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631.MYELOPROLIFERATIVE SYNDROMES AND CHRONIC MYELOID LEUKEMIA: BASIC AND TRANSLATIONAL

Mechanism of Late Recurrences of Chronic Myeloid Leukemia after Discontinuation of TKI Therapy: *BCR::ABL1* ^{Ins35bp} Loss-of-Function Splicing Mutation and Regulatory T Cells

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Introduction: Treatment-free remission (TFR) as one of the goals of treatment of *BCR::ABL*-positive chronic phase chronic myeloid leukemia (CML) has been proposed. Actually, approximately 40-60% of CML patients maintain TFR without recurrence after discontinuation of tyrosine kinase inhibitors (TKIs). In some of such patients, there are fluctuated cases in which *BCR::ABL* positive clones continue to be detected for long periods at levels of international scale (IS) *BCR::ABL* < 0.1%. Such fluctuated cases show no apparent molecular recurrence and maintain TFR for a long time, but they have also been shown to be more likely to eventually develop late recurrence (Haematologica 2022, Blood adv. 2020). We have reported that a higher percentage of PD-1 positive CD8 $^{+}$ T cells before and after TKI discontinuation is associated with molecular recurrence, and together with the possibility of assessing host immune response capacity after TKI discontinuation by using regulatory T (Treg) cells (Fujioka, et al. Cancers 2021). On the other hands, mutations in *BCR::ABL* caused by the alternative splicing, such as *BCR::ABL* ^{Ins35bp}, are known to be one of the TKI resistance in CML. Loss of *BCR::ABL* kinase activity is thought to result in loss of TKI sensitivity and thus failure to achieve deep molecular remission (Yuda, et al. Cancer Science 2017). In this study, we analyzed the mutations that cause splicing variants such as *BCR::ABL* ^{Ins35bp} and host immune response in CML patients to elucidate molecular mechanisms in the long-term maintenance of TFR and late recurrence.
Methods: Twenty-four CML patients who discontinued TKI treatment for any reason at Akita University Hospital were included in the study. The trends of IS level after TKI discontinuation were monitored, and immune profile analysis by multi-color flow cytometry (FCM) was performed in all patients. Mutation analysis of the *BCR::ABL* region including splicing variants was performed in selected patients. Mutation analysis was performed on samples with sufficient cell count and IS > 0.0032% (MR $^{4.5}$), and the *BCR::ABL* region of these samples was amplified by long nested PCR and then analyzed by the next-generation sequencer (NGS).
Results: First, *BCR::ABL* ^{Ins35bp} was evaluated by mutation analysis. Among CML patients who underwent mutation analysis, *BCR::ABL* ^{Ins35bp} was detected in all cases in which the samples could perform analysis at newly diagnosis (ND). Even after TKI discontinuation, a certain percentage of *BCR::ABL* ^{Ins35bp} was detected in all patients, and the percentage of *BCR::ABL* ^{Ins35bp} increased at the time of recurrence regardless of whether early or late. These results suggest that function-dead *BCR::ABL* ^{Ins35bp} clones may remain in CML patients with detectable *BCR::ABL* under and after TKI treatment, and eventually leading to recurrence with transcriptional promotion of native *BCR::ABL*. On the other hand, there were cases in which the *BCR::ABL* ^{Ins35bp} was lost at the time of recurrence. There is a possibility of immunological involvement in such cases. CML patients with detectable *BCR::ABL* in the TFR for a long time had a decreased immunosuppressive Treg cells, while analysis using late recurrence increased Treg cells prior to recurrence (figure). These suggest the presence of cases in which early recurrence is suppressed by host immune responses.
Conclusion: Although the loss of function mutations such as *BCR::ABL* ^{Ins35bp} was previously thought to occur after TKI administration as a cause of TKI resistance, this analysis reveals that the clone is included from the ND before TKI treatment. Examining the *BCR::ABL* ^{Ins35bp} clone at ND may be possible to predict the possibility of recurrence after TFR. Host immune capacity, as assessed by the kinetics of Treg after TKI discontinuation, may contribute to prolonged TFR. Thus Treg monitoring may be important in predicting molecular recurrence, especially in *BCR::ABL* ^{Ins35bp} cases.

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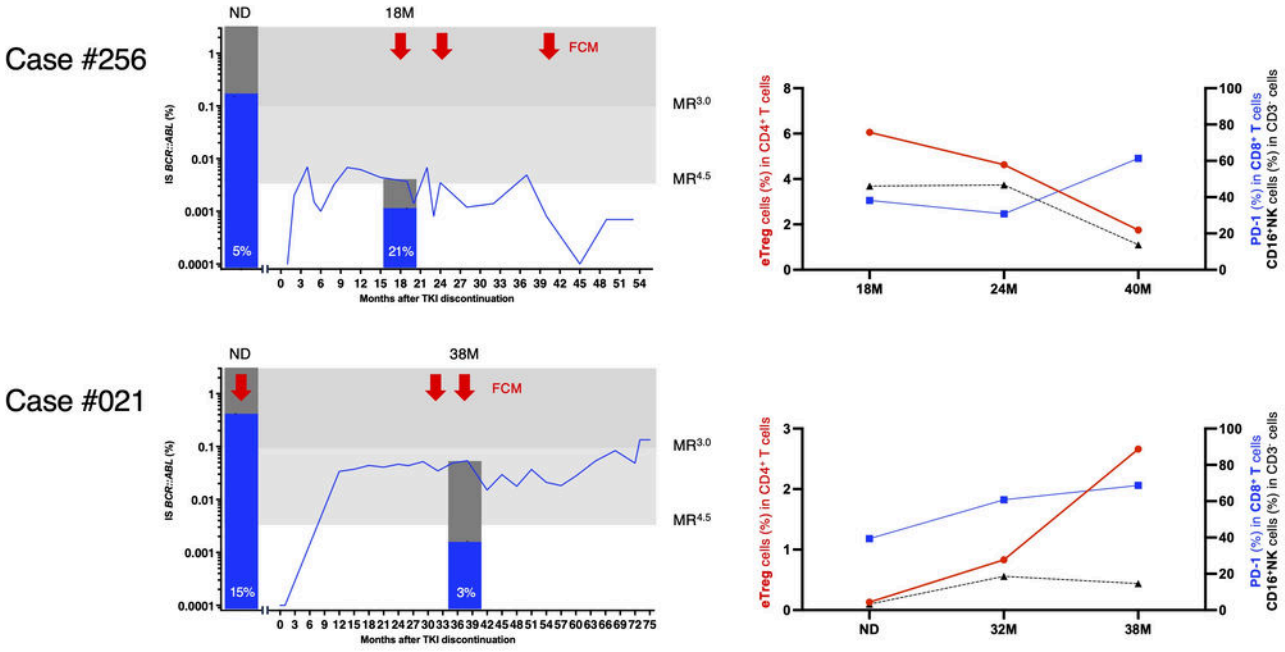

Figure. Kinetics of IS *BCR::ABL* values (left) and immune parameters (right) of representative two cases. In the left diagram, the percentage of *BCR::ABL*^{Ins35bp} (blue) to native *BCR::ABL* (grey) is shown as a bar graph. The red arrow indicate the timepoint of FCM. In the right diagram, the proportions of eTreg (red), PD-1-positive CD8$^+$ T cells (blue) and CD16$^+$ NK cells (black) are shown. Case #256 maintained TFR for 54 months and showed no increase in Treg (upper panel). Case #021 had a late recurrence at 75 months after TKI discontinuation but had shown an increase in Treg cells earlier (lower panel). ND, newly diagnosis.

Figure 1

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