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REVIEW

Standardized definitions of molecular response in chronic myeloid leukemia

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The International Randomized Study of Interferon and STI571 (IRIS) demonstrated long-term cytogenetic responses in patients with chronic-phase chronic myeloid leukemia (CML-CP) treated with the tyrosine kinase inhibitor (TKI) imatinib. However, deep molecular responses (MRs), as measured by reductions in *BCR-ABL* transcript levels below the threshold of major MR, were achieved only by a small proportion of patients. With the advent of the second-generation TKIs nilotinib and dasatinib for the treatment of patients with newly diagnosed CML-CP, the proportion of patients who achieve the deepest levels of MR is likely to increase significantly. With these changes, the potential for patient eligibility in TKI cessations studies is becoming a more widely discussed topic and area for research. These developments highlight the need for robust, standardized and workable definitions of deep MRs. Specifically, it is critical that the measurement of MR is standardized in a manner to withstand both intra- and inter-laboratory variability, as well as new methodological developments. This review summarizes the relevant clinical background and proposes a framework within which standardization of MR can be taken forward.

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BACKGROUND

Most patients with chronic-phase chronic myeloid leukemia (CML-CP) on imatinib therapy achieve a complete cytogenetic response (CCyR) that is maintained for long term. As observed in the International Randomized Study of Interferon and STI571 (IRIS) trial, 86% of patients achieved CCyR on study, which was stable in the majority of cases.[1] Many patients responding to imatinib will also achieve a major molecular response (MMR), defined in the IRIS trial as a 3-log reduction, in *BCR-ABL* transcript levels from a standardized baseline representing the median value of *BCR-ABL/BCR* present at diagnosis.[2] This 3-log reduction has subsequently been defined as $\leqslant$0.1% *BCR-ABL* on the International Scale.[3] Only a minority of imatinib-treated patients achieve what has been termed complete MR (CMR), defined initially by the European LeukemiaNet as undetectable *BCR-ABL* mRNA transcripts by quantitative reverse transcriptase PCR (qRT-PCR) and/or nested PCR in two consecutive high-quality samples with a sensitivity $>10^4$.[4] Recent data, however, has highlighted the shortcomings of this definition. This is a pressing issue because, as detailed below, second-generation tyrosine kinase inhibitors (TKIs) result in a significantly greater proportion of cases who achieve deeper responses compared with imatinib. Furthermore, there is a considerable interest in clinical trials to assess the possibility of stopping TKI therapy once sustained undetectable disease is achieved.

CURRENT DEFINITIONS OF CMR

Any definition of MR needs to take into account the fact that assay sensitivity varies from center to center and that even within established laboratories the sensitivity may vary substantially from sample to sample. The definition needs to be applicable to different assay formats, relate to the International Scale and be adaptable in the face of future technologies that may further improve disease detection. Because of these considerations, there is a general consensus that it is not possible to have a single workable definition of CMR, but rather the level of response needs to be defined by an upper boundary. So, just as MMR corresponds to $\leqslant$0.1% *BCR-ABL*^{IS}, the terms CMR[4], CMR[4.5] and CMR[5] have started to be used to indicate levels of disease that are $\leqslant$0.01% *BCR-ABL*^{IS} ($\geqslant$4-log reduction from IRIS baseline), $\leqslant$0.0032% *BCR-ABL*^{IS} (4.5-log reduction from IRIS baseline) and $\leqslant$0.001% *BCR-ABL*^{IS} (5-log reduction from IRIS baseline), respectively.

SECOND-GENERATION TKI'S IN THE FRONT-LINE SETTING: MOLECULAR MONITORING IS REQUIRED TO MEASURE DEEPER RESPONSES

Data from phase 2 trials of nilotinib conducted by the MD Anderson Cancer Center (MDACC) group and the Gruppo Italiano Malattie Ematologiche dell'Adulto indicate that CCyR is achieved on nilotinib in over 95% of patients with newly diagnosed CML-CP by 12 months.[5,6] Similarly, the Southwestern Oncology Group and MDACC trials of dasatinib in patients with newly diagnosed CML-CP demonstrated CCyR rates of 82–98% by 12 months.[7,8] The pivotal phase 3 nilotinib (ENESTnd)[9] and dasatinib (DASISION)[10] trials showed rates of CCyR of 80% and 83% for nilotinib and dasatinib, respectively, by 12 months (rates of CCyR in the phase 3 studies are likely lower than those in phase 2 studies due to the

