan	87	Imatinib, nilotinib	2 years	MR <sup>4,5</sup>	2 years	60.9% (90% CI 51.6–69.7)
EURO-SKI <sup>117</sup>	2018	Europe	755	Imatinib, nilotinib, dasatinib	3 years	MR <sup>4</sup>	1 year	50% (95% CI 46–54) at 24 months
JALSG-STIM231 <sup>118</sup>	2018	Japan	68	Imatinib	3 years	MR <sup>4</sup>	2 years	67.6% at 12 months
D-STOP <sup>119</sup>	2018	Japan	54	Dasatinib	2 years	DMR	2 years	62.9% (95% confidence interval: 48.5%-74.2%) at 12 months
STAT2 <sup>120</sup>	2018	Japan	78	Nilotinib	2 years	MR <sup>4,5</sup>	2 years	62.8% (95% CI: 51.1–73.5%) at 24 months
DOMEST <sup>121</sup>	2018	Japan	99	Imatinib	2 years	MR <sup>4</sup>	2 years	64.3% at 24 months
Hernandez-Boluda et al <sup>122</sup>	2018	Spain	236	Imatinib, Nilotinib, Dasatinib, Bosutinib, Ponatinib	3 years	MR <sup>4,5</sup>	2 years	64% (95% CI: 55–72) at 4 years
Mahon <sup>123</sup>	2018	Multiple countries	126	Imatinib, nilotinib	3 years	MR <sup>4,5</sup>	1 year	53% (95% CI: 44% to 62%) at 96 weeks

(Continued)

Table 4 (Continued).

Trials	Year of Publication	Country	Sample Size (n)	TKI Used	Minimum TKI Treatment Duration	DMR Definition Used	Minimum DMR Duration	Results of TFR Rate After Stopping Treatment
Long-term STIMI <sup>7</sup>	2017	France	100	Imatinib	3 years	Undetectable minimal residual disease by quantitative PCR	2 years	38% (95% CI, 29% to 47%) at 60 months
ENESTfreedom <sup>124</sup>	2017	Multiple countries	190	Nilotinib	2 years	MR <sup>4,5</sup>	1 year	51.65 (95% CI: 44.2–58.9) at 48 weeks
STOP 2G <sup>125</sup>	2017	France	60	Nilotinib, dasatinib	3 years	MR <sup>4,5</sup>	2 years	53.57% (95% CI, 40.49% to 66.65%) at 48 months
KID <sup>106</sup>	2016	Korea	90	Imatinib	3 years	MR <sup>4,5</sup>	2 years	58.5% ± 5.2% at 24 months
TRAD <sup>126</sup>	2016	Canada	67	Imatinib, dasatinib	3 years	MR <sup>4,5</sup>	2 years	64.7% (48.7–76.7%) at 6 months
DADI (2015) <sup>127</sup>	2015	Japan	63	Dasatinib	1 year	DMR	3 years	49% (95% CI 36–61) at 6 months
TWISTER <sup>128</sup>	2013	Australia	40	Imatinib	3 years	Undetectable minimal residual disease by quantitative PCR	2 years	47.1% at 24 months
ASTIM <sup>129</sup>	2013	France	80	Imatinib	3 years	Undetectable minimal residual disease by quantitative PCR	2 years	61% (95% CI, 51% to 73%) at 36 months
HOVON <sup>130</sup>	2013	Netherlands	15	Imatinib	2 years	MR <sup>4,5</sup>	2 years	33% (CI 44–88%) at 24 months
STIM2 <sup>131</sup>	2013	France	124	Imatinib	3 years	DMR	2 years	61.2% at 12 months
Ho-Young Yhimetal <sup>132</sup>	2012	South Korea	14	Imatinib	2 years	DMR	Unknown	28.6% at 12 months
Kieo STIM <sup>133</sup>	2012	Japan	40	Imatinib	2 years	DMR	2 years	55.4% at 12 months
STIMI <sup>105</sup>	2010	France	100	Imatinib	3 years	Undetectable minimal residual disease by quantitative PCR	2 years	41% at 24 months

achieve a DMR, LSCs are not removed completely. It can be speculated that the patients that achieve TFR have an adequate immunological response in suppressing residual LSCs.

