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NCCN Guidelines Panel: Acute Myeloid Leukemia

On behalf of Illumina, I respectfully request the NCCN Acute Myeloid Leukemia Guideline Panel to review the enclosed to include Next-Generation Sequencing (NGS) when referring to various "molecular" related terminology and applications in the guidelines as described in detail below.

Specific Requested Changes:

We propose that the following figures/terminology are clarified to include NGS as the term "molecular" has become more inclusive of technologies outside of Polymerase Chain Reaction (PCR), when interrogating gene mutations in the AML NCCN Guidelines v 3.2017 – June 6, 2017 : AML-1 (molecular analyses, molecular abnormalities), AML-8 (targeted molecular abnormalities), AML- 9 (molecular studies, molecular abnormalities), AML-10 (molecular abnormalities), AML-11 (molecular markers), AML-13 (molecular markers), AML-14 (molecular profiling), AML-A (molecular abnormalities), and include in the Initial Evaluation (Molecular Markers and Risk Stratification, Workup) section of the discussion.

Note: This recommendation does exclude the terms "Molecular testing, Molecular Assessment, Molecular Remission, Molecular Relapse, Molecular CR" when referring to usage in Acute Promyelocytic Leukemia or Minimal Residual Disease as there is not sufficient evidence to support the use of NGS in these settings.

FDA Clearance:

The recommendation of the NGS technique does not have any one specific product associated with it and, therefore, is not FDA cleared.

Rationale:

As more data and techniques become available to evaluate gene mutations used to identify a patient's mutational profile, a more prescriptive approach is needed to direct laboratories on which techniques are appropriate in the clinical setting.

Previously, PCR was the predominate molecular technology with the capabilities for detecting known gene mutations in AML. Due to this, we have commonly referred to interrogating gene mutations with synonymous terms such as *"molecular abnormalities, molecular analyses, molecular studies, molecular markers and molecular profiling*", as is done in the AML NCCN Guidelines. However, molecular testing has grown into several technologies with different capabilities. We believe a more prescriptive approach is appropriate in identifying which technologies would be suitable, when the term "molecular" is used synonymously with identifying gene mutations.

As an example, in Figure AML-1 (Excerpt 1), *"cytogenetics"* is clarified by labeling the karyotype and FISH technique in *"cytogenetics (Karyotype ± FISH)"*, as the term *"cytogenetics"* encompasses multiple techniques. We believe that the NGS technique should be called out in Figure AML-1 (Excerpt 1), *"Molecular analyses (KIT, FLT3-ITD, NPM1, CEBPA, and other mutations)"* as NGS is a suitable technique to identify these gene mutations, and AML-1 Footer "a" (Excerpt 2), where "*Multiplex gene panels and sequencing assays are available for the assessment of other <u>molecular abnormalities</u>*" are described[5-9].

Additionally, for the same reasons, we believe that the term *"sequencing assays"* in AML-1 Footer "a", should be clarified to *"Next-Generation Sequencing Assays*", as the term "sequencing" would be solely referring to Sanger sequencing techniques.

Excerpt 1: Figure AML-1: Evaluation for Acute Leukemia

"Bone marrow with cytogenetics (karyotype ± FISH) and <u>molecular analyses</u> (KIT, FLT3-ITD, NPM1, CEBPA and other mutations)[a]"

Excerpt 2: Footer "a" of Figure AML-1: Evaluation for Acute Leukemia

"[a] Molecular abnormalities (KIT, FLT3-ITD, NPM1, CEBPA, and other mutations) are important for prognostication in a subset of patients (category 2A) and may guide therapeutic intervention (category 2B) (See AML-A). Multiplex gene panels and sequencing assays are available for the assessment of other molecular abnormalities that have prognostic impact in AML or eligibility for clinical trial (see discussion)"

