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Chronic myeloid leukemia - Section 6

NGS in CML - New standard diagnostic procedure?

Susan Branford[1,2,3,4], Naranie Shanmuganathan[1,2,3,5]

[1]Department of Genetics and Molecular Pathology, Centre for Cancer Biology, SA Pathology, Adelaide, Australia; [2]School of Pharmacy and Medical Science, University of South Australia, Adelaide, Australia; [3]School of Medicine, University of Adelaide, Adelaide, South Australia; [4]School of Biological Sciences, University of Adelaide, Adelaide, Australia; [5]Department of Haematology, Royal Adelaide Hospital, Adelaide, Australia

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 diagnosis[4,5,6,*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,[15] 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

Funding/support: None.
Disclosure: The authors have indicated they have no potential conflicts of interest to disclose.

HemaSphere (2019) 3:S2
Received: 29 January 2019 / Received in final form: 1 March 2019 / Accepted: 5 March 2019
Citation: Branford S, Shanmuganathan N. NGS in CML—New Standard Diagnostic Procedure? *HemaSphere*, 2019;3:S2. http://dx.doi.org/10.1097/HS9.0000000000000199.

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.[16,*17] Damaging variants in known hematologic cancer associated genes[*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[*7] 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.-[*11] *ASXL1* is one of the most commonly mutated genes detected with age-related clonal hematopoiesis, which is associated with increased cardiovascular risk.[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.[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

BCR-ABL1 kinase domain mutation testing is recommended for TKI resistance, and mutations are detected in ~50% of patients. NGS is increasingly used for the detection of *BCR-ABL1* mutations and provides improved sensitivity for early detection of drug resistance compared with Sanger sequencing.[21] Furthermore, sensitive detection of *BCR-ABL1* mutants after TKI resistance can guide therapy decisions.[22] *BCR-ABL1* mutations are rarely the cause of primary resistance and NGS has the potential to characterize mutations associated with *BCR-ABL1* independent resistance. Recent NGS studies at TKI resistance/transformation have generated a higher yield of damaging variants compared to diagnosis.[*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 *RUNX1*, *IKZF1*, and *ASXL1*.[5,*11,*12,14] Importantly, gene fusions were detected at blast crisis. These included known fusions such as *CBFB-MYH11* and *MLL* fusions, plus novel and cytogenetically cryptic fusions where one of the fusion partners was a cancer gene such as *RUNX1*, *MECOM*, and *IKZF1*.[*11] Interestingly, in NGS studies, *BCR-ABL1* kinase domain mutations have rarely been reported without the detection of other mutated genes of potential clinical significance.[*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

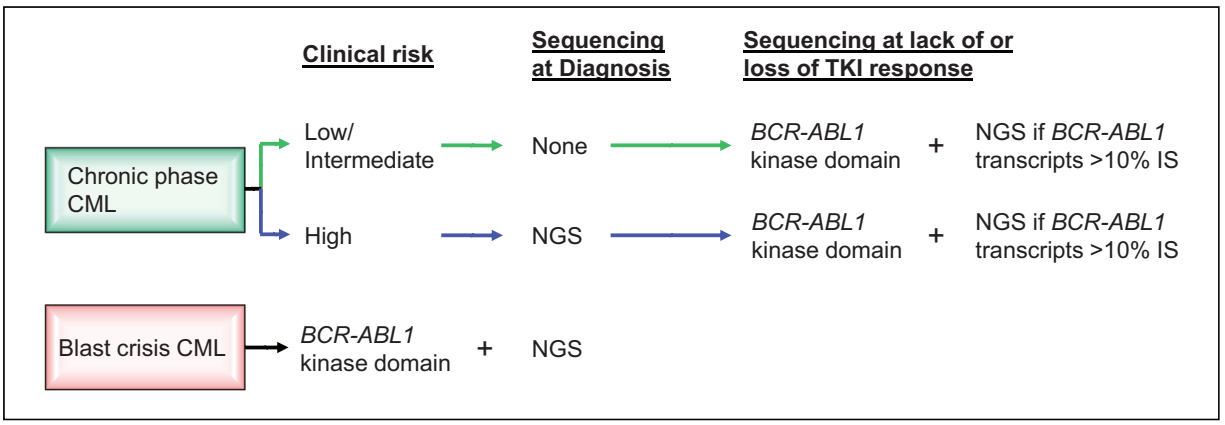

Figure 1. Potential future NGS strategy. Ongoing NGS analysis of patients with various disease phases who are treated with different TKIs will establish the clinical need for sequencing of additional genes. The data to date suggest that NGS at diagnosis for some patients may aid risk stratification. NGS testing at TKI resistance will supplement *BCR-ABL1* kinase domain mutation testing and will yield a higher frequency of mutations. Sequencing of the *BCR-ABL1* kinase domain involves isolation of the leukemic clone and will therefore detect mutants when *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.[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.

