Fluctuation of BCR-ABL1 qPCR IS level beyond 0.1% IS after stopping tyrosine kinase inhibitor in chronic myeloid leukaemia patients with deep molecular response for at least two years

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SUMMARY
Fluctuation of BCR-ABL1 real-time quantitative polymerase chain reaction in International Scale (qPCR IS) level below major molecular response (MMR) (0.1% IS) is a known phenomenon after stopping tyrosine kinase inhibitor (TKI) in chronic myeloid leukaemia (CML) patients who are attempting treatment free remission (TFR). We report here four cases of fluctuation beyond MMR during conduct of a Malaysia Stop TKI Trial (MSIT) to examine the validity of the commonly used relapse criterion – loss of MMR for one reading – aiming to provide evidence in setting relapse criteria for future CML patients who want to attempt TFR.

KEYWORDS:
chronic myeloid leukemia, treatment free remission, BCR-ABL1, tyrosine kinase inhibitor, major molecular response

INTRODUCTION
Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm that originates from an abnormal pluripotent bone marrow stem cell and is consistently associated with BCR-ABL1 fusion gene, which can be quantitated using real-time quantitative polymerase chain reaction (qPCR) and standardized using International Scale (IS) (qPCR IS). One of the many advancements in the field of CML is the concept of treatment free remission (TFR), arguing the necessity of life long tyrosine kinase inhibitor (TKI). About 40% of CML patients, who had achieved deep molecular response (DMR) (molecular response (MR) of 4-log reduction (MR4) (0.01% IS) or deeper) for at least two years, were able to stop TKI safely and remain in TFR, while 60% relapsed molecularly. Criteria of relapse used in majority of stopping TKI trials are loss of major molecular response (MMR) (0.1% IS) for one reading. Fluctuation of qPCR level below MMR (0.1% IS) is a known phenomenon after stopping TKI, probably due to interplay between the persistence of leukaemic stem cells and immunosurveillance. To our knowledge, there no detail report on fluctuation that exceeding MMR, which is probably the reason it is recommended as a criterion of relapse and used in most of the stop TKI trials. During the conduct of Malaysia Stop TKI Trial (MSIT), we observed fluctuation of qPCR IS levels beyond MMR that we feel think it is worth reporting to define safe and practical relapse criteria in CML patients who attempt TFR. Four cases of fluctuation exceeding MMR here.

MATERIALS AND METHODS
MSIT (Malaysia National Medical Research Register (NMRR): NMR-13-1186-15491; ClinicalTrials.gov: NCT02381379) is a multi-center trial in Malaysia aiming to compare the outcomes of peginterferon (pegIFN)-α-2a for a year followed by observation versus observation after stopping TKI in CML patients with DMR for two years or more. Relapse was defined as: 1) one reading of loss of MMR (0.1% IS), or 2) positivity of BCR-ABL1 transcripts in qPCR IS, as confirmed by a second analysis point, indicating the increase (≥ 1 log) in relation to the first analysis point at two successive assessments. The qPCR IS test was sent monthly for the first 12 months, 2-monthly for subsequent 12 months, and 3-monthly thereafter and done in a central laboratory.

RESULTS
Two patients (P1 and P2) in the observation arm, both from the same study site (Sultanah Aminah Hospital) relapsed according to the relapse criteria no.1, i.e. loss of MMR (see Table I). TKI was reinitiated as per protocol. However, a repeated qPCR IS, which was not prohibited in the study protocol, was done prior to the initiation of TKI, which showed DMR. Investigations showed no evidence of wrong sampling or laboratory error. After discussion, investigators decided to stop their TKI after two months of TKI intake. These two “relapse” cases challenge MMR as a relapse criterion and raise doubt on the four relapse cases (R1 to R4, see Table I) prior to the incidence. We re-examined these four cases and could only truly confirm relapse in one case, in which the previous two successive readings showed 1-log increment, fulfilled our trial relapse criterion no.2, before loss of MMR.
Table I: The four cases with fluctuation of qPCR\(^*\) beyond MMR (P1 to P4) and four relapse cases prior to P1 and P2 (R1 to R4)

