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NCCN Guidelines Cutaneous Melanoma

On behalf of Illumina, I respectfully request the NCCN Guideline Panel for Cutaneous Melanoma to consider the enclosed information for the inclusion of liquid biopsy-based DNA testing as a complimentary alternative to tissue testing for the assessment of genetic variants when tumor tissue is unavailable.

Specific Changes (in red text)**:**
(ME-C 3 of 7) Add new sub-bullet point to Principles of Molecular Testing section under Methods of mutation testing, Mutation testing may be performed using tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended.

Rationale:
Studies have demonstrated the use of ctDNA, or 'liquid biopsy', as a viable modality of molecular biomarker testing when tumor tissue is unavailable/insufficient or when a patient is medically unfit for invasive tissue sampling. Recommendations for use of ctDNA have been incorporated into breast, non-small cell lung, esophageal, and gastric cancer guidelines[1-4]. Advanced melanoma patients could also benefit from this less invasive biopsy option when tissue is not available. The genomic alterations of solid cancers may be identified by evaluating cell-free or circulating tumor DNA (cfDNA/ctDNA) in the blood[5, 6]. Being less invasive, liquid biopsy-based genomic analysis is used more frequently in patients with advanced disease who may be unable to have a clinical biopsy for disease surveillance and management. This allows more of these patients to be matched to targeted therapies and clinical trials[5,7-9].

- Molecular profiling for genomic alterations can reinforce the selection of patients for targeted therapies (BRAF and/or MEK inhibitors) and clinical trials (for NRAS-targeting drugs)[9].
    - Approximately 50% and 10%-15% of melanoma patients harbor BRAF and NRAS mutations, respectively[10].
    - These biomarkers can be evaluated by ctDNA testing.
- Tumor tissue is often unavailable or insufficient for biomarker testing[9]; this is particularly true for cutaneous melanoma patients[9]. Liquid biopsy assays can evaluate a spectrum of biomarkers, including those observed in melanoma.[5, 6]
- Several melanoma-focused clinical performance studies have demonstrated good concordance between ctDNA/cfDNA analysis and tissue-derived DNA testing[8, 11, 12].
    - In a prospective study of 60 metastatic melanoma patients, ctDNA sequencing of the *BRAF* gene showed a sensitivity, specificity, and overall percent agreement of 86.8%, 100%, and 90.9%, respectively, compared to tissue-based analysis[8].
    - In 732 patients with *BRAF* mutation-positive melanoma[11], a comparison of cfDNA analysis and tissue DNA testing showed an overall positive percent agreement of 76% (504/661) for V600E and 81% (69/85) for V600K. Overall negative percent agreement was 98% (83/85) for V600E and 99% (659/662) for V600K.

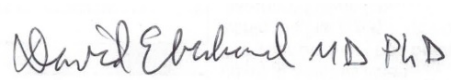
    - In a study of 187 stage III/IV melanoma patients, ctDNA mutational assessment of the *BRAF* gene showed a positive agreement, negative agreement, and overall agreement of 90.3% (56/62), 91.2% (114/125), 90.9% (170/187), respectively, to tissue-based analysis[12].
- Liquid biopsy-based DNA sequencing and tissue-based methods have shown good concordance in several multi-cancer studies[6, 13-15]. CtDNA concordance was higher in tissue metastases than primary tumors[16] and also when an NGS-based method was used to analyze tissue samples[7].
    - In an observational analysis of 165 patients with several different cancers (melanoma n=18)[6], ctDNA analysis had a clinical sensitivity of 85%, specificity of 99.6%, and diagnostic accuracy of 99.3% for all mutated oncogenes, compared with DNA sequencing of matched tissue. Similar but smaller studies showed the positive predictive value to be 100% (melanoma n=14; total n=59)[13] and an overall concordance of 85.9% (melanoma n=13; total n=61)[15].
    - In a *BRAF* mutational study in 160 patients with 18 different cancers (melanoma n=36)[14], the overall agreement between plasma cfDNA and tissue DNA analyses was 88%. The sensitivity, specificity, positive predictive value, and negative predictive value of cfDNA testing were 73%, 98%, 96%, and 85%, respectively.

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

1. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Breast Cancer. Version 4.2021. 2021; https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Accessed May 4, 2021.
2. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Esophageal and Esophagogastric Junction Cancers. Version 2.2021. 2021; https://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf. Accessed May 4, 2021.
3. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Gastric Cancer. Version 2.2021. 2021; https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf. Accessed May 4, 2021.
4. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer. Version 4.2021. 2021; https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf. Accessed May 4, 2021.
5. Rothwell DG, Ayub M, Cook N, et al. Utility of ctDNA to support patient selection for early phase clinical trials: the TARGET study. *Nature Medicine*. 2019/05/01 2019;25(5):738-743.
6. Lanman RB, Mortimer SA, Zill OA, et al. Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA. *PloS one*. 2015;10(10):e0140712.
7. Willis J, Lefterova MI, Artyomenko A, et al. Validation of Microsatellite Instability Detection Using a Comprehensive Plasma-Based Genotyping Panel. *Clinical Cancer Research*. 2019;25(23):7035.
8. Rowe SP, Luber B, Makell M, et al. From validity to clinical utility: the influence of circulating tumor DNA on melanoma patient management in a real-world setting. *Mol Oncol*. 2018;12(10):1661-1672.
9. Boyer M, Cayrefourcq L, Dereure O, et al. Clinical Relevance of Liquid Biopsy in Melanoma and Merkel Cell Carcinoma. *Cancers*. 2020;12(4):960.
10. Tsao H, Chin L, Garraway LA, Fisher DE. Melanoma: from mutations to medicine. *Genes Dev*. 2012;26(11):1131-1155.

11. Santiago-Walker A, Gagnon R, Mazumdar J, et al. Correlation of *BRAF* Mutation Status in Circulating-Free DNA and Tumor and Association with Clinical Outcome across Four BRAFi and MEKi Clinical Trials. *Clinical Cancer Research*. 2016;22(3):567.
12. Haselmann V, Gebhardt C, Brechtel I, et al. Liquid Profiling of Circulating Tumor DNA in Plasma of Melanoma Patients for Companion Diagnostics and Monitoring of BRAF Inhibitor Therapy. *Clinical chemistry*. 2018;64(5):830-842.
13. Cheng J, Cao Y, MacLeay A, et al. Clinical Validation of a Cell-Free DNA Gene Panel. *The Journal of Molecular Diagnostics*. 2019;21(4):632-645.
14. Janku F, Huang HJ, Claes B, et al. *BRAF* Mutation Testing in Cell-Free DNA from the Plasma of Patients with Advanced Cancers Using a Rapid, Automated Molecular Diagnostics System. *Molecular Cancer Therapeutics*. 2016;15(6):1397.
15. Tae Kim S, Suk Lee W, Lanman RB, et al. Prospective blinded study of somatic mutation detection in cell-free DNA utilizing a targeted 54-gene next generation sequencing panel in metastatic solid tumor patients. *Oncotarget; Vol 6, No 37*. 2015;
16. Perkins G, Yap TA, Pope L, et al. Multi-purpose utility of circulating plasma DNA testing in patients with advanced cancers. *PloS one*. 2012;7(11):e47020-e47020.

Thank you for your consideration,



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