Abstract

The prospective French cessation trial, Stop Imatinib trial (STIM) represents a breakthrough in Treatment-Free Remission (TFR) research in Chronic Myeloid Leukemia (CML) treatment with Tyrosine Kinase Inhibitors (TKIs). About 40% had long-term complete molecular response (CMR) after imatinib cessation in STIM trial. It demonstrated that imatinib cessation might be possible in monotherapy if CMR can be achieved and maintained for a certain period of time. TFR is targeted to avoid late side effects and allow fertile females to become pregnant and bear children as well as to save the treatment costs and health economics of TKI treatment. The predictive factors contributing to the success of TFR were reported in prospective clinical trials and retrospective observations. They are as follows: (1) Sokal score, (2) imatinib treatment duration, (3) history of interferon-α treatment, and (4) the duration of CMR before cessation. In addition, (5) polymorphisms of ABCG2 421A/C, (6) the BIM common deletion polymorphism, and (7) the increase in the proportion of NK cells or reduction of regulatory T cells are potential biomarkers for predicting TFR. In this review, we discussed TFR as a treatment target of CML, and some conditions for successful TFR in CML treatment with TKIs.

ABBREVIATIONS

CML: Chronic Myeloid Leukemia; TKIs: Tyrosine Kinase Inhibitors; CCyR: Complete Cytogenetic Remission; MMR: Major Molecular Response; ELN: European Leukemia Net; STIM: Stop Imatinib; bcr-abl: breakpoint cluster region-Abelson 1; PCR: Polymerase Chain Reaction; ASH: American Society of Hematology; TFR: Treatment-Free Remission; QOL: Quality Of Life; MRD: Minimal Residual Disease; BCRP: Breast Cancer Resistance Protein; SNPs: Single Nucleotide Polymorphisms; GIST: Gastrointestinal Stromal Tumor; BIM: BCL2-Like 11

INTRODUCTION

Treatment of Chronic Myeloid Leukemia (CML) with Tyrosine Kinase Inhibitors (TKIs) such as imatinib has dramatically improved the prognosis of this condition. However, the cessation of TKI treatment is considered impossible, because in vitro assays show that CML stem cells cannot be eliminated [1]. Clinically, neither Complete Cytogenetic Remission (CCyR) nor a Major Molecular Response (MMR) is sufficient to prevent recurrence after the cessation of medication [2,3]. Furthermore, progression from chronic to acute-phase disease is considered a major risk factor for treatment cessation. Such a progression is difficult to treat with TKI alone; the European Leukemia Net (ELN) guidelines and the hematopoietic tumor guidelines of the Japanese Society of Hematology prohibit TKI treatment cessation in daily practice outside planned clinical research settings [4,5]. On the other hand, treatment effects are reported to be sometimes maintained after incidental or planned treatment cessation prompted by side effects or pregnancy [6-11].

Previous prospective imatinib cessation trials

The Stop Imatinib (STIM) prospective cessation trial was announced in 2010 by a French group led by Mahon [12]. The subjects were 100 patients with CML maintaining Complete Molecular Response (CMR) without the breakpoint cluster region-Abelson 1 (bcr-abl) mRNA detected by high-sensitivity quantitative Polymerase Chain Reaction (PCR) for over 2 years. Among them, 39% had long-term CMR after imatinib cessation. In contrast, molecular genetic evidence of recurrence was found in 61% of patients within 6 months after imatinib cessation. However, re-administration of imatinib in patients with recurring disease resulted in a rapid molecular genetics effect without disease progression. Follow-up data were published by the American Society of Hematology (ASH) annual meeting in 2013, but no new relapses or disease progressions were observed [13].

The idea for this trial can be traced back to a report on an...
interferon cessation trial by Mahon 10 years earlier. Although extremely rare, CMR can be achieved through interferon treatment; in this trial, 47% of patients maintained CMR after interferon cessation [14]. This successful experience likely inspired the group to conduct a similar trial with patients administered imatinib, which results in a higher rate of CMR achievement. The results of the prospective French cessation trial represent a breakthrough in Treatment-Free Remission (TFR) research. The STIM2 trial, which involves patients without prior interferon treatment, is in progress in France. Despite the short observational period, the results are similar to those of the STIM1 trial [15].