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application of the intention-to-treat principle and that patients with missing or non-evaluable samples were considered as non-responders). In the ENESTnd trial, 55% of patients treated with nilotinib 300 mg twice daily achieved MMR by 12 months, whereas only 27% achieved MMR on imatinib ($P<0.0001$).[9] The difference in nilotinib MMR rates compared with imatinib continued to persist by 24 months, where 71% and 44% of patients achieved MMR on nilotinib 300 mg BID compared with imatinib ($P<0.0001$).[11] By 24 months, $CMR^{4.5}$ was achieved in 25% of patients treated with nilotinib 300 mg twice daily and 9% of patients treated with imatinib ($P<0.0001$).[11–13] A deep level of MR to nilotinib (400 mg twice daily) has also been demonstrated in the phase 2 MDACC trial, with 21% of newly diagnosed CML-CP patients achieving $CMR^{4.5}$ by 18 months,[6] and in the Gruppo Italiano Malattie Ematologiche dell'Adulto trial, with 27% of patients achieving CMR^{4} at 24 months.[14] In the DASISION trial, 46% of patients treated with dasatinib 100 mg once daily achieved MMR by 12 months, whereas only 28% achieved MMR on imatinib ($P<0.0001$).[10] By 24 months, $CMR^{4.5}$ was achieved in 17% and 8% of patients treated with dasatinib or imatinib ($P=0.002$ vs imatinib).[15] Similar response to dasatinib has also been demonstrated in the phase 2 MDACC trial, with 6% of patients achieving $CMR^{4.5}$ by 18 months,[7] and in the Southwestern Oncology Group trial, with 27% of patients achieving CMR^{4} at 12 months in evaluable patients ($P=0.31$ vs imatinib).[8] Taken together, these results demonstrate that the difference in the rates of response between second-generation TKIs and imatinib becomes more pronounced as the depth of MR increases.[16]

Interestingly, although second-generation TKIs yield higher rates of CMR vs imatinib, there is no evidence to support the eradication of CML stem cells. On the contrary, there are data suggesting an inability of TKIs to eliminate precursor CD34+ CML cells.[17] Moreover, there are reports of disease relapse following achievement of CMR on TKI-based therapy.[18–20] Currently, the only treatment modality available to CML patients that is considered potentially curative is allogeneic stem cell transplantation, which may induce remission via elimination of CML stem cells through a graft vs leukemia effect.[21] Still, the morbidity and mortality rate following allogeneic stem cell transplantation remains prohibitively high, precluding allogeneic stem cell transplantation as a first-line or even second-line treatment option for most CML patients. Thus, new treatment strategies are required.

TOWARDS THE PATH TO A CURE

Several trials are evaluating novel combinations of TKI therapy with immunomodulatory agents or agents that target CML stem cells to determine if it is possible to further increase the number of patients who are able to achieve and sustain deep MRs. Interferon alpha (IFN) monotherapy has been shown to induce durable complete remissions in a minority of CML patients, potentially through immunological targeting of CML stem cells, but with the added burden of long-term treatment and adverse events.[22–24] Recent data from a proof of concept study of TKI cessation (with IFN maintenance) following MMR on imatinib plus IFN demonstrated promising results.[25] In this study, imatinib therapy was stopped in 20 patients who had concomitantly been pretreated with imatinib and IFN for over 2 years. With a median of 2.4 years after imatinib withdrawal (range, 0.5–4.0 years), 15 out of 20 (75%) patients remained in remission on IFN. The number of patients in CMR increased with IFN from two patients at baseline to five patients after 2 years. Relapses occurred in five patients, all within the first year, but all patients were able to reestablish molecular remission when imatinib was reintroduced.

A phase I trial of nilotinib in combination with low-dose IFN has been initiated to determine the optimal IFN dose for this combination. The randomized German CML Study V will compare nilotinib 300 mg twice daily with nilotinib 300 mg twice daily plus IFN to induce $\geqslant$18 months of MMR, followed by either continued nilotinib or IFN monotherapy to induce $\geqslant$12 months of CMR, followed by cessation of all therapies. Furthermore, recent *in vitro* data suggest that a combination of nilotinib with stem-cell active drugs, such as Janus kinase (JAK) inhibitors, MEK inhibitors and the hedgehog pathway inhibitors, may have a synergistic effect and could provide a rationale for future combination trials.[26–28]

