

Review Article

Immunological Control of Chronic Myeloid Leukemia Leading to Treatment-Free Remission

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Future Aspects of Chronic Myeloid Leukemia Treatment

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Abstract

Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disorder of hematopoietic stem cells caused by a formation of the *BCR-ABL1* chimeric gene, which encodes an aberrant tyrosine kinase with oncogenic activity. Despite the introduction of Tyrosine Kinase Inhibitors (TKIs) such as imatinib that dramatically improved the treatment of CML, CML remains incurable for the most part, and only allogeneic hematopoietic stem cell transplantation can eradicate and cure CML. This is probably because quiescent leukemic stem cells are resistant to TKIs. However, some CML patients are able to discontinue imatinib treatment after achieving a durable deep molecular response, although they still have very low levels of residual leukemic cells. This implies that immunological control also plays a critical role in minimizing CML cells and helps maintain a complete molecular response. Indeed, recent clinical trials have suggested that the combination of imatinib with interferon- α yields stronger molecular response rates and an improved possibility of treatment-free remission. Moreover, identification of several leukemia-specific antigens is promising for the development of vaccination against CML. As a specific prognostic factor that could determine which patients can safely discontinue the treatment, natural killer cells were recently revealed to be critically important. However, cytotoxic T lymphocytes specific for CML cells could also be a good candidate for this purpose. This review summarizes the recent advances in the novel immunological aspects of CML therapy leading to treatment-free remission under immunological control of CML.

Keywords

- Chronic myeloid leukemia
- Tyrosine kinase inhibitor
- Immunological control
- NK cells
- cytotoxic T lymphocytes
- IFN- α

ABBREVIATIONS

CML: Chronic Myeloid Leukemia; CMR: Complete Molecular Response; CTL: Cytotoxic T Lymphocyte; GIST: Gastrointestinal Stromal Tumor; HSC: Hematopoietic Stem Cell; IFN: Interferon; LGL: Large Granular Lymphocyte; NK: Natural Killer; TKI: Tyrosine Kinase Inhibitor; WT1: Wilms' Tumor Antigen 1.

INTRODUCTION

Chronic Myeloid Leukemia (CML) is a clonal neoplasia of Hematopoietic Stem Cells (HSCs) that causes marked increases in white blood cells and platelets [1]. The disease progresses through chronic, accelerated, and blastic phases, and it results in death within a few years after diagnosis without appropriate

treatment. It is characterized by the presence of the Philadelphia chromosome due to reciprocal translocation between chromosomes 9 and 22 and by the formation of the *BCR-ABL1* chimeric gene, which encodes an aberrant tyrosine kinase with oncogenic activity.

Tyrosine Kinase Inhibitors (TKIs) currently represent the standard treatment for patients with CML. Imatinib (Glivec®) is the first TKI used to treat chronic phase CML patients, and it is currently used as the first line drug [2-4]. Imatinib has significantly prolonged patients' survival, and it has replaced interferon (IFN)- α treatment because it provides high cytogenetic and molecular response rates with fewer side effects [3].

However, approximately 20% of CML patients develop resistance to imatinib during the first 5 years of treatment [3]. Therefore, second generation TKIs such as dasatinib (Sprycel®), nilotinib (Tasigna®), and bosutinib (Bosulif®) were developed, all of which are currently approved for the treatment of CML [5-9]. Since some patients may develop resistance due to the mutations in *BCR-ABL1* kinase domain, third generation TKIs such as ponatinib (Iclusig®) have also been developed [10].

Rapid disease relapse usually occurs after TKI discontinuation probably due to the quiescent leukemic stem cells that are resistant to TKI therapy [11-15]; therefore, TKI-based therapy is considered to be lifelong. However, recent evidence suggests that imatinib discontinuation is possible, despite very low levels of residual leukemic cells, in some patients who achieved a durable deep molecular response [16] or in patients who were previously treated with IFN- α [17]. This possibility is probably due to immunological control of CML [18]. If more CML patients can be cured while safely discontinuing an expensive drug such as imatinib, a dramatic reduction of both personal and governmental medical expenses could be realized without sacrificing patient care.

