SEQUENTA

Submitted by:
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NCCN Guidelines® Panel: Acute Lymphoblastic Leukemia

On behalf of Sequenta, I respectfully request the NCCN Acute Lymphoblastic Leukemia Guidelines Panel to review the enclosed data for inclusion of the ClonoSIGHT™ MRD test in the NCCN recommendations for MRD assessment.

Specific Changes:
Request the following changes to section ALL-F in NCCN Acute Lymphoblastic Leukemia Guidelines Version 1.2014:

- To Bullet 4 ("The most frequently employed methods…") add: *The ClonoSIGHT MRD test is a newer option that uses multiplexed PCR and next-generation sequencing (NGS) to identify, detect and quantify clonal Ig and TCR gene rearrangements.*
- To Bullet 5 ("Current multicolor flow cytometry or PCR methods…") add: *The ClonoSIGHT MRD test can detect leukemic cells at a sensitivity threshold of $<1X10^{-6}$ (<0.0001%) leukocytes. High concordance between the ClonoSIGHT MRD test and both multicolor flow cytometry and RQ-PCR has been demonstrated. The ability of the ClonoSIGHT MRD test to simultaneously track multiple gene rearrangements, multiple clones for each rearrangement and evolved clonal sequences can avoid false-negative results.*
- After Bullet 5, sub-bullet 3 ("RQ-PCR: sampling of bone marrow….") add new sub-bullet: *ClonoSIGHT MRD test: sampling from bone marrow MNCs or peripheral blood; sensitivity is affected by input cell number, which is ideally at least $1X10^{6}$ cells (about 1-3 mL of bone marrow or 5-10 mL of peripheral blood provides a sufficient number of cells for analysis).*
- To Bullet 6 ("High-sensitivity PCR assays…") add: *The ClonoSIGHT MRD test uses a set of universal primers for analysis of Ig and TCR gene rearrangements and therefore has the benefit of being applicable to all patients without reagent customization.*

Request equivalent changes to those noted above be made in the narrative section of the Guidelines, specifically under "Role of MRD Evaluation" (pages MS-40 – MS-41) and "NCCN Recommendations for MRD Assessment" (pages MS-45 – MS-46).

Also request addition of the following information to page MS-40, after the sentence describing the concordance data between flow cytometry and PCR: *In a study comparing the ClonoSIGHT MRD test to both flow cytometry and RQ-PCR in samples from 106 patients, the concordance rates were 90% and 96%, respectively, with all but one discordance attributable to the increased sensitivity of the ClonoSIGHT MRD test compared to the other methods.*

Also request inclusion of a description of the results of Logan, et al. (see summary below) under "MRD Assessment in Adult ALL" (pages MS-43 – MS-45), as this study demonstrates the prognostic value of MRD assessment using the ClonoSIGHT MRD test in the adult ALL post-transplant population.

FDA Clearance:
FDA clearance is not required for this assay because the ClonoSIGHT MRD test is performed in the central laboratory at Sequenta, Inc. that is regulated and certified under the Clinical Laboratory Improvement Amendments (CLIA) and the College of American Pathologists (CAP).

Rationale:
Based on extensive evidence in the literature, the NCCN Acute Lymphoblastic Leukemia Guidelines recognize the prognostic value of MRD assessment in ALL and recommend the use of either multicolor flow cytometry or RQ-PCR for MRD assessment, noting that the two methods have been demonstrated to be concordant. Data in the studies referenced and summarized below demonstrate that the ClonoSIGHT MRD test is concordant with both multicolor flow cytometry and RQ-PCR, therefore supporting addition of this test as a third alternative for NCCN recommendations for MRD assessment. The specific changes requested above reflect technical aspects and advantages of the ClonoSIGHT MRD test that are also supported by the literature referenced below, and are reflective of the level of detail provided for multicolor flow cytometry and RQ-PCR in the Guidelines.

Note: The studies referenced below refer to the "LymphoSIGHT method", not the ClonoSIGHT MRD Test. This is the nomenclature Sequenta uses to distinguish between the use of our technology for research (LymphoSIGHT) and clinical use (ClonoSIGHT). "ClonoSIGHT MRD test" is the name of the commercial product available to clinicians through Sequenta's CLIA-certified CAP-accredited laboratory, and is therefore the recommended language for NCCN guidelines.

The following articles are submitted in support of the changes proposed in this letter:

1. **Faham M, Zheng J, Moorhead M, et al. Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. *Blood* 2012;120:5173-80.**
   This study of bone marrow samples from more than 100 pediatric patients with B-lineage ALL demonstrates quantitative concordance between the ClonoSIGHT MRD test and both multicolor flow cytometry and RQ-PCR. The technical performance data presented demonstrate that sensitivity is limited only by input cell number and thus can detect MRD at levels well below 0.0001%. The ability of the ClonoSIGHT MRD test to detect and follow multiple clones and evolved clones is also demonstrated.

2. **Ladetto M, Bruggemann M, Monitillo L, et al. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. *Leukemia* 2014;28:1299-1307.**
   This study compares the ClonoSIGHT MRD test to RQ-PCR in a series of 55 adult patients: 15 with ALL, 30 with MCL and 10 with MM. Overall 378 samples were analyzed (62 were taken at diagnosis and 316 after treatment; 218 samples were bone marrow and 160 peripheral blood). Correlation of MRD results was observed between the two methods ($R=0.791$, $P<0.001$), with concordance in 79.6% of cases. In ALL, a total of 26 follow-up samples were analyzed using both methods: 20/26 follow-up samples (77%) were concordant between the two methods.

3. **Logan AC, Vashi N, Faham M, et al. Immunoglobulin and T cell receptor gene high-throughput sequencing quantifies minimal residual disease in acute lymphoblastic leukemia and predicts post-transplantation relapse and survival. *Biol Blood Marrow Transplant* 2014;20:1307-13.**
   This study used the ClonoSIGHT MRD test to quantify MRD in 237 peripheral blood samples from 29 adult B cell ALL patients before and after allo-HCT. MRD positivity ($\geq 10^{-6}$) at any time through day +100 post-transplant predicts subsequent relapse (HR 14; 95% CI 4.7 – 44, $p<0.0001$). In serial post-HCT blood samples, MRD$\geq 10^{-6}$ had 100% positive predictive value for relapse with median lead time of 89 days.

Sincerely,

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