An Australian group conducted an imatinib cessation trial with inclusion criteria similar to those of the STIM trial. The inclusion criteria for this study were patients with chronic CML who received more than 3 years of imatinib treatment and maintained CMR without bcr-abl mRNA detected by quantitative PCR for at least 2 years. Out of 40 participants, 18 (42.7%) maintained CMR after imatinib cessation. Among the 22 patients showing molecular relapse, many relapsed within 1 year of treatment cessation, which is similar to patients in the STIM trial. A rapid molecular genetics reaction was confirmed upon retreatment [16].

Analysis of the percentage of stable CMR (i.e., the percentage of patients maintaining stable CMR for over 2 years) among patients administered imatinib was possible because of meticulous quantitative PCR tests performed in Australia [17]. In other words, it is possible to estimate the proportion of new CML patients starting imatinib treatment who are eligible for future treatment cessation. Of 423 patients with chronic CML starting imatinib treatment, 36.5% have maintained stable CMR for 2 years as of 8 years later. Therefore, if all patients were to discontinue imatinib treatment, an estimated 15% of new CML patients would maintain molecular genetics effects without treatment 8 years after beginning treatment. Nevertheless, there is still room for improvement even if some patients achieve TFR.

The French STIM trial and Australian TWISTER trial demonstrate that CMR was maintained following cessation of treatment with imatinib in approximately 40% of CML patients. On the other hand, the According to STIM (A-STIM) trial was a similar prospective cessation study with similar inclusion criteria that relaxed the requirements for retreatment from loss of CMR to loss of MMR. Surprisingly, the results of this trial indicate that MMR can be maintained despite CMR loss after treatment cessation [18]. Despite the small number of patients (N = 58), the median observational period after imatinib cessation was 17.2 months, and 18 patients (31%) maintained MMR after CMR loss. The MMR maintenance rate without imatinib retreatment at 24 months was 65.6%. Although the reason why molecular genetics exacerbation was not detected in the presence of remaining lesions is unknown, some sort of tumor immunity is thought to be involved. The A-STIM trial shows that disease progression is not observed owing to retreatment after loss of MMR and that the STOP trial is in fact being conducted safely within planned clinical research settings [4,5]. However, in the 2013 ASH follow-up data for A-STIM, 1 patient who had lost MMR showed disease progression despite achieving MMR again after retreatment [19], this patient represents the first case of disease progression in prospective cessation trials. However, this was considered a “spontaneous disease progression” after re-achieving MMR with imatinib, and further examination did not reveal point mutations in ABL. Further exacerbation was prevented by administration of nilotinib (a second-generation TKI) and allogeneic bone marrow transplantation. In any case, these 3 prospective trials demonstrate imatinib cessation is possible in monotherapy if CMR can be achieved and maintained for a certain period of time.

### Treatment targets for CML with TKI

TKIs reduce the number of CML cells in a time-dependent manner. Michor et al. performed a detailed investigation of changes in bcr-abl mRNA copy number and found bcr-abl kinetics can be divided into 2 phases [20]. The most dramatic reduction of CML cells occurs in phase I and is likely a reduction of differentiated granulocyte-type cells. Meanwhile, in phase II, the reduction likely occurs in progenitor cells and cells near CML stem cells. For levels below the limit of sensitivity by PCR (i.e., unanalyzable levels), if we assume the gradual reduction of cells in phase II continues in a time-dependent manner, the number of CML clones will eventually approach 0. On the basis of observations in the STIM trial wherein nearly all molecular genetics relapse occurred within 6 months and the fact that the duration to relapse is dependent on the completeness of remission, Deininger predicts a “sustenance limit” whereby TFR can be obtained [21]. In other words, only patients achieving treatment effects below the sustenance limit, which exists in an unknown domain below the limits of PCR sensitivity, can successfully discontinue drug administration.

There are 4 classifications of treatment targets for CML. The first treatment target for all CML patients is the prevention of progression from chronic to acute-phase disease. The advent of imatinib has resulted in a long-term prognosis without progression for over 90% of patients. The goal is to achieve and maintain MMR, which represents a 3-log reduction of bcr-abl mRNA copy number and is considered a safe haven, through continued imatinib treatment [22].

The second treatment target is Quality Of Life (QOL). The aforementioned first target is achieved through continued imatinib treatment; mild side effects of the treatment are tolerated as an inevitability of anticancer agents. Therefore, varying degrees of reduced QOL are possible in many patients with CML. Clinical trials for second-generation TKIs confirm the loss of QOL due to imatinib treatment can be recovered by switching to other TKIs [23]. Furthermore, patient QOL in CML treatment has been possibly underestimated by clinicians [24]. More options for TKIs that can be used according to patient age or complications could allow treatment continuation without decreasing the QOL of all patients. In such cases, MMR or CCyR (depending on age) may be adequate molecular genetics treatment effects.