This review summarizes the recent advances in novel immunological aspects of CML therapy aimed at treatment-free remission.

Immunostimulatory effects of TKIs

TKIs are known to block immune responses such as activation of T cells and Natural Killer (NK) cells, owing to the inhibition of off-target src kinases including c-kit and lck, which are important in the activation and proliferation of T cells and B cells [19-25]. Although imatinib seems to have almost no direct effect on the reactivity of NK cells, second generation TKIs have broader inhibition patterns [19,25]. Dasatinib was reported to abrogate cytotoxicity and cytokine production of NK cells in vitro [20,21,23], and nilotinib impaired cytokine production by NK cells at high concentrations [19,24,25]. However, in vivo immunological effects of TKIs, especially dasatinib, seem to be quite different from the in vitro effects [26,27].

In addition to BCL-ABL1, imatinib is also an inhibitor of c-kit as one of the off-target TK receptors, which is required for the malignant transformation of stromal cells of the gut in Gastrointestinal Stromal Tumors (GISTs) [28]. Since imatinib can control the disease progression and induce stable disease in more than 80% of GIST patients, imatinib is now a standard treatment for advanced GISTs [29]. However, several reports demonstrated that imatinib may mediate antitumor effects by an alternate mode of action instead of a direct effect on tumoral c-kit mutations in GISTs [30-32]. Intriguingly, it was previously reported in mice that imatinib acts on host dendritic cells to promote activation of NK cells and their antitumor effects [31]. In addition, most GIST patients who were treated with imatinib showed activation of NK cells, which positively correlated with clinical outcome [31]. Therefore, the IFN- γ production level of NK cells after 2 months of treatment with imatinib is considered a possible independent predictor for long-term survival in advanced GISTs [32]. Moreover, imatinib was recently demonstrated to inhibit the immunosuppressive protein, indoleamine 2,3-dioxygenase,

resulting in activation of CD8⁺ T cells and induction of apoptosis of regulatory T cells in both murine and human tumors [33]. Thus, imatinib may be able to stimulate NK cells and CD8⁺ T cells indirectly through off-target TK receptors even in CML patients.

In CML or Philadelphia chromosome-positive acute lymphoblastic leukemia, approximately one half of patients under dasatinib therapy have been shown to have induced lymphocytosis, which correlates with good therapeutic response; however, it also correlates with autoimmune-like adverse effects such as pleural effusions and colitis [26,27,34-39]. The lymphocytosis is due to the increased number of Large Granular Lymphocytes (LGLs), consisting of clonally expanding CD8⁺ T cells or NK cells [27,39]. Although there is evidence that the dasatinib-associated expansion of LGLs is linked to the reactivation of cytomegalovirus [34], the molecular mechanisms whereby dasatinib induces expansion of LGLs are not well understood. Thus, dasatinib seems to have distinctly opposite in vitro and in vivo effects. To clarify the contradictory observations, detailed in vitro experiments were performed. Dasatinib added directly to functional assays of NK cells (e.g., cytotoxicity or proliferation) inhibited effector functions of NK cells, while 24 h pretreatment of NK cells followed by washout of dasatinib led to dose-dependent enhancement of their effector functions [40]. These results suggest that the opposite effects of dasatinib may be ascribed to timing and dosing of imatinib; the in vivo effects of dasatinib are highly likely to reflect only the short-term exposure of dasatinib in vitro because of its very short half-life in vivo [41].