With the new treatment modalities being developed to target CML stem cells, it would be beneficial if a method that optimally measured residual CML disease at the stem cell level was established. Currently, qRT-PCR is used to measure treatment response; however, a recent study demonstrated that low expression levels of *BCR-ABL* in a stem cell population are associated with resistance to imatinib.[29] These cells would be difficult to detect using qRT-PCR and therefore alternative methods to monitor residual disease may be useful. One possibility is the measurement of *BCR-ABL* fusion junctions from genomic DNA, an approach that is technically demanding and labor intensive because the genomic breakpoints need to be characterized for each patient and individual detection assays designed and validated.[20,30,31] Notwithstanding these technical hurdles, one study reported that all patients ($n=10$) who lost CMR after imatinib cessation had detectable levels of *BCR-ABL* on analysis of genomic DNA, and patients who maintained CMR displayed a stable level of *BCR-ABL* DNA.[20] These data may provide a rationale to use genomic DNA as a methodology to monitor residual disease, at least on a research basis.

CONSIDERATIONS FOR FUTURE DISCONTINUATION STUDIES

Several groups are assessing the discontinuation of TKIs following the achievement of sustained CMR. The Stop Imatinib (STIM) study, the most mature of its kind, prospectively assessed imatinib discontinuation in 100 CML patients in complete molecular remission for >2 years in duration.[19] The investigators claimed a sensitivity of the assay of 5 logs, but this was not standardized and thus difficult to compare with other studies. In the interim analysis (median 17 months follow-up after imatinib discontinuation), 42 out of 69 (61%) patients relapsed, with 98% of relapses occurring in the first 7 months following discontinuation of imatinib. At 12 months, the probability of persistent CMR was 41% (95% CI 29–52). All patients who relapsed responded to reintroduction of imatinib, with 26 achieving sustained CMR. Interestingly, the kinetics of response in these patients was heterogeneous. Along with factors identified in a univariate analysis for prediction of relapse (risk score, gender and duration of imatinib therapy), perhaps the time to and duration of prior CMR might also influence the rate and kinetics of relapse in patients on discontinuation studies. In addition, some studies have suggested that patients may discontinue TKIs but use IFN as a maintenance therapy.

Interestingly, the type of TKI therapy may have an important role in determining if patients are able to discontinue therapy. It may be ideal for patients undergoing treatment to achieve the fastest and deepest MRs possible. Therefore, it may be beneficial if patients are first treated with second-generation therapies or if patients that experience imatinib intolerance or suboptimal response to imatinib be switched to second-generation therapies immediately. In 2012, the EURO-SKI (STOP kinase inhibitors) study will begin across several European countries and will include patients with sustained CMR who were previously treated with imatinib or second-generation TKIs following imatinib intolerance and may begin to address some of these outstanding questions.

The STIM study required patients to have maintained CMR for at least 2 years before discontinuing imatinib. With that requirement, approximately 40% of patients with CML were able to sustain CMR

for at least 12 months of therapy. However, only approximately 10% of patients treated with imatinib would be eligible for this type of study based on published literature and experience in clinical trials and clinical practice.[13,19,32] As only about 10% of patients treated with imatinib are able to achieve CMR, there have been efforts to increase the proportion of patients who achieve the deepest levels of sustained response. The ENESTcmr study is a randomized study of nilotinib 400 mg twice daily vs imatinib that seeks to determine the rate of undetectable *BCR-ABL* in patients who did not achieve this response on prior long-term (>2 years) imatinib therapy. Results from the ENESTcmr study demonstrated that significantly more patients achieved CMR^4 and $CMR^{4.5}$ by 12 months on nilotinib vs those patients remaining on imatinib.[33] More patients also achieved CMR (undetectable *BCR-ABL* with $\geqslant$4.5-log assay sensitivity) on nilotinib (23%, $P=0.02$) vs imatinib (11%). At 12 months, more patients also achieved CMR confirmed on two consecutive samples when switched to nilotinib (12.5%, $P=0.108$) vs remaining on imatinib (5.8%). Thus, at this 12 month time point, the results suggest the achievement of the deepest levels of measurable response on nilotinib, and it will be interesting to see how much the results improve with longer follow-up.

THE NEED FOR IMPROVED DEFINITIONS OF MR AND LABORATORY STANDARDIZATION

The clinical studies described above highlight the need for robust, standardized and workable definitions of deep MRs. However, there are two immediate problems with the current definitions of CMR^4, $CMR^{4.5}$ etc. The first is semantic: the fact that they are defined by an upper boundary means that disease may still be detectable by qRT-PCR or nested PCR at a lower level, which does not sit well with the term 'complete'. We therefore propose that the terms are modified to 'MR', that is, MR^4, $MR^{4.5}$ etc (Figure 1). Logically then MMR would correspond to MR^3, however, because the term MMR is so well established, we suggest it is retained. The second problem concerns laboratory standardization: how comparable is MR^4, $MR^{4.5}$ etc across different laboratories? We have undertaken preliminary analysis that suggests there is in fact substantial variation that is caused principally by technical differences but is exacerbated by differences in laboratory definitions.

