Chronic myeloid leukemia - Section 6

NGS in CML - New standard diagnostic procedure?

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Take home messages

• The detection of specific variants at diagnosis of CML may contribute to risk of treatment failure.
• Approximately 50% of patients with TKI resistance acquire BCR-ABL1 kinase domain mutations and NGS will identify other somatically mutated cancer-associated genes that contribute to resistance.

Introduction

Genomic profiling of hematologic malignancy over the last decade using next-generation sequencing (NGS) has revealed an abundance of acquired gene variants and some of these signify prognostic risk groups.1 Diseases that appear morphologically similar are now known to be molecularly diverse. Treatment decisions for individual patients will likely benefit from the incorporation of genomic information with clinical variables and laboratory parameters to classify risk. These new prognostic models have been suggested for acute myeloid leukemia where a large share of prognostic information is determined by genomic factors, and revised genetic categories now define risk.1 In myelodysplastic syndromes, the incorporation of mutational data into the Revised International Prognostic Scoring System improved outcome prediction.2 In myeloproliferative neoplasms, where most patients share a limited set of phenotypic driver mutations, there is significant heterogeneity in outcome and models for predicting poor risk in chronic phase are limited. Recent genomic studies identified specific cooccurring variants that modified outcome, and combining genomic data with clinical parameters improved prognostication and disease classification.3

CML is initiated by a single genomic event, the BCR-ABL1 fusion, and remarkable treatment success is achieved with tyrosine kinase inhibitor (TKI) therapy. Whether there is a role for NGS at diagnosis of CML in terms of risk stratification remains to be established. Effective therapy for most patients with CML may have limited the genomic investigation at CML diagnosis, which has markedly progressed in other hematologic malignancies. However, data is emerging that additional somatic variants in hematologic malignancy associated genes are present in some patients at diagnosis4,5,6,7,8 and may contribute to poor prognostic risk.5,9,10,11 Additional genomic abnormalities are required for transformation of CML to an acute leukemia and, no doubt, NGS for specific patients with TKI resistance will be informative.5,11,12,13,14

Current state-of-the-art

Role for NGS at diagnosis of CML

Measurement of BCR-ABL1 transcript decline during TKI therapy using quantitative PCR is now established as the cornerstone of patient monitoring for outcome prediction and treatment intervention, with BCR-ABL1 kinase domain mutation analysis reserved for patients with lack or loss of response. There is limited prognostic relevance of the BCR-ABL1 transcript level at diagnosis,17 and BCR-ABL1 mutation analysis is not required at this time. Therefore, risk is primarily based on clinical parameters at diagnosis. With reduced cost of NGS and its incorporation into standard clinical practice in many laboratories, it is timely to examine whether there is a role for NGS for risk stratification in CML.

We recently studied patients at chronic phase diagnosis using whole-exome sequencing, copy number variation and whole-transcriptome sequencing (RNA-Seq).11 The strategy proved useful for the detection of diverse variant types, including single-nucleotide and small insertion/deletion variants, focal deletions, large copy number gains and losses, and gene fusions. Samples at diagnosis of patients with extreme responses were tested: major...
molecular response at a median of 6 months of imatinib or blast crisis at a median of 6 months after commencing TKI therapy. The majority of patients had a corresponding nonleukemic control sample tested to establish the somatic status of variants. We focused on variants of potential or known clinical relevance using established classification systems.\textsuperscript{16,17} Damaging variants in known hematologic cancer associated genes\textsuperscript{18} were more frequently detected at diagnosis in patients with subsequent transformation compared to those with an optimal outcome: 56% vs 16%. There was also an association with high Sokal risk score and relevant variants at diagnosis. The recurrently mutated genes at diagnosis were ASXL1, RUNX1, IKZF1, TP53, and a novel gene SETD1B. Furthermore, a novel class of variant was detected at diagnosis, which was genomic rearrangement involving genes located on the chromosomes involved in the Philadelphia translocation, which we termed Ph-associated rearrangements. These were primarily detectable from RNA-Seq data. The rearrangements frequently involved genomic inversions and some patients had evidence of chromosome fragmentation and imperfect sequence reassembly. The Ph-associated rearrangements were more frequently detected in patients with a poor outcome vs an optimal response: 33% vs 11%. Their role in treatment outcome requires further analysis.