Immunostimulatory effects of IFN- α

IFN- α has multiple activities including antiviral, antiproliferative, and immunostimulatory effects through activation of several transcription factors that regulate cell proliferation, maturation, and apoptosis [42]. IFN- α was introduced for treatment of CML patients in the early 1980s and subsequently became the treatment of choice, but it was associated with severe adverse effects and TKI therapy later replaced it [43]. Although only a small proportion of CML patients treated with IFN- α achieved a complete cytogenetic response, interestingly, these patients had prolonged survival [44,45]. IFN- α can down-regulate the expression of the *BCR-ABL1* gene [46-50] and also induce the immune system to recognize and eliminate CML cells [51-53]. Indeed, IFN- α was demonstrated to stimulate autologous Cytotoxic T Lymphocytes (CTLs), which specifically recognize BCR-ABL1 antigens and BCR-ABL1-dependent antigens [54]. One of the BCR-ABL1-dependent antigens is the leukemia-associated antigen serine protease, proteinase-3 [55,56]. PR1-specific CTLs, which recognize the proteinase-3 peptide in an HLA-A2-restricted manner, are able to specifically eliminate CML progenitors [55,57,58]. The induction of a PR1-specific T-cell response by IFN- α was reported to contribute to improved molecular response in patients treated with combination therapy using imatinib and IFN- α [59]. In addition, the PR1-specific CTLs were demonstrated to mediate antitumor immunity after IFN- α withdrawal and to contribute to continued cytogenetic remission without subsequent treatment by IFN- α [57]. Thus, long-term persistence of tumor-reactive CTLs may be necessary to control the outgrowth of residual leukemic cells and prevent relapse.

Recent clinical studies have shown promising results that

combining IFN- α with imatinib improves the therapeutic outcome [60,61]. In addition, after prior combination therapy with imatinib and IFN- α , IFN- α monotherapy induced deeper molecular responses and enabled discontinuation of imatinib in most patients [59]. These results indicate that IFN- α could be a maintenance treatment after imatinib discontinuation, and many ongoing clinical trials are currently investigating combination therapy based on different TKIs and pegylated IFN- α . Although IFN- α is not currently used as the first-line treatment in CML, the findings to date indicate that combination therapy with IFN- α and TKIs has great potential to sustain immunological control of residual leukemic cells, particularly including CTLs against leukemic cells. Whether these CTLs could be a good biological marker to predict patients who can safely discontinue the treatment remains to be clarified in a large-scale study of patients.

IFN- α has two additional unique properties: the ability to re-achieve Complete Molecular Response (CMR) against CML with the most TKI-resistant mutation (T315I) and to promote the cell cycling of dormant HSCs. Although TKIs have greatly improved the outcome for CML patients, a small number of patients develop resistance to the treatment. The most common *BCR-ABL1* mutation is T315I, which affects the contact site of TKI to BCR-ABL1 and confers resistance to imatinib, nilotinib, and dasatinib [62]. Recently, CML patients with the T315I mutation were shown to re-achieve a CMR with IFN- α treatment or a combination of IFN- α and TKI [63-65]. Moreover, IFN- α was recently demonstrated to promote the cell cycling of normal quiescent HSCs [66,67]. If a similar mechanism of action occurs with dormant leukemic stem cells in the combination therapy of IFN- α with TKIs, IFN- α may also promote their cell cycling and thereby expose them to attack by TKIs.

Vaccination against CML

The BCR-ABL1 oncoprotein is a therapeutic target for CML, and several immunotherapies such as vaccination against CML are being investigated [68,69]. There are unique sequences of amino acids specific for CML cells in the junction region of constitutively active tyrosine kinase [68,70,71]. Anti-leukemic effect against these unique sequences may be achieved by vaccination with a suitable adjuvant or dendritic cells. Promising data from clinical trials with BCR-ABL1 peptide vaccines demonstrated the clinical usefulness of provoking anti-CML immune responses [68,69]. In addition to the BCR-ABL1, there are several antigens selectively expressed or overexpressed in CML cells. Among them, Wilms' Tumor Antigen 1 (WT1), a zinc finger transcription factor, is an attractive target for immunotherapeutic approaches for CML [72-75]. The qualities that make WT1 attractive are that it is overexpressed in most leukemias as well as in solid tumors and it is present in bone marrow HSCs but not in somatic cells [76]. In addition to WT1, several other antigens have also been reported in CML, including proteinase-3, hTERT (human telomerase reverse transcriptase), and PRAME (preferentially expressed antigen of melanoma). The specific antitumor vaccination strategy is a promising therapeutic option in combination with TKIs and/or IFN- α , and it could possibly lead to the complete eradication of CML. Indeed, IFN- α treatment was demonstrated to increase proteinase-3 expression in peripheral blood and to

induce the increase of its specific CTLs, which may contribute to sustained remission after imatinib discontinuation [59].

