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NCCN Guidelines Panel: T-Cell Lymphomas Panel

On behalf of Illumina, I respectfully request the NCCN T-Cell Lymphomas Guideline Panel to review the enclosed information for inclusion of next-generation sequencing (NGS) as an additional technique in the diagnosis of T-cell lymphomas (TCLs).

**Specific Changes:**

We ask the panel to recommend NGS as a molecular testing method for the detection of clonal T-cell receptor (TCR) gene rearrangements. NGS methods can assess T-cell clonality, identifying the exact nucleotide sequence of each clone and its relative frequency in the sample tested.

**FDA Clearance:**

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

**Rationale:**

Traditional techniques for detection of clonal TCR gene rearrangements include flow cytometry and polymerase chain reaction (PCR) coupled with capillary electrophoresis (CE), with the latter being the most widely used method [1]. CE measures PCR product size, which serves as a surrogate for TCR sequence.

NGS is an attractive test option because it can be performed on small inputs of nucleic acid from many different specimen types, including formalin-fixed paraffin-embedded (FFPE) tissues. In addition, NGS methods are sensitive and can detect targets present at very low frequencies. For detection of clonal TCR gene rearrangements, NGS methods can identify the full range of clonal populations in a sample, the relative frequency of each clone, and the specific nucleotide sequence of each clone [1]. The unique sequence of the clone(s) can be used as a personalized marker to monitor treatment response, assess minimal residual disease, and identify recurrence [1, 3-5]. NGS may also guide selection of targeted therapies [1].

The NCCN panel recognizes that molecular analysis to detect clonal TCR gene rearrangements is essential in the diagnosis of mycosis fungoides (MF) and Sezary syndrome (SS). It is particularly helpful in the diagnosis of MF, which can resemble benign inflammatory skin disorders. NGS-based methods may be more accurate than current methods in diagnosing cutaneous TCL (CTCL), even in the early stages of disease.

- In a study evaluating MF patient samples, NGS was more sensitive than TCR $\gamma$  PCR plus CE and enabled tracking of specific clones to accurately identify recurrent MF [1].
  - 85% (29/34) of samples were deemed clonal at the TCR $\gamma$  locus by NGS whereas only 44% (15/34) were deemed clonal using PCR/CE.
  - 3 patients deemed as clonal by NGS had subsequent samples (1 to 4 years apart). In these follow-up samples, NGS detected TCR sequences that were identical to those identified during the initial NGS testing. For example, 1 patient presented with an arm lesion; one year later, a new abdominal lesion harbored the same clonal TCR gene rearrangement.

- In a study evaluating samples from CTCL patients (with SS or MF) and non-CTCL patients, NGS was more sensitive and specific than TCRγ PCR plus CE and was effective in distinguishing early recurrence from benign inflammation [6].
  - In skin lesion samples from 46 patients with CTCL (based on histologic diagnosis), NGS of the TCRβ CDR3 regions detected clonal TCR gene rearrangements in 100% (46/46) of samples.
    - PCR/CE evaluation of a subset of these cases detected clonal TCR gene rearrangements in 70% (27/39) of samples. Of the 12 patients who did not have clonal TCR gene rearrangements detected by PCR/CE, 10 had early stage (IA or IB) disease.
  - NGS distinguished CTCL from benign inflammatory skin disorders and healthy skin.
  - NGS identified CTCL recurrences and facilitated early diagnosis of recurrence.
- In a study of blood and skin tissue pretherapy samples from 10 patients with SS, NGS identified clonal TCRβ sequences in 100% (10/10) of samples [3]. The specificity of the NGS assay was 99.9%.
- In a prospective study of patients with stage IA-IIA CTCL treated with resiquimod, NGS of the CDR3 of TCRβ genes identified clonal TCR gene rearrangements in 91% (10/11) of skin tissue samples [5]. The clonal sequences were used as a marker to track response, with 90% (9/10) showing reductions in the percentage of malignant T cells. Improvement in skin inflammation tended to lag behind reduction of the clonal populations.

NGS-based sequencing is also suitable for detecting clonal TCR gene rearrangements in patients with other T-cell lymphomas [2, 4, 7-11].

- In a study evaluating peripheral blood, bone marrow, and FFPE tissue samples, 27 clonal, 19 polyclonal, and 2 oligoclonal (as determined by PCR/CE) samples were analyzed by NGS of the TCRγ locus [2]. NGS results agreed with PCR/CE results in 93% (43/46) of samples identified as clonal or polyclonal by PCR/CE.
- In a study of lymph node cell suspension samples from peripheral TCL not otherwise specified (PTCL-NOS) and angioimmunoblastic T-cell lymphoma (AITL) patients, NGS was more sensitive than flow cytometry in detecting clonal TCR gene rearrangements [4].
  - NGS detected clonal TCR gene rearrangements in 96% (45/47) and 80% (16/20) of samples deemed as clonal and non-clonal, respectively, by flow cytometry. The samples deemed clonal by NGS but not flow cytometry generally had a dominant clone and a larger background T-cell repertoire, similar to the diverse repertoire of healthy controls.