<table>
<thead>
<tr>
<th>Patient (sex)</th>
<th>Arm</th>
<th>Four successive readings of qPCR(^*) (% IS) as per protocol prior to relapse as loss of MMR</th>
<th>Relapse as loss of MMR</th>
<th>Retrospectively confirm relapse</th>
<th>Outcome in Nov 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>qPCR(^*) (%)</td>
<td>Months after stopping TKI</td>
<td>Date</td>
<td></td>
</tr>
<tr>
<td>P1 (F)</td>
<td>Observation</td>
<td>0.0003</td>
<td>0.0070</td>
<td>0.0133</td>
<td>0.0001</td>
</tr>
<tr>
<td>P2 (F)</td>
<td>Observation</td>
<td>0.0057</td>
<td>0.0035</td>
<td>0.0222</td>
<td>0.0051</td>
</tr>
<tr>
<td>P3 (M)</td>
<td>pegIFN</td>
<td>0.0098</td>
<td>0.0274</td>
<td>0.0108</td>
<td>0.0337</td>
</tr>
<tr>
<td>P4 (M)</td>
<td>pegIFN</td>
<td>0.0286</td>
<td>0.0541</td>
<td>0.0816</td>
<td>0.0795</td>
</tr>
<tr>
<td>R1 (M)</td>
<td>Observation</td>
<td>-</td>
<td>-</td>
<td>0.0059</td>
<td>0.0896</td>
</tr>
<tr>
<td>R2 (F)</td>
<td>Observation</td>
<td>0.0300</td>
<td>0.0637</td>
<td>0.0117</td>
<td>0.0774</td>
</tr>
<tr>
<td>R3 (F)</td>
<td>pegIFN</td>
<td>0.0281</td>
<td>0.0354</td>
<td>0.0225</td>
<td>0.0680</td>
</tr>
<tr>
<td>R4 (F)</td>
<td>pegIFN</td>
<td>0.0083</td>
<td>0.0121</td>
<td>0.0203</td>
<td>0.0664</td>
</tr>
</tbody>
</table>

DMR, deep molecular response, also equivalent to 0.01\(^\%\) or better; F, female; M, male; MMR, major molecular response, also equivalent to 0.1\(^\%\); pegIFN, peginterferon; qPCR\(^*\), real-time quantitative polymerase chain reaction in International Scale; TFR, treatment free remission; TKI, tyrosine kinase inhibitor.
Following the incidence, study protocol was amended to include a repeated qPCR\(^6\) on the time of restarting TKI after loss of MMR, which means relapse criteria no. 1 – loss of MMR – must be confirmed by two successive readings. The TKI would be given for two months and re-stopped if the repeated qPCR\(^6\) does not confirm the loss of MMR. After the change of protocol, we had two more patients (P3 and P4) who experienced fluctuation of qPCR\(^6\) beyond MMR. In Jan 2020, P3 in pegIFN arm experienced such fluctuation (see Table I). He was restarted on TKI for two months, just like the previous two cases of fluctuation, but had not returned to us for review due to the Malaysia and Singapore lock-down during COVID-19. In May 2020, P4 in pegIFN arm experienced such fluctuation, too. However, the treating physician decided to continue his TKI after two months and withdrawn from trial.

**DISCUSSION**

From the four cases reported here, the fluctuation of qPCR\(^6\) occurred after 12 months of stopping TKI. This is probably the phenomenon caused by interplay between leukaemic stem cells and immunosurveillance\(^7,8\) compared to fast rising qPCR\(^6\) without fluctuation in most relapse cases within 6 months of stopping TKI. Retrospectively, patients R2 and R4 were probably experiencing the same fluctuation beyond MMR.

Is there an outcome difference between the loss of MMR for one reading and two readings? Up to Nov 2020, P1 and P2 have been follow-up for 47 months. P1 had true relapse (0.1235%\(^6\)) at 45 months after stopping TKI with prior increasing trend of qPCR\(^6\) and the repeated qPCR\(^6\) after relapse was 0.5324%\(^5\). There was differences of opinion among the investigators that maybe it does not matter whether loss of MMR for two readings is needed to confirm relapse because maybe loss of MMR for one reading predicts the relapse later. This awaits more data. For Malaysia setting at the moment, considering the availability and turnaround-time of qPCR\(^6\) result in hospitals of Ministry of Health of Malaysia outside of clinical trial, we would not recommend attempting TFR in our eligible CML Malaysian patients outside of clinical trial.

**CONCLUSION**

In view of the four cases reported, treating physician could consider fluctuation of qPCR\(^6\) beyond MMR before diagnosing relapse in CML patients who are attempting TFR and already stopping TKI for 12 months or more. It is safer to restart TKI once there was a loss of MMR while awaiting the result of the repeat qPCR\(^6\).

**REFERENCES**