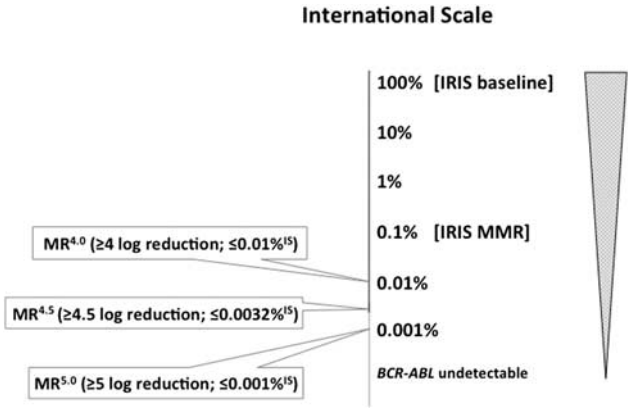

Figure 1. Definitions of MR. The International Scale for *BCR-ABL* qRT-PCR measurement is expressed as a percentage and is fixed to two key points: 100% corresponds to the IRIS standardized baseline and 0.1% corresponds to the upper limit of MMR. MR^4 corresponds to $\leqslant$0.01% $BCR\text{-}ABL^{IS}$, $MR^{4.5}$ corresponds to $\leqslant$0.0032% $BCR\text{-}ABL^{IS}$ etc. Note that all log reductions are from the IRIS baseline and not from individual pretreatment levels.

The international, collaborative effort to standardize molecular testing for CML to date has largely concerned detectable residual disease, with a particular emphasis on determining whether a patient has or has not achieved MMR. The focus has been on the derivation of laboratory-specific conversion factors (CFs) that enable locally derived results to be converted to the International Scale. In an important proof of principle study, this process has been demonstrated to work well for about 50% of testing laboratories.[34,35] However, there are a number of obvious issues with CFs, for example, (i) the process is lengthy and costly; (ii) because of the requirement to involve an established reference laboratory, the process is only open to a limited number of laboratories at any given time; (iii) many centers struggle to accrue sufficient numbers of suitable samples; (iv) it is unclear as to how often CFs need to be revalidated; (v) it is unclear as to what happens to the 50% of laboratories who fail to achieve the defined performance criteria.[34] Some of these issues may be addressed by the recent commercial development of secondary reference reagents calibrated to the World Health Organization primary *BCR-ABL* standards.[36]

With regard to definitions of MR, however, a new problem arises in how to define assay sensitivity in a standardized manner when *BCR-ABL* mRNA is undetectable. Assay sensitivity was initially considered by the Europe Against Cancer Group,[37] but the criteria they established only works for assays using an internal control gene that is independent of the fusion being tested. For CML, by far the most widely used internal control gene is normal *ABL*, which is not independent. We therefore suggest that the following criteria should be used to define MR:

- MR^4 = either (i) detectable disease $\leqslant$0.01% $BCR\text{-}ABL^{IS}$ or (ii) undetectable disease in cDNA with $\geqslant$10 000 *ABL* transcripts*
- $MR^{4.5}$ = either (i) detectable disease $\leqslant$0.0032% $BCR\text{-}ABL^{IS}$ or (ii) undetectable disease within cDNA with $\geqslant$32 000 *ABL* transcripts*

*numbers of *ABL* transcripts in the same volume of cDNA used to test for *BCR-ABL*.

These working definitions depend critically on the ability of testing laboratories to measure absolute numbers of *ABL* control gene transcripts in a comparable manner, as well as their ability to achieve the requisite sensitivity. In addition, there is a considerable variation in the way in which testing laboratories define undetectable or low-level disease. Currently, the extent of variation in *ABL* measurement and laboratory definitions is not known and so the European Treatment and Outcome Study (EUTOS) group is undertaking a detailed international performance evaluation with the aim of recommending specific laboratory definitions, protocols and internal quality assurance across different assay formats (including other control genes) that will facilitate standardization of MR and thus provide greater comparability between centers. This collaboration has already successfully coordinated the measurement of the primary endpoint from the ENEST1st trial, which recently reported the results of the first interim analysis where MR^4 was the primary endpoint at 18 months.[38] Thus, this collaboration may be applied as a future model for standardization of molecular monitoring.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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