Prognostic factors for treatment-free remission

Although indefinite TKI therapy is currently the recommended standard in CML, permanent TKI intake raises concerns about the evolution of drug resistance, long-term safety, tolerability, and costs. Therefore, there is a significant need to find novel effective treatment options to cure CML patients, as well as specific prognostic factors that could determine which patients can discontinue the therapy without relapse. To date, several factors have been reported, including shorter time to *BCR-ABL1* negativity, male sex, low Sokal risk score, longer duration of imatinib therapy, and longer duration of CMR prior to discontinuation [16,17,77]. However, further investigation of this issue requires larger clinical studies with many more patients.

It is noteworthy that a recent study suggested that 41% of imatinib-treated CML patients with a CMR lasting more than 2 years are able to discontinue the treatment without relapse [16]. Moreover, a subset of CML patients was also demonstrated to maintain a CMR after imatinib cessation, but a highly sensitive quantitative PCR assay revealed that these patients had persistent *BCR-ABL1* DNA [18,78]. These studies clearly indicate that imatinib therapy may not need to be continued indefinitely and that some CML patients can discontinue imatinib without apparent molecular relapse despite having persistent residual CML cells. In the former study, most of the patients who experienced a molecular relapse did so within 6 months after discontinuation of imatinib, and the relapsed patients exhibited a molecular response after restarting imatinib [16]. This evidence strongly suggests that TKI therapy contributes to minimizing *BCR-ABL1*-positive CML cells, but eradicating CML cells is difficult, and therefore other factors should also be involved in maintaining the minimization of CML cells. One such factor is currently considered to be immunological control, and increasing evidence suggests that NK cells are important in controlling leukemic growth and sustaining a CMR [79,80]. It was recently demonstrated that CML patients who sustain a CMR after imatinib discontinuation have higher levels of functional NK cells than do normal subjects or patients who did not have a sustained CMR but maintained a major molecular response for more than 2 years under imatinib therapy [80]. Similarly, patients treated with IFN- α who have been able to discontinue the treatment without relapse showed increased NK cell counts [81]. Indeed, NK cells were demonstrated to be able to control the CML cells *in vivo* through absent self-recognition after implantation in irradiated bone marrow of the recipient mice [82]. The NK cell-mediated effect was based at least in part on the targeting of leukemia-initiating stem cells [82]. Although imatinib-mediated off-target effects may be involved in triggering activation of NK cells as shown in GIST patients [30-32], molecular mechanisms and determinants whereby NK cells are activated in CML patients with a deep molecular response under imatinib treatment remain to be elucidated.

Although CTL responses are also attractive candidates as a predictive factor of relapse risk after TKI discontinuation, there are almost no reports on this so far. One likely reason is because

generation of CTL responses might be more highly affected by the TKI-mediated inhibition of off-target kinases than activation of NK cells. For instance, one of these off-target kinases, lck, binds to CD4 and CD8 and plays a critical signaling role in the selection and maturation of responding T cells [83]. Therefore, CML patients have lower numbers of CD8⁺ T cells under imatinib treatment, and this number returns to the level of healthy controls after imatinib discontinuation [80]. However, results from allogeneic HSC transplantation have shown curative anti-leukemic effects mediated by alloreactive CTLs. One of several mechanisms by which IFN- α might contribute to control of CML is also considered to be mediated by increasing the expression of proteinase-3 and augmenting its CTL response [59]. In some dasatinib-treated patients, moreover, clonal T-cell proliferation occurs, and this phenomenon has been associated with a good response to therapy [26,35,39]. Thus, studies of CTL responses in CML patients, especially specific for CML antigens such as proteinase-3, would further help to improve the selection of candidates for a trial of TKI discontinuation.

