Prognosis and Molecular Monitoring in Chronic Myeloid Leukemia

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Abstract

Tyrosine kinase inhibitors with activity against *BCR-ABL* form the cornerstone of CML therapy, and are particularly effective in those with chronic-phase disease. Because some patients exhibit primary resistance or secondary failure to TKI therapy, it is recommended that continued monitoring of disease burden be performed. In this article, we review methods of detecting the Philadelphia chromosome and *BCR-ABL* transcript, and discuss the correlation of response with patient outcomes. Expert guidelines that incorporate definitions and milestones of response are referenced to aid in clinical decision-making.

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Introduction

Tyrosine kinase inhibitors (TKIs) active against *BCR-ABL* have become integral to the treatment of chronic myelogenous leukemia (CML) and are particularly effective in patients with chronic-phase disease. However, some patients will nonetheless demonstrate primary or secondary resistance to such therapy and will require an alternative therapeutic strategy consisting of a switch to a different TKI, or possibly stem cell transplantation for patients who have advanced to blastic-phase disease. Therefore, continued monitoring of the burden of disease is warranted for all patients. In CML, the therapeutic target of *BCR-ABL* is also a direct biomarker of disease burden, so that sensitive assessment of disease response can easily be performed. Perhaps not surprisingly, there is growing evidence that deeper and more rapid reduction in disease burden is associated with improved outcomes. This brief review addresses where we are currently in regard to disease monitoring in CML, and where we should be going.

What Is the Best Method for Monitoring CML?

Conventional metaphase cytogenetics and fluorescence in-situ hybridization (FISH) are 2 complementary methods of establishing the presence of disease, determining disease stage, and evaluating response to therapy. Cytogenetic analysis has the added ability to screen for additional chromosomal abnormalities, which would be indicative of accelerated-phase disease. Unfortunately, cytogenetic testing requires bone marrow cells capable of proliferative capacity and thus is associated with the financial and invasive procedural burden of a marrow aspiration. On the other hand, FISH is a more sensitive method, with approximately 10 times as many cells (~200) analyzed per assay, and can be performed on either bone marrow or peripheral blood, although a major limitation is that additional abnormalities may only be detected through the use of specific probes, and thus it suffers as a diagnostic test compared to cytogenetics.

Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) testing is the most sensitive method for detection of *BCR-ABL* mRNA, with current methods able to detect as few as a single CML cell in a background of up to at least 100,000 normal cells. This high level of sensitivity allows routine disease monitoring to be performed on peripheral blood. *BCR-ABL* transcript levels determined by qRT-PCR are highly correlated with disease burden as determined by cytogenetics or FISH (at least, in the range that all 3 can be comparatively measured), and many centers with expertise in qRT-PCR use *BCR-ABL* transcript monitoring to monitor patients instead of cytogenetics, once cytogenetics have been performed at diagnosis to establish the diagnosis and stage of disease.[1,2]

One problem with *BCR-ABL* measurement is that these assays historically sprung up as home-brew assays in centers performing allogeneic transplantation before the TKI era. With the advent of TKI therapy, multiple laboratories developed their own assays. The laboratory-to-laboratory variations are myriad, most notably in the variability in which housekeeping genes are used as controls. In

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addition, there is a lack of a widely available *BCR-ABL* reference standards upon which to base the designation of relative or absolute transcript levels. In the IRIS study, a baseline *BCR-ABL* transcript level (measured as *BCR-ABL/BCR*) was determined through PCR testing of peripheral blood samples from 30 untreated, chronic-phase CML patients in each of the 3 IRIS laboratories.[3] Median values for the 30 samples served as the baseline *BCR-ABL/BCR* level for each laboratory, to which subsequent patient samples would be compared. Thus, the *BCR-ABL* log reduction value for each patient was performed by comparing a patient's result to the median value of the diagnostic reference group. In the IRIS study, a 3-log reduction from the median baseline, referred to as a major molecular response (MMR), correlated with an excellent progression-free survival and thus became established as a response metric with prognostic importance.[4-7]

Unfortunately, the original specimen pool that was used to determine the baseline *BCR-ABL/BCR* transcript levels in the IRIS study has since been depleted. However, before the consumption of these specimens, an equivalent measure of *BCR-ABL* transcript levels was engineered, and thus a standard for *BCR-ABL* has been established, known as the international scale (IS).[8] Through exchange of samples with an IS reference laboratory, an IS conversion factor can be established for a particular laboratory, which will then allow for standardization of results to the IS. The IS has been conveniently aligned with important milestones for treatment, with a value of 1% IS correlating with a complete cytogenetic response (CCyR), and an IS of 0.1% indicating the level of MMR.