The third target is TFR. TFR refers to a state of Minimal Residual Disease (MRD) after TKI cessation without molecular genetics relapse. The results of the aforementioned prospective cessation trials indicate that it is an achievable target for some patients. Achievement of CMR and maintenance of MMR for a fixed period are required as the molecular genetics treatment effect;
the goal is to provide more patients a chance of TFR through TKI induction and reinforcement therapy or maintenance therapy with concomitant drugs besides TKIs [25].

The last treatment target is cure. In theory, there is no risk of relapse if MRD is absent. Until now, this was thought to be achievable only through stem cell transplantation; however, research on CML stem cells may lead to the development of TKI monotherapies or combination therapies capable of achieving this treatment objective. On the other hand, relapse has occurred several years after stem cell transplantation in patients thought to be cured; therefore, cure can only be determined through long-term observation.

**Reasons for targeting TFR**

TFR is targeted to avoid late side effects and allow fertile females to become pregnant and bear children, because imatinib can delay infant development [26]. Moreover, pregnancy is a frequently cited reason for discontinuation in patient reports [27-30]. The long-term late side effects of second-generation TKIs remain unknown, which could become an issue for all patients regardless of age or sex.

Meanwhile, there are concerns about the treatment costs and health economics of TKI treatment. Kantarjian along with other CML experts worldwide recently discussed these problems in Blood. Although TKIs have doubtlessly dramatically improved patient prognosis, the medical costs of continued treatment are astronomical. In Japan, the cost of imatinib, which is the least expensive option, for 1 year for 1 CML patient is approximately \$4.5 million (US$43,500). In the USA, this cost doubles to US$92,000 [31]. If TKI treatment can be successfully terminated, continuing to administer TKIs to such patients can be considered overtreatment from a health economics perspective. Indeed, in the 50-month long-term observation period of the STIM trial (a 100-patient cessation trial), an estimated €5.5 million (approximately US$7.5 million) in medical costs was saved [13].

The authors reported on an epidemiological study of patients discontinuing imatinib treatment in Japan in which 42 CML patients had terminated imatinib treatment for over 6 months for the following reasons: side effects (18 patients, 43%), economic reasons (14 patients, 33%), pregnancy (3 patients, 7%), and termination request prompted by long-term CMR (8 patients, 19%) [30]. In a retrospective study of patients discontinuing TKIs by MD Anderson, 35 patients discontinued treatment for reasons including side effects (18 patients, 51%), termination request prompted by long-term CMR (8 patients, 23%), economic reasons (6 patients, 17%), and pregnancy (3 patients, 9%) [29]. Although these 2 epidemiological studies were conducted in different countries, the reasons for cessation were similar. The aforementioned third treatment target of achieving TFR is one that addresses drug side effects and medical costs in addition to the second target of improving QOL.

**Conditions for successful TFR**

Several conditions are inferred to be required for successful TFR. The first condition for TFR is the achievement of CMR. However, several factors must be investigated, including the length of CMR maintenance before treatment cessation, whether pretreatment with interferon-α contributes to successful TFR, and whether characteristic biomarkers exist for patients who can achieve TFR.

We investigated the differences between patients who achieved CMR and those who did not after long-term imatinib treatment [32]. Evaluating the molecular genetics effects of long-term imatinib treatment in 157 CML patients achieving MMR with international scale-PCR revealed that 75 patients (approximately 50%) had achieved MR4.5. When we compared the backgrounds of the 75 patients in the CMR group (i.e., MR4.5 achieved) with the non-CMR group (i.e., MMR achieved, MR4.5 not achieved), there were no differences with respect to age, sex, physique, CML disease duration, previous treatments, treatment intensity, or treatment duration. However, the Sokal score at the time of onset (p = 0.0367) and time to MMR (median value: 18.6 months vs. 13.3 months, p = 0.0239) were significantly correlated with CMR achievement. In addition, possession of the A allele of ABCG2 at 421C/A, the gene that encodes the imatinib drug transporter BCRP (breast cancer resistance protein), was significantly correlated with CMR achievement (p = 0.0276). On the basis of the lack of difference in imatinib trough blood concentrations, we inferred differences in excretory drug transporter activation affect imatinib concentrations in leukemia cells, regulating the treatment effect. Multivariate analysis revealed imatinib treatment duration, time to MMR, and ABCG2 421C/A polymorphism were all independently associated with CMR achievement. In particular, Single Nucleotide Polymorphisms (SNPs) in ABCG2, which are observed in about 20% of the Japanese population, also contribute to MMR achievement [33], therefore, these SNPs may have affected successful TFR in the Japanese population.