CONCLUSION

Since allogeneic HSC transplantation is well known to cure CML patients, CML is considered to be one of the diseases most sensitive to immunological manipulation (Figure 1). Among

various TKIs, dasatinib is the most prominent. Specifically, it has strong inhibitory activity with broader specificities than other TKIs, and it is unique among the TKIs in inducing an expansion of LGLs consisting of clonally expanding CD8⁺ T cells and NK cells, which correlates with better prognosis in CML patients [26,35,39]. Therefore, it would be very interesting to identify the molecular targets of dasatinib that allow it to induce such phenomena; this might lead to the development of a new therapeutic intervention. Since TKIs were introduced into CML therapy, IFN- α treatment was almost completely abandoned, but recent clinical trials have indicated that combination therapy with IFN- α and TKIs induces higher response rates [60,61]. In addition, IFN- α treatment is highly likely to augment the possibility of safely discontinuing imatinib without subsequent relapse by enhancing responses of NK cells and CTLs [57,59]. Vaccine strategy is also very attractive, and several promising leukemia-specific antigens have been identified, such as BCR-ABL1 [68,69] and WT1 [72-75]. In addition, there are two promising new types of cancer therapy, immune checkpoint blockade therapy and engineered T-cell therapy. The former uses antibodies against immune checkpoint molecules such as CTLA-4 (cytotoxic T-lymphocyte antigen-4) or PD1 (programmed death 1), PD-L1 (programmed cell death 1 ligand 1) [84]. These antibodies inhibit immune system tolerance and exhaustion to tumors and thereby provide potentially better