**Proposed Changes: Page TCEL-1, footnote c; page MFSS-1, footnote f; page PCTLD-2, footnote j; page LGLL-1, footnote g; page TPLL-1, footnote c; page NKTL-1, footnote g.**

#### **Current Statement**

*“Such as FISH, karyotype, genomic analysis”*

#### **New Statement**

*“Such as FISH, karyotype, **next-generations sequencing, other** genomic analysis”*

The following articles are submitted in support of this proposed change.

- [1] K. E. Sufficool, C. M. Lockwood, H. J. Abel, I. S. Hagemann, J. A. Schumacher, T. W. Kelley and E. J. Duncavage, "T-cell clonality assessment by next-generation sequencing improves detection sensitivity in mycosis fungoides," *J Am Acad Dermatol*, vol. 73, no. 2, pp. 228-236, 2015.
- [2] J. A. Schumacher, E. J. Duncavage, T. L. Mosbrugger, P. M. Szankasi and T. W. Kelley, "A comparison of deep sequencing of TCRG rearrangements vs traditional capillary electrophoresis for assessment of clonality in T-cell lymphoproliferative disorders," *Am J Clin Pathol*, vol. 141, pp. 348-359, 2014.
- [3] W. K. Weng, R. Armstrong, S. Arai, C. Desmarais, R. Hoppe and Y. H. Kim, "Minimal residual disease monitoring with high-throughput sequencing of T cell receptors in cutaneous T cell lymphoma," *Sci Transl Med*, vol. 5, no. 214, p. 214ra171, 2013.
- [4] S. D. Brown, G. Hapgood, C. Steidl, A. P. Weng, K. J. Savage and R. A. Holt, "Defining the clonality of peripheral T cell lymphomas using RNA-seq," *Bioinformatics*, vol. 33, no. 8, pp. 1111-1115, 2017.
- [5] A. H. Rook, J. M. Gelfand, M. Wysocka, A. B. Troxel, B. Benoit, C. Surber, R. Elenitsas, M. A. Buchanan, D. S. Leahy, R. Watanabe, I. R. Kirsch, E. J. Kim and R. A. Clark, "Topical resiquimod can induce disease regression and enhance T-cell effector functions in cutaneous T-cell lymphoma," *Blood*, vol. 126, no. 12, pp. 1452-1461, 2015.
- [6] I. R. Kirsch, R. Watanabe, J. T. O'Malley, D. W. Williamson, L. Scott, C. P. Elco, J. E. Teague, A. Gehad, E. L. Lowry, N. R. LeBouef, J. G. Krueger, H. S. Robins, T. S. Kupper and R. A. Clark, "TCR sequencing facilitates diagnosis and identifies mature T cells as the cell of origin in CTCL," *Sci Trans Med*, vol. 7, no. 308, p. 308ra158, 2015.
- [7] R. Marks, S. Kock, C. Wehr, J. Finke, M. Werner, J. Duyster and P. Fisch, "High throughput T cell receptor sequencing augments diagnosis and response assessment in patients with angioimmunoblastic T cell lymphoma [abstract]," *Blood*, vol. 130, p. 1526, 2017.
- [8] Q. Gong, C. Wang, W. Zhang, J. Iqbal, Y. Hu, T. C. Greiner, A. Cornish, J. Kim, R. Rabadan, F. Abate, X. Wang, G. G. Inghirami, T. W. McKeithan and W. C. Chan, "Assessment of T-cell receptor repertoire and clonal expansion in peripheral T-cell lymphoma using RNA-seq data," *Sci Rep*, vol. 7, no. 1, p. 11301, 2017.
- [9] S. Hendry, H. Pham, D. Constantin, S. Chang and V. Rathi, "Validation of TCR gene rearrangement studies for lymphoma clonality assessment by next generation sequencing [Pathology Update abstract]," *Pathology*, vol. 50, no. Supplement 1, p. S74, 2018.
- [10] E. Mahe, D. Mulder, M. Dowar, M. Sukhai, L. Nguyen, P. Ohashi, J. Delabie, T. L. Stockley, T. Pugh and S. Kamel-Reid, "Improving T-cell receptor clonotyping of T-cell lymphomas using hybrid-capture and next-generation sequencing [AACR 107th Annual Meeting 2016 abstract]," *Cancer Res*, vol. 76, no. 14 Suppl, p. Abstract nr 846, 2016.
- [11] M. H. Syed, C. Ho, K. Petrova-Drus, J. Yao, W. Yu, A. Zehir, K. Nafa and M. E. Arcila, "Clinical utility of clonality testing by next generation sequencing in the monitoring of B-cell and T-cell malignancies [ASCO Meeting abstract]," in *ASCO Meeting Library*, Orlando, 2017.

Thank you for your consideration,



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