We conducted a retrospective investigation of the background of patients who successfully discontinued imatinib in daily medical care in Japan [30]. Questionnaires were sent to 780 council members of the Japan Society of Hematology, and 181 (23%) responses were received. Of the 3,242 CML patients undergoing imatinib treatment (estimated to be one third of all Japanese patients with CML), 50 (1.5%) had discontinued imatinib treatment for 6 months or more for various reasons. A secondary investigation uncovered the detailed backgrounds of 43 patients, 47% of whom maintained long-term CMR after imatinib cessation. Comparing patients with and without molecular relapse within 12 months revealed significant differences with respect to median imatinib treatment duration (26.3 vs. 51.7 months, p = 0.0228), total imatinib dose (275.4 vs. 576.8 g, p = 0.0042), and history of interferon treatment (36% vs. 77%, p = 0.0102). In addition, the median duration of CMR maintenance before cessation was significantly longer in the group without relapse (6 vs. 32.5 months); therefore, we inferred these patients achieved a deeper level of remission than can be detected by PCR in a time-dependent manner. Receiver-operating characteristic curve analysis indicated successful TFR requires at least 24 months of CMR maintenance before imatinib cessation. This is coincidentally the same duration as in the patient recruitment criteria in the STIM trial. Sokal scores were low risk for most patients, and there was no significant difference between groups.
Bebjamini et al. investigated the background of patients who discontinued TKI in MD Anderson’s study [29]. Although only 27 patients achieved CMR at the time of cessation, the duration of CMR before discontinuation was 64 months, which resulted in the substantial success of TFR. Furthermore, high-dose imatinib and second-generation TKIs also contributed significantly to the success of TFR. These results suggest successful TFR requires a deep molecular genetic effect.

The predictive factors contributing to the success of treatment cessation in the STIM trial as determined by stratified subanalysis were a low-risk Sokal score and an imatinib treatment duration exceeding 50 months. This suggests that adequate treatment of chronic early-phase patients can lead to TFR in over 50% of cases [12].

The predictive factors contributing to the success of treatment cessation in the TWISTER trial were Sokal scores and pretreatment with interferon-α for over 12 months [16]. Interestingly, the presence of MRD, which was determined by DNA-PCR in the present trial, did not affect TFR prognosis. Furthermore, although the study included many patients who received long-term imatinib treatment, treatment duration was not a significant predictor of TFR when patients with treatment durations longer and shorter than the median duration of 70 months were compared.

Ohyashiki et al. performed a subset analysis of peripheral blood T/NK cells and compared them to controls; they found that NK cells had increased significantly in patients who successfully discontinued imatinib [34,35]. According to Mizoguchi et al., these patients interestingly maintain such trends for more than 3 years after cessation. The relationship between NK cells and successful TKI cessation was reported at [ASH2013] by the European-Ski and several other groups [36,37]. The theory that tumor immunity centered on NK cells continues after imatinib cessation is intriguing; it will be interesting to determine if the same observation holds true for fluctuating patients who have lost CMR but maintain MMR (i.e., patients whose bcr-abl copy number fluctuates below MMR). On the other hand, the proportion of CD8+ effector memory T cells increases during imatinib treatment and therefore contributes to neither the treatment effect nor TFR. In contrast, a German study indicates molecular relapse is unlikely if PRI-specific cytotoxic T cells increase as a result of interferon-α maintenance therapy. Our CMR study revealed regulatory T cells were suppressed in the CMR group, suggesting tumor immunity during imatinib treatment possibly contributes to both, NK- and T cell-mediated immunity [32]. A similar phenomenon is reported regarding imatinib treatment for Gastrointestinal Stromal Tumor (GIST) [38]. Immunological research on CML is limited to T- and NK cell profiling as well as observations of changes in cell numbers. Therefore, further studies are required to determine the mechanism of tumor immunity in CML.

BCL2-like 11 (BIM) is a protein associated with apoptosis and is required to induce TKI-mediated apoptosis in tumor cells. The common deletion polymorphism of intron 2 of the BIM gene is found in 20% of the Asian population (including the Japanese population), resulting in the expression of an isoform without the BH3 domain. This is known to contribute to primary resistance to TKI in CML [39]. Katagiri et al. investigated the BIM common deletion and imatinib TFR in a few patients [40]. A French group recently showed that the T allele of BIM c465C>T (rs7247110) is associated with Sokal score, resulting in longer time to MMR achievement [41]. Moreover, BIM mRNA expression is reduced in peripheral blood mononuclear cells in healthy persons with this SNP, suggesting polymorphisms of BIM may influence TKI treatment effects in non-Asians as well.

