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 NCCN Guidelines Panel: Chronic Myeloid Leukemia Panel

On behalf of Illumina, I respectfully request the NCCN Chronic Myeloid Leukemia Guideline Panel to review the enclosed information for inclusion of next-generation sequencing (NGS) for BCR-ABL1 mutation analysis.

Specific Changes:

We ask the panel to recommend NGS for BCR-ABL1 mutation analysis. NGS can detect low-level BCR-ABL1 mutations that may be missed by the current standard of care, allowing for appropriate tyrosine kinase inhibitor (TKI) therapy selection.

FDA Clearance:

The recommendation to use an NGS-based technique is not associated with any specific FDA-cleared product/s.

Rationale:

Sanger sequencing is the standard method for BCR-ABL1 mutation analysis. It is expected that methods used for BCR-ABL1 mutation analysis would be developed and performed by a laboratory that is Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists (CAP)-accredited.

Although Sanger sequencing is widely accepted, it is unable to detect mutations at frequencies less than 10% to 20% (ie, mutations present in less than 10% to 20% of cells) [1]. Recently, a growing body of literature demonstrates that NGS detects TKI-resistant mutations present at low levels that can be missed by Sanger sequencing, which enables earlier detection and appropriate TKI therapy selection.

- NGS detects low-level TKI-resistant mutations that are missed by Sanger sequencing.
 - A prospective multicenter study showed that low-level TKI-resistant mutations were detected by NGS (with a limit of detection of 3% mutation level) but missed by Sanger sequencing in CML patients with Failure or Warning* [2]. Of 211 patients, 20% (42/211) were positive for any point mutation by Sanger sequencing versus 45% (94/211) positive by NGS; 25% (52/211) of patients had low-level mutations detected by NGS that were missed by Sanger sequencing. Longitudinal follow-up data, available for 27 patients with low level mutations, showed that TKI-resistant mutations were detected and tended to increase in burden at later timepoints unless treatment was changed to an appropriate TKI therapy.
 - Several additional studies support that NGS detects low-level mutations (as low as 1%–4% mutation level) that are missed by Sanger sequencing.
 - In heavily pretreated CML patients treated with ponatinib, NGS detected mutations in 61% (163/267) of patients, with 266 mutations detected overall. In comparison, Sanger sequencing detected mutations in 49% (131/267) of the patients, with 161 mutations detected overall. NGS detected all 161 mutations detected by Sanger sequencing and detected 105 low-level mutations (frequencies of 1%–11%) missed by Sanger sequencing (present in 27% [73/267] of patients) [1]. In 12% (32/267) of patients, only low-level mutations were found.
 - In 141 CML patients failing on imatinib, 33% (47/141) had at least 1 mutation detected by NGS [3]. Samples from a subset (n=10) of the 47 patients with mutations detected by NGS were tested by Sanger sequencing; 5 low-level mutations (frequencies of 10%–20%) in 40% (4/10) of these patients were missed by Sanger sequencing.

* Per the European LeukemiaNet (ELN) definitions

- In 107 CML patients on first- or second-line TKI therapy with no evidence of mutations by Sanger sequencing, 13% (14/107) had low-level mutations (frequencies of 1.5%–11.7%) detected by NGS [4]. The low-level mutations missed by Sanger sequencing included a T315I mutation in 2 patients with Warning on second-line therapy.
- By detecting low-level mutations, NGS can identify relevant mutations earlier than Sanger sequencing, possibly changing TKI therapy decisions.
 - Soverini et al studied 51 imatinib-resistant CML patients on second-line therapy with dasatinib or nilotinib (guided by Sanger sequencing BCR-ABL1 mutation analysis) who later acquired dasatinib- or nilotinib-resistant mutations (as determined by Sanger sequencing) [5, 6]. NGS detected the dasatinib- or nilotinib-resistant mutations earlier than Sanger sequencing in 45% (23/51) of patients. Relevant mutations were detected by NGS 3 to 9 months (median 3 months) earlier than Sanger sequencing. In 5 patients, mutations were detected by NGS at baseline.
 - Another study examined 60 imatinib-resistant patients (45 with CML and 15 with Ph+ ALL) who failed second-line therapy with dasatinib or nilotinib and acquired BCR-ABL1 mutations [7]. Switchover to second-line therapy was performed after BCR-ABL1 mutation analysis by Sanger sequencing, so patients known positive for a T315I mutation were not included in this evaluation. NGS detected 90 mutations overall in 80% (48/60) of patients at time of switchover (to second-line therapy). Prior Sanger sequencing detected 30 mutations overall in 47% (28/60) of patients but missed 60 low-level mutations. Of the 60 low-level mutations, 30 conferred dasatinib and/or nilotinib resistance, including T315I mutation (n = 13). NGS detected dasatinib- or nilotinib-resistant mutations earlier than Sanger sequencing in 43% (26/60) of patients. Thus, low-level mutations detected by NGS in this population were relevant for TKI choice at switchover.
 - In a retrospective longitudinal analysis of samples from 12 CML patients failing first-line imatinib therapy, NGS detected TKI resistant mutations 2 to 11 months earlier than Sanger sequencing in 75% (9/12) of patients [8].
 - In 40 CML patients who failed first- to third-line TKI therapy and had high levels of T315I mutation previously detected by Sanger sequencing, earlier samples were tested by NGS [9]. In 50% (20/40) of patients, NGS detected low-level T315I mutations a median of 3 months before the mutation reached levels ($\geq 15\%$) that could be detected by Sanger sequencing. In the other 20 patients, the earliest measurable T315I mutation frequency was 27% or more, thus, the T315I mutation was potentially detectable by both NGS and Sanger.