Proposed Changes

Excerpt 1: Figure AML-1: Evaluation for Acute Leukemia

"Bone marrow with cytogenetics (karyotype ± FISH) and molecular analyses (PCR ±NGS) (KIT, FLT3-ITD, NPM1, CEBPA and other mutations).[a]"

Or

"Bone marrow with cytogenetics (karyotype ±FISH) and mutational analysis (PCR ±NGS) for genes KIT, FLT3-ITD, NPM1, CEBPA and other mutations.[a] "

And

"Multiplex gene panels and next-generation sequencing assays are available for the assessment of other molecular abnormalities that have prognostic impact in AML or eligibility for clinical trial (see discussion)"

These minor changes would be more prescriptive to techniques that are appropriate for looking at "molecular abnormalities" when using the term "molecular analyses".

Additional Figure Changes: AML-8, AML-9, AML-10, AML-11, AML-13, AML-14, AML-A and Discussion

Throughout the Acute Myeloid Leukemia NCCN guidelines, there are additional instances of the terms *"molecular abnormalities*, *molecular analyses*, *molecular studies*, *molecular markers and molecular profiling"*, being used synonymously when referring to gene mutations. We are recommending similar changes, with the addition of NGS as an appropriate molecular technique in these instances. We also recommend any caveats for appropriate usage of NGS should be described in the discussion section.

The following articles are submitted in support of using NGS as a technique to test molecular abnormalities. We would like to acknowledge the contributions of NCCN panel members who are also co-authors or co-contributors of some of these publications.

1) Lin P, Li H, Fan S, et al. A targeted next-generation sequencing in the molecular risk stratification of adult acute myeloid leukemia: implications for clinical practice. *Cancer Medicine*. 2017;6(2):349-360. doi:10.1002/cam4.969.
2) Arber D, Orazi A, Hasserjian R, Thiele J, Borowitz M, Lebeau M, Bloomfield C, Cazzola M, Vardiman J et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood, 127(20), 2391-2405.
3) Reinig E, Yang F, Traer E, Arora R,Brown S,Rattray R, Braziel R, Fan G, Press R, Dunlap J, et al. Targeted Next-Generation Sequencing in Myelodysplastic Syndrome and Chronic Myelomonocytic Leukemia Aids Diagnosis in Challenging Cases and Identifies Frequent Spliceosome Mutations in Transformed Acute Myeloid Leukemia. *Am J Clin Pathol* 2016; 145 (4): 497-506. doi: 10.1093/ajcp/aqw016
4) Cancer Genome Atlas Research N. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368:2059–74.
5) Luthra R, Patel KP, Reddy NG, et al. Next-generation sequencing-based multigene mutational screening for acute myeloid leukemia using MiSeq: applicability for diagnostics and disease monitoring. *Haematologica*. 2014;99(3):465-473. doi:10.3324/haematol.2013.093765.
6) Haslam, K., Catherwood, M.A., Dobbin, E. et al. Mol Diagn Ther (2016) 20: 457. doi:10.1007/s40291-016-0222-3
7) McCourt CM, McArt DG, Mills K, et al. Validation of Next Generation Sequencing Technologies in Comparison to Current Diagnostic Gold Standards for *BRAF*, *EGFR* and *KRAS* Mutational Analysis. Devaney J, ed. *PLoS ONE*. 2013;8(7):e69604. doi:10.1371/journal.pone.0069604.
8) Au CH, Wa A, Ho DN, Chan TL, Ma ESK. Clinical evaluation of panel testing by next-generation sequencing (NGS) for gene mutations in myeloid neoplasms. *Diagnostic Pathology*. 2016;11:11. doi:10.1186/s13000-016-0456-8.
9) Hiemenz MC, Kadauke S, Lieberman DB, et al. Building a Robust Tumor Profiling Program: Synergy between Next-Generation Sequencing and Targeted Single-Gene Testing. Mallick B, ed. *PLoS ONE*. 2016;11(4):e0152851. doi:10.1371/journal.pone.0152851.

Sincerely,