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 NCCN Guidelines Panel: Myelodysplastic Syndromes (MDS)

On behalf of Illumina, I respectfully request the NCCN Myelodysplastic Syndromes guideline panel to review the enclosed to include next-generation sequencing (NGS) when referring to “sequencing” in the guidelines as described in detail below.

### **Specific Requested Changes:**

We propose that the following terminology is clarified to include NGS, when interrogating gene mutations in the MDS NCCN Guidelines v1.2018: MDS-C 1 of 4 (Frequent Mutations in MDS-Associated Genes Likely to Indicate Clonal Hematopoiesis); and MS-18 (Molecular Abnormalities in MDS) section of the discussion.

### **FDA Clearance:**

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

### **Rationale:**

MDS is a clonal disease characterized by the subsequent and progressive acquisition of somatic mutations, mostly in recurrently involved genes[1]. In MDS, clonal evolution is dynamic, with an increase in the number, and variability, of driver mutations as the disease progresses[1, 2]. As the nature of acquired driver mutations is relevant in the progression of the disease[2-6], early detection of such mutations can be of important prognostic value[2, 7]. The improved sensitivity offered by NGS facilitates identification of somatic mutations[4, 5, 9] and evaluation of clonal complexity[6], which would be missed by a low coverage method such as Sanger sequencing. Also, NGS allows rapid and simultaneous analysis of hundreds to thousands of genomic regions at once[3, 10]. In comparison, approaches applying a combination of polymerase chain reaction (PCR) methods and capillary Sanger sequencing are time consuming, labor intensive and less informative[10]. Furthermore, PCR-based methods are often limited by the necessity to design sequence specific primers on potentially evolving sequences, as clonal mutations may arise or be lost in different phases of the disease[11].

As more molecular techniques become available to evaluate genomic alterations and establish tumor mutational profiles, a more prescriptive approach is needed to direct laboratories to the most clinically relevant techniques. NGS has been particularly effective in the study of somatic mutations and clonal hematopoiesis in MDS[2-5, 9, 11-15] for the reasons listed above. We suggest that the MDS Guidelines are clarified to mention NGS, where applicable, in the analysis of MDS mutations for prognostic purposes. Specifically, we believe that the term “DNA sequencing” in MDS-C Footer “1”, should be clarified to “NGS”, as the term “sequencing” refers solely to Sanger sequencing.

### **Proposed Changes**

#### **Excerpt 1: MDS-C (page 1 of 4) Table: Frequent Mutations in MDS-Associated Genes**

##### **Change 1**

*“The specific mutations listed in this table are likely to be somatic if found in tumor material.”*

*“The specific mutations listed in this table are likely to be somatic if found in tumor material and should be evaluated by next-generation sequencing”*

##### **Change 2**

*“Known gene polymorphisms frequent in the population should be excluded from DNA sequencing results as they are likely germline variants and not evidence of clonal hematopoiesis.”*

*“Known gene polymorphisms frequent in the population should be excluded from next-generation sequencing results as they are likely germline variants and not evidence of clonal hematopoiesis.”*

#### **Excerpt 2: Discussion - Prognostic Stratification – Molecular Abnormalities section (page MS-19)**

### **Change 3**

*“Mutations identified in peripheral blood samples can accurately reflect mutations detected in the bone marrow of patients of MDS when more sensitive sequencing techniques are used to detect them”*

*“Mutations identified in peripheral blood samples can accurately reflect mutations detected in the bone marrow of patients of MDS when **next-generation sequencing techniques** are used to detect them”*

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. Walter MJ, Shen D, Ding L, Shao J, Koboldt DC, Chen K, et al. Clonal architecture of secondary acute myeloid leukemia. *N Engl J Med*. 2012;366(12):1090-8. doi: 10.1056/NEJMoa1106968. PubMed PMID: 22417201; PubMed Central PMCID: PMC3320218.
2. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med*. 2011;364(26):2496-506. doi: 10.1056/NEJMoa1013343. PubMed PMID: 21714648; PubMed Central PMCID: PMC3159042.
3. Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371(26):2477-87. doi: 10.1056/NEJMoa1409405. PubMed PMID: 25426838; PubMed Central PMCID: PMC4290021.
4. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia*. 2014;28(2):241-7. doi: 10.1038/leu.2013.336. PubMed PMID: 24220272; PubMed Central PMCID: PMC3918868.
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8. Bejar R. Clinical and genetic predictors of prognosis in myelodysplastic syndromes. *Haematologica*. 2014;99(6):956-64. doi: 10.3324/haematol.2013.085217. PubMed PMID: 24881041; PubMed Central PMCID: PMC4040892.
9. Bejar R, Stevenson KE, Caghey B, Lindsley RC, Mar BG, Stojanov P, et al. Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation. *J Clin Oncol*. 2014;32(25):2691-8. doi: 10.1200/JCO.2013.52.3381. PubMed PMID: 25092778; PubMed Central PMCID: PMC4207878.
10. Kohlmann A, Bacher U, Schnittger S, Haferlach T. Perspective on how to approach molecular diagnostics in acute myeloid leukemia and myelodysplastic syndromes in the era of next-generation sequencing. *Leuk Lymphoma*. 2014;55(8):1725-34. doi: 10.3109/10428194.2013.856427. PubMed PMID: 24144312.
11. Duncavage EJ, Tandon B. The utility of next-generation sequencing in diagnosis and monitoring of acute myeloid leukemia and myelodysplastic syndromes. *Int J Lab Hematol*. 2015;37 Suppl 1:115-21. doi: 10.1111/ijlh.12361. PubMed PMID: 25976969.
12. Yoshizato T, Nannya Y, Atsuta Y, Shiozawa Y, Iijima-Yamashita Y, Yoshida K, et al. Genetic abnormalities in myelodysplasia and secondary acute myeloid leukemia: impact on outcome of stem cell transplantation. *Blood*. 2017;129(17):2347-58. doi: 10.1182/blood-2016-12-754796. PubMed PMID: 28223278; PubMed Central PMCID: PMC5409449.
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15. Malcovati L, Papaemmanuil E, Bowen DT, Boulwood J, Della Porta MG, Pascutto C, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. *Blood*. 2011;118(24):6239-46. doi: 10.1182/blood-2011-09-377275. PubMed PMID: 21998214; PubMed Central PMCID: PMC3236114.

Sincerely,

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