EUROPEAN HEMATOLOGY ASSOCIATION

Chronic myeloid leukemia - Section 2

Molecular work up and monitoring of chronic myeloid leukemia patients

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Take-home messages

- Standardized MRD analysis is the best molecular predictor of outcome in CML.
- Biomarkers for advanced disease and pharmacokinetic variables may help to predict response.
- Increasing evidence of the role of the immune system in the response of CML to treatment.

Diagnosis of CML

All cases of chronic myeloid leukemia (CML) are, by definition, positive for the *BCR-ABL* fusion. About 95% of cases exhibit a visible Philadelphia chromosome on conventional cytogenetic analysis, the smaller derivative of the t(9;22)(q34;q11), or a variant that typically involves one or more additional chromosomes. The remaining 5% of cases have a cytogenetically cryptic *BCR-ABL* fusion and often have a normal karyotype. Such cases may be picked up by FISH to detect aberrant juxtaposition of the *BCR* and *ABL* genes or RT-PCR to detect *BCR-ABL* mRNA. The great majority (97-98%) of CML cases expresses a chimeric mRNA in which *BCR* exon 13 or exon 14 is joined to *ABL* exon 2 (e13a2 and e14a2 fusions, respectively, also commonly referred to as b2a2 and b3a2). The remaining 2-3% of cases express diverse, atypical fusions involving other exons of *BCR* and/or *ABL*. For all cases, it is important to determine the *BCR-ABL* mRNA transcript type prior to treatment to enable effective molecular monitoring.

Definition of prognosis prior to treatment

Considerable efforts have been made to identify pre-treatment biomarkers that can distinguish patients destined to perform well on therapy from those who will perform poorly. These biomarkers can be considered within four basic categories: (i) intrinsic differences in disease biology, (ii) markers of disease progression (iii) pharmacokinetic variables that influence the effectiveness of therapy, and (iv) the immune environment.

Intrinsic disease biology

Although atypical *BCR-ABL* fusions and complex *BCR/ABL* rearrangements are not thought to be strong indicators of prognosis (except possibly the rare occurrence of the p190 fusion), it has been suggested that individuals expressing e13a2 *BCR-ABL* have marginally inferior cytogenetic and molecular responses to tyrosine kinase inhibitors (TKIs) compared to e14a2 cases. Although no effect is discernible on survival, differences in the rate and the depth of molecular response could potentially impact on TKI discontinuation.[1-4] Emerging data suggest that in some cases *BCR-ABL* may be acquired on a background of clonal hematopoiesis driven by mutations in genes such as *TET2* or *ASXL1*, but at the current time there appears to be no clear impact of this finding on clinical course or outcome.[5]

Disease progression

Advanced phase CML responds poorly to therapy and it is no surprise that markers of disease progression are associated with an adverse prognosis.[6] Detection of additional cytogenetic abnormalities, particularly major route abnormalities (+Ph, trisomy 8, isochromosome 17q or trisomy 19), suggests progression to accelerated phase or blast crisis and these abnormalities at diagnosis are associated with a negative impact on survival.[7] Gene expression profiling may indicate advanced disease in some individuals who would otherwise be categorised as chronic phase.[8] At the stem cell level, there is marked heterogeneity in the relative proportion of *BCR-ABL* positive and (presumed) normal stem cells, with a greater proportion of leukemic stem cells suggesting more advanced disease and correlating with an inferior outcome.[9]

Pharmacokinetics

Like all other drugs, the effectiveness of TKIs is influenced by ADME: absorption, distribution, metabolism and excretion. *CYP2C8* genotype significantly alters imatinib metabolism in patients through gain- and loss-of-function mechanisms.[10] Polymorphic variants, expression levels and, more convincingly, functional activity of the transporter OCT1 (encoded by *SLC22A1*), correlate with clinical outcome for patients treated with imatinib (but not other TKIs).[11] Similarly, high expression levels of *ABCB1* (which encodes the multidrug resistance protein MDR1 implicated in TKI export) has been linked to initiation of TKI resistance[12] and polymorphic variants of this genes linked to molecular response.[13]

Immune environment

There is increasing evidence that immunologic surveillance mechanisms impact on the response of CML to therapy. Killer immunoglobulin-like receptors (KIRs) profiles on natural killer (NK) cells have been shown to predict for response to TKIs and the polymorphic variants KIR2DL5B and KIR2DS1 are associated with outcome.[14,15] Low numbers of L-selectin (CD62L)-expressing CD4+ and CD8+ T cells correlated with adverse clinical features and both reduced CD62L expression on T cells and increased soluble CD62L levels predicted molecular response to TKI therapy.[16] Finally, a high proportion TNF-α/IFN-γ secreting mature NK cells is associated with successful imatinib discontinuation, whereas high expression of the CTLA-4 ligand CD86 on plasmacytoid dendritic cells is associated with a higher risk of relapse after TKI discontinuation.[17]

Despite these advances it is sobering to appreciate that no biomarker has thus far been proven to outperform simple, cheap clinical scoring systems (Sokal, Hasford, EUTOS, ELTS). In addition to the markers above, behavioral factors that influence treatment compliance are also relevant to prognosis.[18]

Definition of prognosis and response on treatment

Plasma levels of imatinib correlate with clinical response and changes in *ABCB1* expression may help to predict response,[13] but by far the strongest prognostic indicator is the measurement of residual disease levels on treatment by reverse transcription – quantitative PCR (RT-qPCR) using methods aligned to the International Scale.[19] Optimal response as defined by the European LeukaemiaNet is strongly associated with better outcomes, and rising *BCR-ABL* mRNA levels on sequential analysis suggest disease relapse, usually either due to biological resistance or inadequacy of therapy due to compliance issues. In routine practice, resistance is associated with secondary *BCR-ABL* mutations in up to a third of cases and the finding of such mutations may help to guide subsequent therapy. Mutations may be detected down to levels of 1-2% variant allele frequency (VAF) using next generation sequencing (NGS),[20] and with even greater sensitivity using targeted approaches, but it is not yet clear if these increased levels of detection afford any clinical advantage over standard Sanger sequencing, which detects mutations down to a level of 10-20%. Other mechanisms of resistance such as *BCR-ABL* overexpression and *LYN* kinase overexpression are difficult to discern on a routine basis.

Interpreting molecular responses is helped by assessing sequential trends rather than just considering specific time-dependent milestones. In this regard measurement of the rate of decline of *BCR-ABL* transcripts from months 0-3 on treatment may be useful to decide whether a patient should be considered as an early molecular response (EMR) failure or not. Standardized measurement of deep molecular responses (MR^4, $MR^{4.5}$ etc.)[19] is particularly important when considering stopping therapy. Digital RT-PCR may provide greater accuracy for measurement of low levels of disease but it is not yet clear if this is of any clinical benefit. Similarly, DNA based PCR approaches are of interest to increase the limit of detection of CML cells, but it seems unlikely that this will become routine practice.

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