

Submitted by:

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NCCN Guidelines Panel: Acute Lymphoblastic Panel

As a pediatric oncologist and on behalf of Adaptive Biotechnologies, I would like to request that the NCCN Acute Lymphoblastic Leukemia Guidelines Panel consider the inclusion of the use of the multiplex PCR and high throughput sequencing (HTS) platform for determination of MRD for inclusion in the clinical practice guidelines for ALL.

Specific Changes: We respectfully request the acknowledgment of this platform as an option to assess MRD in ALL at all points in treatment where MRD testing is currently proposed using ASO-PCR or multicolor flow based methods.

We would like to request the following inclusions into the current treatment guidelines:

- Revised ALL-2 to include a footnote referencing collection of 0.5-2 mls of fresh BMA collected in EDTA or (or 2-5 mls peripheral blood) for testing to identify the dominant clone and clonal distribution for subsequent MRD assessment.
- Revise ALL-F fourth bullet to describe the available methods for MRD assessment including high-throughput sequencing (HTS or "next generation sequencing, NGS") based MRD assessment. Supporting references are attached.
- Revise ALL-F fifth bullet to indicate that while current conventional flow cytometric methods can detect leukemic cells at a sensitivity threshold around 1×10^{-4} , the sensitivity and limit of detection of HTS-based MRD analyses are essentially only a function of the number of cells that are analyzed. If one million cells are analyzed the sensitivity of the HTS platform approaches one in one million. (1×10^{-6}).
- Revise ALL-F bullet 5 sub bullets, indicating that;
 - MRD assessment upon completion of initial induction is recommended.
 - MRD testing should also be considered at the time of suspected complete response.

Supporting the requests above, we also seek revisions to the Discussion section to include a description of the evidence supporting the use of HTS-based MRD assessment. Updates to the discussion section would ideally include an addition to MS-7 referencing the collection of an identification specimen used to determine the dominant clone (see references below). The **Role of MRD** discussion on MS-44 – MS-45 would also include a summary of the evidence which supports the changes proposed above and define HTS-MRD as an option (see the enclosed references). Finally, the **NCCN Recommendation for MRD Assessment** on MS-50 would also be revised to reflect the updates proposed in the bullet points above.

Laboratory Certification: The HTS-based MRD assay is performed in a Clinical Laboratory at Adaptive Biotechnologies and is certified and regulated by the Clinical Laboratory Improvement Amendments (CLIA) and the College of American Pathologists (CAP).

FDA Clearance: Adaptive is seeking regulatory clearance for the clonoSEQ HTS-based MRD assay in the United States in 2017.

Rationale: HTS-based MRD assessment has been evaluated in more than eight clinical ALL trials which included approximately 1000 subjects. These studies have demonstrated that the HTS-based platform is at least as, if not more, accurate and standardized than flow cytometry and allele-specific oligonucleotide PCR and also more sensitive (2-11). These studies have also proven that increased sensitivity is relevant to and correlated with patient outcome (1, 8-11). Currently, the assay has been incorporated into three ongoing cooperative group trials for ALL and has been incorporated into drug development plans by a number of large and small biotech/pharma companies. At least as importantly, the assay is currently being used by over 300 clinicians (including 180 clinicians specializing in the treatment of patients with ALL at over 75 institutions). Over 2000 samples from ALL patients have been received by Adaptive's commercial CLIA/CAP certified testing laboratory for the purpose of real-time patient management. In addition, nearly 3000 samples from ALL patients have been evaluated by Adaptive for MRD-related research projects with academic institutions and cooperative groups. The rapidly growing body of evidence demonstrates the growing acceptance of the HTS-based MRD assay which was recently recognized in the v1.2017 Multiple Myeloma Clinical Practice Guidelines.

Supporting Information: Immunosequencing is a multiplex PCR-based method that amplifies rearranged Immunoglobulin (Ig) and T-cell receptor (TCR) hypervariable region (CDR3) nucleotide sequences. When applied to genomic DNA, the frequency of each CDR3 sequence identified is highly representative of the relative frequency of each lymphocyte containing that CDR3 sequence in the biologic sample. Thus, the immunosequencing assay captures both the full Ig or TCR repertoire and specific individual clones. Given the capacity of HTS, this approach is extremely sensitive and only limited by the amount of DNA that is analyzed. Routinely, if one million cells' worth of DNA is analyzed, the assay can detect clones at a sensitivity that approaches 1:1,000,000. Thus, this technology provides a highly accurate and standardized method for assessment of lymphoid clonality in healthy, diseased, or malignant tissues and for identifying and tracking the presence and frequency of common and rare clones within the total adaptive immune system.

The data generated by the immunosequencing assay is a combination of receptor sequences and their frequencies. For each sample, DNA is extracted and the relevant immune receptor CDR3 region is amplified and sequenced. In brief, bias-controlled V and J gene primers are used to amplify rearranged V(D)J segments for high throughput sequencing at ~10x coverage. Next, sequencing errors in the raw sequence data are corrected via a clustering algorithm, and the primary nucleotide sequence of the amplified regions from the immune receptors' unique CDR3 segment is determined, quantified, and annotated according to the International ImMunoGeneTics collaboration (12), identifying which V, D, and J genes contributed to each rearrangement.

If you have any questions or require additional information, please do not hesitate to contact me at 206-693-2160 or via e-mail at lkirsch@adaptivebiotech.com. Thank you for your time and consideration.

Sincerely,

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

1. Stow P, Key L, Chen X, et al. Clinical significance of low levels of minimal residual disease at the end of remission induction therapy in childhood acute lymphoblastic leukemia. *Blood* 2010; 115:4657-4663
2. 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.
3. Wu D, Sherwood A, Fromm J, et al. High throughput sequencing detects minimal residual disease in acute T lymphoblastic leukemia. *Science Translational Medicine* 2012; 4:134ra63
4. Carlson C, Emerson R, Sherwood A, et al. Using synthetic templates to design an unbiased multiple PCR assay. *Nature Communications* 2013; 4:2680 | DOI: 10.1038/ncomms3680
5. 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.
6. 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
7. Wu D, Emerson R, Sherwood A, et al. Detection of minimal residual disease in B lymphoblastic leukemia by high-throughput sequencing of IgH. *Clinical Cancer Research* 2014; DOI: 10.1158/1078-0432.CCR-13-3231
8. Pulsipher M, Carlson C, Langholz B, et al. IgH-V(D)J NGS-MRD measurement pre- and early post-allo transplant defines very low- and very high-risk ALL patients *Blood* 2015; 125:3501-3508.
9. Kirsch I, et al. High throughput sequencing as a measure of early response to therapy in childhood ALL. European Hematology Association Annual Meeting 2016 (Copenhagen, Denmark) Oral Presentation (EHA-1844) abstract code S496
10. Kirsch I, et al. High throughput sequencing as a measure of early response to therapy in childhood ALL International Society for Pediatric Oncology Oral Presentation **SIOP Abstract 2016; O-031**
11. Wood B, Wu D, Kirsch I, et al. **Residual Disease Monitoring By High Throughput Sequencing Provides Risk Stratification in Childhood B-ALL and Identifies a Novel Subset of Patients Having Poor Outcome.** 2016. American Society of Hematology Annual Meeting. 2016; Publication Number: 1086 Oral Presentation Submission ID: 98596
12. Yousfi Monod, M., Giudicelli, V., Chaume, D., Lefranc, M.P., 2004. IMGT/JunctionAnalysis: the first tool for the analysis of the immunoglobulin and T cell receptor complex V-J and V-D-J JUNCTIONs. *Bioinformatics* 20 Suppl 1, i379-385.