What Is the Best Definition of Response to Therapy in CML?

The most recent guidelines pertaining to CML from the National Comprehensive Cancer Network (NCCN), version 1.2015, and the 2013 European Leukemia Net (ELN) define similar, albeit slightly different, response criteria (Table 1).[9,10] A complete hematologic response is defined as a normalization in peripheral blood counts, including total leukocyte count of less than 10×10^9/L, platelet count of less than 450×10^9/L, absence of palpable splenomegaly, and absence of immature myeloid cells in the peripheral blood. ELN guidelines suggest the additional criterion of basophils comprising less than 5% of the peripheral blood differential. Cytogenetic responses are based on sequential bone marrow cytogenetic analyses, with minor cytogenetic response indicating presence of t(9;22) in greater than 35%, or more than 7 out of 20, metaphases. A partial cytogenetic response (PCyR) indicates between 1% and 35%, or up to 7 out of 20, metaphases. A CCyR indicates the absence of detectable t(9;22). Notably, NCCN and ELN guidelines are based on cytogenetics rather than FISH, though use of FISH results is acceptable should cytogenetic results be unavailable.

Table 1 Response Criteria in CML

Level of Response	Definition
Complete hematologic response	Normal CBC and differential, absence of palpable splenomegaly
Minor cytogenetic response	35%-90% Ph metaphases[a]
Partial cytogenetic response	1%-34% Ph metaphases[a]
Complete cytogenetic response	0% Ph metaphases[a]
Major molecular response	$\geq$ 3-log reduction of *BCR-ABL* mRNA[b] or BCR-ABL $\leq$ 0.1% IS
Complete molecular response	Negative PCR with at least 4.5-log sensitivity

[a]At least 20 metaphases must be studied to assess cytogenetic response.
[b]Reduction in BCR-ABL mRNA compared to laboratory-specific baseline level of pooled, untreated, chronic-phase patients.

Molecular responses are defined by qRT-PCR for detection of *BCR-ABL* mRNA transcript levels. Fortunately, peripheral blood may be used for such monitoring by qRT-PCR. MMR, a milestone that correlates with long-term prognosis, is defined by a transcript level less than 0.1% by qRT-PCR on the International Scale (IS) or a greater than 3-log scale reduction in BCR-ABL1mRNA from the laboratory-specific standardized baseline, if qRT-PCR (IS) not available. More stringent responses, such as MR^4 and $MR^{4.5}$, suggest molecular remission with undetectable transcripts at 4-log and 4.5-log scale reductions, respectively, from the standard baseline.

Both major groups have published suggested schedules of response assessment, with response milestones designated to suggest adequate response versus treatment failure (Table 2).[9,10]

Why Are CCyR and MMR Considered Such Important Milestones?

Cytogenetic response is one of the most important indicators of therapeutic success, and many studies have shown the achievement of CCyR as favorably associated with overall and progression-free survival. For example, in a long-term follow-up analysis of patients treated in the IRIS randomized trial, the achievement of a CCyR at 6 months was associated with a decreased risk of disease progression to advanced phase, compared to those patients who did not experience a CCyR (10% vs. 25% risk of progression) at a median of 42 months of follow-up.[11] Comparing various treatment strategies for newly diagnosed chronic-phase CML, Jabbour et al[12] found higher rates of CCyR in patients receiving high-dose imatinib or second-generation TKIs compared to those receiving low-dose imatinib, but importantly, patients who did experience CCyR, irrespective of the means, demonstrated similar rates of 3-year event-free survival (97%-98%) and overall survival (99%). By contrast, those who did not experience CCyR demonstrated a 3-year event-free survival of 67% to 83% and overall survival of 90% to 94%, highlighting the importance of CCyR.[12] Both the ELN and NCCN recognize the CCyR within a year of therapy as an extremely important milestone.[9,10]

Although CCyR remains a major therapeutic milestone, the further achievement of MMR seems to be a safe haven, as secondary resistance and progression are relatively unusual once MMR has been achieved. In the IRIS trial, considering only those patients with a CCyR, there was a 97% progression-free survival at 54 months in the subset with more than a 3-log reduction (MMR) at 12 months, as opposed to an 89% progression-free survival in the subset with less than a MMR at 12 months.[13] Additional