## Predictors and Biomarkers for TFR

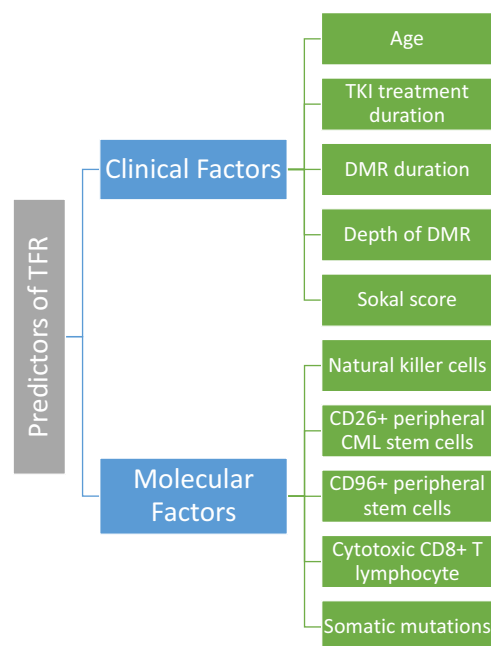
Clinical parameters and biomarkers that can be used to predict TFR in CML patients are still under investigation and this remains a topic of interest (Figure 2). Vigón et al identified several immunological parameters that predict relapse.<sup>134</sup> These parameters are low levels of natural killer cells, natural killer T cells, and CD8<sup>±</sup>TCR $\gamma\beta$ <sup>+</sup> T cells.<sup>134</sup> Similarly, Rea et al found that higher number of natural killer (NK) cells were observed in CML patients that did not relapse.<sup>135</sup> Both CD26 and CD93 which are circulating CML stem cell markers appear to be correlated with TFR as well.<sup>51,136,137</sup> Other possible markers include regulatory T cells, cytotoxic CD8<sup>+</sup> T lymphocyte (CTL), and somatic mutations.<sup>134,138–140</sup> Type of BCR-ABL1 transcript also has a role; in a study by D'Adda et al, TFR maintenance was observed to be higher in CML patients with e14a2 transcript when compared with e13a2 transcripts.<sup>141</sup> However, all of these biomarkers are not readily available for routine clinical use, and thus, clinical parameters remain the most widely used or accessible parameters for TFR.<sup>142</sup>

Many studies have data that can be used to identify clinical parameters that can be used for TFR predictions.<sup>115,143–145</sup> In general, longer duration of TKI therapy, longer DMR duration, older age, undetectable BCR-ABL1 3 months after TKI discontinuation, and deeper DMR status are observed as predictors for TFR.<sup>115,143,144,146,147</sup> Recent study by Kim et al proposed six years imatinib treatment and 4.5 years of MR<sup>4</sup> response as the optimal duration for starting TFR.<sup>147</sup>

It is speculated that adding concomitant ruxolitinib or pembrolizumab to patients' TKI therapy may produce a beneficial outcome.<sup>148</sup> In the future, it is hoped that treatment-free remission can become more achievable for CML patients and can also become a treatment outcome for other cancers as well.

## Side Effects of TKI Discontinuation

Several long-term CML patients who have been treated with a TKI have had side effects when discontinuing their TKI. Side effects of TKI discontinuation include mostly muscle and joint pain. These side effects can last up to a few months and can be treated with supportive care such as NSAIDs. Around 30% of patients may experience musculoskeletal pain after TKI discontinuation.<sup>143,149,150</sup> Musculoskeletal pain may be experienced in different regions of the body such as the



**Figure 2** Known predictors of treatment-free remission.

shoulders, extremities, hips, hands, and feet.<sup>150</sup> A study by Hernandez-Boluda et al revealed that patients that stopped TKI had elevated cholesterol level after discontinuing with the exception of nilotinib.<sup>122</sup>

Currently, the mechanisms of TKI withdrawal symptoms are unclear.<sup>106</sup> It is speculated that TKIs inhibit c-kit and platelet-derived growth factor receptor (PDGFR), which results in an increased number of osteoclasts and osteoblasts.<sup>149,150</sup> A small study by Katagiri et al found that patients with symptoms of musculoskeletal pain after discontinuing TKI treatment had a lower body weight and lower BMI, but other factors that can predict whether patients will develop musculoskeletal pain are still unknown.<sup>149</sup>

Generally, the side effects of TKI discontinuation are not a major concern, as most patients' symptoms resolve within 3 months and most of the symptoms are mild.<sup>151</sup> However, clinicians should be aware of the side effects and educate patients as needed to prepare them for this possibility.

## Conclusion

Many advances have been made in improving our understanding of the pathophysiological processes of CML. However, the etiology of the Philadelphia chromosome and mechanisms of LSC persistence are still under investigation. Somatic mutations and additional chromosomal abnormalities are associated with CML progression into blast phase. The frontline treatment of CML currently is TKI as it is more effective than previous therapies. The current aim of CML treatment is to achieve TFR, and CML patients who achieve a DMR and receive long-term TKI therapy have a higher likelihood of achieving this. In the future, it is hoped that more patients can achieve treatment-free remission.

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