The predictive factors for TFR identified by these trials are as follows: (1) Sokal score, (2) imatinib treatment duration, (3) history of interferon-α treatment, and (4) the duration of CMR before cessation. In addition, polymorphisms of ABCG2 421A/C and the BIM common deletion polymorphism at the time of onset are potential biomarkers for predicting TFR. Finally, peripheral blood T/NK cell profiling during treatment or after TKI cessation, such as the increase in the proportion of NK cells or reduction of regulatory T cells, can act as predictors of TFR. Nevertheless, these predictive factor candidates will need to be studied in further detail in future prospective trials.

CONCLUSION

The results of the French STIM trial demonstrate the possibility of discontinuing TKI treatment in a portion of CML patients. Several TKI cessation trials under careful molecular-genetics monitoring are in progress. Although eradication of CML stem cells by TKIs was thought to be impossible, clinical studies have shown otherwise. Nevertheless, basic confirmation is required to determine whether such eradication of CML stem cells occurs as a result of TKIs alone or immunological effects on MRD play a role. Furthermore, predetermination of CML patients who are likely to succeed in cessation will increase the probability of safe cessation. Those with a lower probability of successful cessation or those in whom relapse occurs after discontinuation can also wait for effective drugs that can be used in combination with TKIs. Advancements in research for effective second attempts (i.e., reattempts at cessation) could make TFR an achievable goal for more CML patients.

CONFLICT OF INTEREST

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REFERENCES

1. Varet BR, et al. Loss Of Major Molecular Response As a Trigger For
Rousselot P, Charbonnier A, Cony-Makhoul P, Agape P, Nicolini FE,
oncology. 2013.

2. Imatinib After Durable Undetectable Disease. Journal of clinical
2013; 121: 381.

3. Deininger M. Hematology: curing CML with imatinib—a dream come

4. Hughes TP, Branford S. Monitoring disease response to tyrosine
kinase inhibitor therapy in CML. Hematology Am Soc Hematol Educ
Program. 2009.

5. Cortes JE, Lipton JH, Miller CB, Alawadhi S, Akard L, Pinilla-Ibarz
J, et al. Change in Chronic Low-Grade Nonhematologic Adverse
Events (AEs) and Quality of Life (QoL) in Adult Patients (pts) with
Philadelphia Chromosome-Positive (Ph+) Chronic Myeloid Leukemia
in Chronic Phase (CML-CP) Switched From Imatinib (IM) to Nilotinib

Patient-versus physician-reporting of symptoms and health status in

7. Ahmed W, Van Etten RA. Alternative approaches to eradicating the
malignant clone in chronic myeloid leukemia: tyrosine-kinase
inhibitor combinations and beyond. Hematology Am Soc Hematol

8. Bansal D, Shava U, Varma N, Trehan A, Marwaha RK. Imatinib has
adverse effect on growth in children with chronic myeloid leukemia.

delivery after planned discontinuation of imatinib in a patient with
chronic myeloid leukemia. See comment in PubMed Commons below

al. Pregnancy under treatment of imatinib and successful labor in
a patient with chronic myelogenous leukemia (CML). Outcome of
discontinuation of imatinib therapy after achieving a molecular

al. Patient-driven discontinuation of tyrosine kinase inhibitors: single
institution experience. See comment in PubMed Commons below

Patient-driven discontinuation of imatinib in Japanese patients with
chronic myeloid leukemia. See comment in PubMed Commons below

13. Experts in Chronic Myeloid Leukemia. The price of drugs for chronic
myeloid leukemia (CML) is a reflection of the unsustainable prices of
cancer drugs: from the perspective of a large group of CML experts.
See comment in PubMed Commons below Blood. 2013; 121: 4439-
4442.

H, et al. A multicenter clinical study evaluating the confirmed complete
molecular response rate in imatinib-treated patients with chronic
phase chronic myeloid leukemia by using the international scale of
real-time quantitative polymerase chain reaction. See comment in

15. Takahashi N, Miura M, Scott SA, Kagaya H, Kameoka Y, Tagawa H,
et al. Influence of CYP3A5 and drug transporter polymorphisms on
imatinib trough concentration and clinical response among patients
with chronic phase chronic myeloid leukemia. See comment in


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