References

1. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129:424–447.
2. Nazha A, Narkhede M, Radivoyevitch T, et al. Incorporation of molecular data into the Revised International Prognostic Scoring System in treated patients with myelodysplastic syndromes. *Leukemia*. 2016;30:2214–2220.
3. Grinfeld J, Nangalia J, Baxter EJ, et al. Classification and personalized prognosis in myeloproliferative neoplasms. *N Engl J Med*. 2018;379:1416–1430.
4. Roche-Lestienne C, Marceau A, Labis E, et al. Mutation analysis of TET2, IDH1, IDH2 and ASXL1 in chronic myeloid leukemia. *Leukemia*. 2011;25:1661–1664.
5. Menezes J, Salgado RN, Acquadro F, et al. ASXL1, TP53 and IKZF3 mutations are present in the chronic phase and blast crisis of chronic myeloid leukemia. *Blood Cancer J*. 2013;3:e157.
6. Schmidt M, Rinke J, Schafer V, et al. Molecular-defined clonal evolution in patients with chronic myeloid leukemia independent of the BCR-ABL status. *Leukemia*. 2014;28:2292–2299.

*7. Kim T, Tyndel MS, Kim HJ, et al. Spectrum of somatic mutation dynamics in chronic myeloid leukemia following tyrosine kinase inhibitor therapy. *Blood*. 2017;129:38–47.

Study of 100 patients described variants in known hematologic cancer genes in a proportion of patients at CML diagnosis.

8. Togasaki E, Takeda J, Yoshida K, et al. Frequent somatic mutations in epigenetic regulators in newly diagnosed chronic myeloid leukemia. *Blood Cancer J*. 2017;7:e559.
9. Sloma I, Mitjavila-Garcia MT, Feraud O, et al. Whole-genome analysis reveals unexpected dynamics of mutant subclone development in a patient with JAK2-V617F-positive chronic myeloid leukemia. *Exp Hematol*. 2017;53:48–58.
10. Mologni L, Piazza R, Khandelwal P, et al. Somatic mutations identified at diagnosis by exome sequencing can predict response to imatinib in chronic phase chronic myeloid leukemia (CML) patients. *Am J Hematol*. 2017;92:E623–E625.

*11. Branford S, Wang P, Yeung DT, et al. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. *Blood*. 2018;132:948–961.

Patients with mutated cancer genes at diagnosis had a poorer outcome and various types of mutations were detected at disease transformation, including focal gene deletions and a high frequency of gene fusions.

*12. Grossmann V, Kohlmann A, Zenger M, et al. A deep-sequencing study of chronic myeloid leukemia patients in blast crisis (BC-CML) detects mutations in 76.9% of cases. *Leukemia*. 2011;25:557–560.

By sequencing only 12 genes in patients at blast crisis, a high frequency of mutated genes was detected and most patients with BCR-ABL1 kinase domain mutations had other mutated genes.

13. Soverini S, Score J, Iacobucci I, et al. IDH2 somatic mutations in chronic myeloid leukemia patients in blast crisis. *Leukemia*. 2011;25:178–181.
14. Kim T, Tyndel MS, Zhang Z, et al. Exome sequencing reveals DNMT3A and ASXL1 variants associate with progression of chronic myeloid leukemia after tyrosine kinase inhibitor therapy. *Leuk Res*. 2017;59:142–148.
15. Hanfstein B, Shlyakhto V, Lauseker M, et al. Velocity of early BCR-ABL transcript elimination as an optimized predictor of outcome in chronic myeloid leukemia (CML) patients in chronic phase on treatment with imatinib. *Leukemia*. 2014;28:1988–1992.
16. Sukhai MA, Craddock KJ, Thomas M, et al. A classification system for clinical relevance of somatic variants identified in molecular profiling of cancer. *Genet Med*. 2016;18:128–136.

*17. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn*. 2017;19:4–23.

This joint consensus paper outlined the critical factors for the appropriate interpretation of variants in terms of their clinical relevance.

*18. Sondka Z, Bamford S, Cole CG, et al. The COSMIC Cancer Gene Census: describing genetic dysfunction across all human cancers. *Nat Rev Cancer*. 2018;18:696–705.

COSMIC is an important reference database when assessing the relevance of mutated genes.

19. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med*. 2017;377:111–121.
20. Ernst T, Busch M, Rinke J, et al. Frequent ASXL1 mutations in children and young adults with chronic myeloid leukemia. *Leukemia*. 2018;32:2046–2049.
21. Soverini S, De Benedittis C, Castagnetti F, et al. In chronic myeloid leukemia patients on second-line tyrosine kinase inhibitor therapy, deep sequencing of BCR-ABL1 at the time of warning may allow sensitive detection of emerging drug-resistant mutants. *BMC Cancer*. 2016;16:572.
22. Parker WT, Lawrence RM, Ho M, et al. Sensitive detection of BCR-ABL1 mutations in patients with chronic myeloid leukemia after imatinib resistance is predictive of outcome during subsequent therapy. *J Clin Oncol*. 2011;29:4250–4259.