Proposed Changes

Page CML-C, Monitoring Response to TKI Therapy and Mutational Analysis Chart

Current Statement

“BCR-ABL kinase domain mutation analysis”

New Statement

*“BCR-ABL kinase domain mutation analysis **by Next-Generation Sequencing**”*

New Statement

Add footnote: *“Next-generation sequencing (NGS) assays can detect low-level mutations that would be missed by Sanger sequencing. Detection of low-level mutations enables earlier detection, allowing for appropriate therapy selection.”*

The following articles are submitted in support of this proposed change. We would like to acknowledge the contributions of NCCN panel members who are also authors of some of these publications.

1. Deininger MW, Hodgson JG, Shah NP, Cortes JE, Kim DW, Nicolini FE, et al. Compound mutations in BCR-ABL1 are not major drivers of primary or secondary resistance to ponatinib in CP-CML patients. *Blood*. 2016;127(6):703-12. Epub 2015/11/26. doi: 10.1182/blood-2015-08-660977. PubMed PMID: 26603839; PubMed Central PMCID: PMC4760131.
2. Soverini S, Bavaro L, De Benedittis C, Martelli M, Stella S, Vigneri P, et al. What's Next in CML - a Prospective Study Evaluating Sanger Sequencing and Next Generation Sequencing (NGS) for BCR-ABL1 Kinase Domain (KD) Mutation Screening. *Blood*. 2017;130(Suppl 1):248-.
3. Duong CQ, Nguyen C, Nguyen TT, Nguyen LV, Pham HQ, Trinh HTT, et al. Utilization of next-generation deep sequencing to analyze BCR-ABL1 kinase domain mutation for Imatinib-resistant chronic myeloid leukemia patients in Vietnam. *J Leuk (Los Angel)*. 2017;5(2). doi: 10.4172/2329-6917.1000235.
4. Soverini S, De Benedittis C, Castagnetti F, Gugliotta G, Mancini M, Specchia G, et al. BCR-ABL Mutations in Chronic Myeloid Leukemia (CML) Patients (pts) with Failure and Warning to First- and Second-Line Tyrosine Kinase Inhibitor (TKI) Therapy: What Is the Advantage of Next-Generation Sequencing (NGS) over Conventional Sequencing? *Blood*. 2015;126(23):346-.
5. Soverini S, De Benedittis C, Zazzeroni L, Machova Polakova K, Castagnetti F, Gugliotta G, et al. In Chronic Myeloid Leukemia Patients on 2nd-Line Tyrosine Kinase Inhibitor Therapy, Deep Sequencing at the Time of Warning May Allow Sensitive Detection of Emerging BCR-ABL1 Mutants. *Blood*. 2014;124(21):815-.
6. Soverini S, De Benedittis C, Castagnetti F, Gugliotta G, Mancini M, Bavaro L, 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. Epub 2016/08/04. doi: 10.1186/s12885-016-2635-0. PubMed PMID: 27485109; PubMed Central PMCID: PMC4970247.
7. Soverini S, De Benedittis C, Polakova KM, Linhartova J, Castagnetti F, Gugliotta G, et al. Next-generation sequencing for sensitive detection of BCR-ABL1 mutations relevant to tyrosine kinase inhibitor choice in imatinib-resistant patients. *Oncotarget*. 2016;7(16):21982-90. Epub 2016/03/17. doi: 10.18632/oncotarget.8010. PubMed PMID: 26980736; PubMed Central PMCID: PMC4908338.
8. Machova Polakova K, Kulvait V, Benesova A, Linhartova J, Klamova H, Jaruskova M, et al. Next-generation deep sequencing improves detection of BCR-ABL1 kinase domain mutations emerging under tyrosine kinase inhibitor treatment of chronic myeloid leukemia patients in chronic phase. *Journal of cancer research and clinical oncology*. 2015;141(5):887-99. Epub 2014/11/05. doi: 10.1007/s00432-014-1845-6. PubMed PMID: 25367136.
9. Baer C, Kern W, Koch S, Nadarajah N, Schindela S, Meggendorfer M, et al. Ultra-deep sequencing leads to earlier and more sensitive detection of the tyrosine kinase inhibitor resistance mutation T315I in chronic myeloid leukemia. *Haematologica*. 2016;101(7):830-8. Epub 2016/04/23. doi: 10.3324/haematol.2016.145888. PubMed PMID: 27102501; PubMed Central PMCID: PMC4904462.

Thank you for your consideration,



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