Kim et al\textsuperscript{17} also reported variants at CML diagnosis using a DNA-based targeted gene panel in a study of 100 patients with various outcomes to TKI therapy. Among damaging variants in hematologic malignancy associated genes were nonsense/frameshift variants in ASXL1 exon 12, in 8 patients. Five of these patients progressed or had TKI resistance, which is consistent with our study where 7 of 9 patients with the same type of ASXL1 variant at diagnosis had a poor outcome.\textsuperscript{11,12} ASXL1 is one of the most commonly mutated genes detected with age-related clonal hematopoiesis, which is associated with increased cardiovascular risk.\textsuperscript{19} Interestingly, a recent targeted sequencing study of 21 CML patients aged 0 to 27 years detected ASXL1 exon 12 frameshift/nonsense variants in 6 patients (29%) as the sole mutated gene.\textsuperscript{20} These were historical samples, which precluded correlation with TKI therapy outcome. However, their detection in young patients suggests against a general age-related mechanism of mutated ASXL1 acquisition.

Role for NGS at TKI resistance

\textit{BCR-ABL1} kinase domain mutation testing is recommended for TKI resistance, and mutations are detected in \textasciitilde 50% of patients. NGS is increasingly used for the detection of \textit{BCR-ABL1} mutations and provides improved sensitivity for early detection of drug resistance compared with Sanger sequencing.\textsuperscript{21} Furthermore, sensitive detection of \textit{BCR-ABL1} mutants after TKI resistance can guide therapy decisions.\textsuperscript{22,23} \textit{BCR-ABL1} mutations are rarely the cause of primary resistance and NGS has the potential to characterize mutations associated with \textit{BCR-ABL1} independent resistance. Recent NGS studies at TKI resistance/transformation have generated a higher yield of damaging variants compared to diagnosis.\textsuperscript{11,12,14} These studies are starting to fill a knowledge gap of resistance-associated lesions. The most frequently mutated genes at resistance were those commonly mutated in other hematologic malignancies such as \textit{RUNXI}, \textit{IKZF1}, and \textit{ASXL1}.\textsuperscript{11} Importantly, gene fusions were detected at blast crisis. These included known fusions such as \textit{CBFB-MYH11} and \textit{MLL} fusions, plus novel and cytogenetically cryptic fusions where one of the fusion partners was a cancer gene such as \textit{RUNXI}, \textit{MECOM}, and \textit{IKZF1}.\textsuperscript{11} Interestingly, in NGS studies, \textit{BCR-ABL1} kinase domain mutations have rarely been reported without the detection of other mutated genes of potential clinical significance.\textsuperscript{11,12} To date, the data indicate that sequencing of an expanded set of genes will aid the detection of mutations that contribute to drug resistance.

Future perspectives

The use of NGS has significantly enhanced cancer causing gene discovery, functional characterization and drug development. Furthermore, NGS for patients with hematologic malignancy has been progressively integrated into patient management strategies for diagnosis, risk stratification and to aid treatment approaches. Expanded studies for patients with CML are required to synthesize the significance of specific variants and mutated genes that are being discovered in patients at diagnosis and disease progression. It is essential that these studies carefully annotate somatic variants and appropriately evaluate their clinical relevance as set out in recent joint consensus

### Figure 1. Potential future NGS strategy

Ongoing NGS analysis of patients with various disease phases who are treated with different TKIs will establish the clinical need for sequencing of additional genes. The data to date suggest that NGS at diagnosis for some patients may aid risk stratification. NGS testing at TKI resistance will supplement \textit{BCR-ABL1} kinase domain mutation testing and will yield a higher frequency of mutations. Sequencing of the \textit{BCR-ABL1} kinase domain involves isolation of the leukemic clone and will therefore detect mutants when \textit{BCR-ABL1} transcript levels are still low. However, the sensitivity of mutant detection using an NGS gene panel is reduced since sequences are derived from both leukemic and nonleukemic cells. Therefore, higher leukemic load is required before attempting NGS.
recommendations from experts in the field.\textsuperscript{16,17} Otherwise, there is a risk of overinterpretation, which could dilute the true significance of additional variants in patients with CML in terms of prognosis. If the data are appropriately managed, I suggest we will uncover a clinical role for NGS testing for select patients at diagnosis and to complement BCR-ABL1 kinase domain mutation analysis at TKI resistance, where the highest yield of mutated genes may be identified (Fig. 1). NGS will likely not be required for all patients. Future testing may identify the clinical parameters at diagnosis when NGS for some patients could provide prognostic information. We expect that NGS will become important for patients with treatment failure or suboptimal response to TKIs and aid the characterization of resistance. Genomic analysis may contribute to the selection of appropriate therapy, drive drug development and ultimately lead to better outcomes for patients with CML.

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