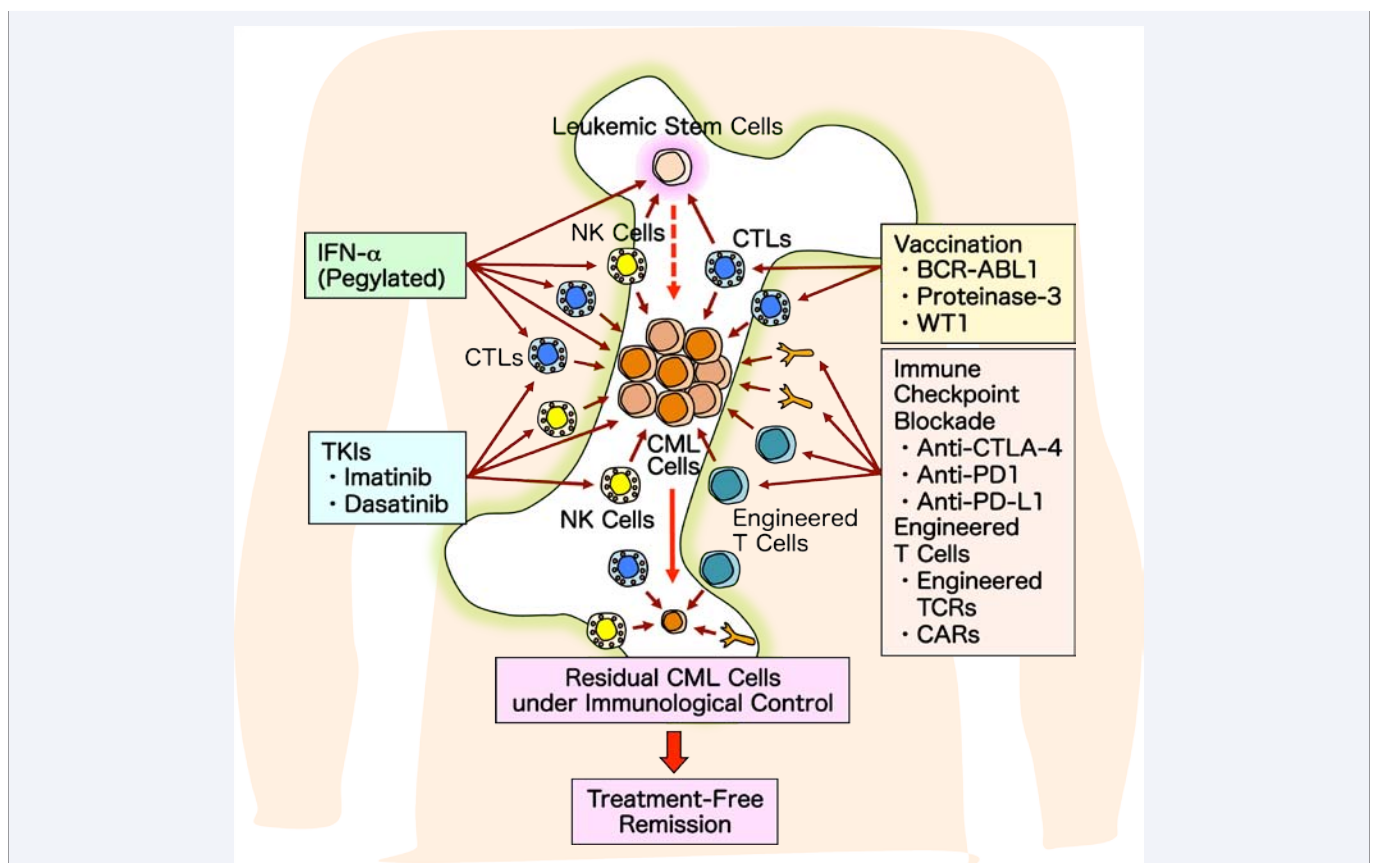


Figure 1 Immunological control of CML leading to treatment-free remission. CML is considered to be one of the diseases most sensitive to immunological manipulation. Combined and integrated immunological interventions of TKIs, IFN- α , vaccines, immune checkpoint blockade antibodies, and engineered T cells will open a new avenue to a treatment-free remission, that is, a cure. Therefore, NK cells and CML-specific CTLs could be good candidates as prognostic markers for safe discontinuation of treatment. CAR: chimeric antigen receptor; CTLA-4: cytotoxic T-lymphocyte antigen-4; PD1: programmed death 1; PD-L1: programmed cell death 1 ligand 1; TCR: T-cell receptor.

situations for augmentation of antitumor immune responses. The latter is performed by adoptive transfer using T cells engineered by introduction of genes that encode T-cell receptors or chimeric antigen receptors of desired specificity and affinity for tumors [85,86]. Notably, chimeric antigen receptors can recognize cell surface antigens in an HLA-independent fashion. These new promising cancer immunotherapies may also significantly contribute to eradicating and curing CML in near future.

In conclusion, combined and integrated immunological interventions of TKIs, IFN- α , vaccines, immune checkpoint blockade antibodies, and engineered T cells will open a new avenue to a treatment-free remission, that is, a cure (Figure 1). Although there is currently increasing evidence that NK cells are a good prognostic marker for safe discontinuation of imatinib [79,80], CTL specific for CML cells such as BCR-ABL1 or proteinase-3 would also be a good candidate for a prognostic marker in addition to the depth of CMR achieved and genetic background [87,88]. Further clinical studies using several sensitive HLA tetramer-peptide complexes, which recognize leukemic specific CTLs, are necessary in a large number of patients before and after discontinuation of TKIs.

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Conflict of Interest

Kazuma Ohyashiki received research support from Bristol-Myers Squibb KK and Novartis KK, and served as consultant and advisor of Novartis KK, Bristol-Myers Squibb KK and Ariad, honoraria for lecture fees from Novartis KK and Bristol-Myers Squibb KK.

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