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NCCN Guidelines Panel: Colon Cancer Panel

On behalf of Illumina, I respectfully request the NCCN Colon Cancer guideline panel to review the enclosed information regarding extended RAS molecular testing in metastatic colorectal cancer and ask for inclusion of an FDA-approved next-generation sequencing (NGS)-based *in vitro* diagnostic for panitumumab eligibility, which targets 56 mutations in *KRAS* exons 2, 3, and 4 and *NRAS* exons 2, 3, and 4. In addition, physicians need to be informed that the current FDA approved PCR-based assays for RAS testing only examine *KRAS* exon 2.

Specific Requested Changes:

We ask the panel to emphasize that *KRAS* and *NRAS* testing in mCRC needs to cover exons 2, 3, and 4 of both genes. This level of extended RAS testing is available through a new FDA-approved NGS-based Extended RAS Panel assay. This assay determines RAS mutation status in exons 2, 3, and 4 of both *KRAS* and *NRAS* in colorectal cancer patients being considered for panitumumab therapy.

FDA Clearance:

On 29 June 2017, the NGS-based Praxis™ Extended RAS Panel was approved for the detection of 56 specific mutations in RAS genes [*KRAS* (exons 2, 3, and 4) and *NRAS* (exons 2, 3, and 4)] in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue samples. The test is indicated to aid in the identification of patients with colorectal cancer for treatment with Vectibix® (panitumumab) based on a no mutation detected test result [1].

Rationale:

- A growing body of literature has established that mutations in *KRAS* exons 2, 3 and 4, and *NRAS* exons 2, 3, and 4 are predictive of a lack of patient benefit with panitumumab and cetuximab in mCRC, with a negative impact on progression-free survival and overall survival [1-9].
  - The PRIME study (20050203) was a phase 3, multicenter, open-label, randomized trial that evaluated panitumumab plus FOLFOX4 compared with FOLFOX4 alone as first-line therapy in 1183 patients with wild-type *KRAS* exon 2 mCRC [10]. *KRAS* status was determined by the investigational use only *TheraScreen KRAS* Mutation kit (QIAGEN®), which targeted 7 somatic mutations in *KRAS* exon 2. Later, banked PRIME samples that were characterized as wild-type *KRAS* exon 2 were evaluated for mutations in *KRAS* exons 3 and 4, and *NRAS* exons 2, 3, and 4 using Sanger sequencing. The ascertainment rate of tumor RAS status was 90%; **17%** of tumor samples carried other *RAS* mutations (other than *KRAS* exon 2) [3]. In the PRIME cohort, extended RAS analysis would have saved 201 patients from an ineffective therapy and associated adverse events.
  - In 2017, a joint guideline was published and explicitly stated that extended RAS mutational assessment (including KRAS and NRAS exons 2, 3, and 4) should become standard of care for metastatic CRC [5].
- To determine the accuracy of the Praxis Extended RAS Panel, NGS-based assay results were compared to results from Sanger sequencing in samples from 441 patients from the PRIME study. The positive percent agreement (PPA) was 98.7% and the negative percent agreement (NPA) was 97.6% [1]. The Sanger methodology was more labor intensive, time consuming, and required a much higher DNA input, as opposed to NGS which simultaneously detected 56 potentially activating mutations in a single experiment using only 40 nanograms of DNA.
- Per the FDA labeling for panitumumab [8] and cetuximab [9], treatment is not indicated in patients with RAS mutant colorectal cancer or when RAS mutation status is unknown.
  - The other FDA-approved tests, which are PCR-based, detect mutations in *KRAS* exon 2 **only**, missing KRAS exons 3 and 4 and any NRAS mutations, thereby not establishing the full RAS mutation status (ie, RAS mutation status is unknown).
    - The cobas® *KRAS* Mutation Test is approved for the detection of seven somatic mutations in codons 12 and 13 of the *KRAS* gene. The test is intended to be used as an aid in the identification of CRC patients for whom treatment with Vectibix (panitumumab) or with Erbitux® (cetuximab)) may be indicated based on a no mutation detected result [11].
    - The therascreen *KRAS* RGQ PCR Kit is approved for the detection of seven somatic mutations in the human *KRAS* oncogene (in codons 12 and 13 of exon 2). The test is intended to aid in the identification of CRC patients for treatment with Vectibix (panitumumab) and Erbitux (cetuximab) based on a *KRAS* no mutation detected test result [12].

Current Recommendation

COL-A 4 of 5: *KRAS*, *NRAS*, and *BRAF* Mutation Testing

- All patients with metastatic colorectal cancer should have tumor tissue genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations. Patients with any known *KRAS* mutation (exon 2 or non-exon 2) or *NRAS* mutation should not be treated with either cetuximab or panitumumab.[43, 44, 45] *BRAF* V600E mutation makes response to panitumumab or cetuximab highly unlikely. [46-48]

- Testing for *KRAS*, *NRAS*, and *BRAF* mutations should be performed only in laboratories that are certified under the clinical laboratory improvement amendments of 1988 (CLIA-88) as qualified to perform *high complexity* clinical laboratory (molecular pathology) testing. No specific methodology is recommended (eg, sequencing, hybridization).

Proposed Changes

COL-A 4 of 5: *KRAS*, *NRAS* and *BRAF* Mutation Testing

- All patients with metastatic colorectal cancer should have tumor tissue genotyped for *KRAS* exons 2, 3, and 4; *NRAS* exons 2, 3, and 4; and *BRAF* mutations. Patients with any known RAS mutation or unknown RAS mutation status should not be treated with either cetuximab or panitumumab.[43, 44, 45] BRAF V600E mutation makes response to panitumumab or cetuximab highly unlikely. [46-48]
- Testing for *KRAS* (exons 2, 3, and 4); *NRAS* (exons 2, 3, and 4); and BRAF mutations should be performed only in laboratories that are certified under the clinical laboratory improvement amendments of 1988 (CLIA-88) as qualified to perform *high complexity* clinical laboratory (molecular pathology) testing. No specific methodology is recommended for *BRAF* mutation testing (eg, sequencing, hybridization). Determination of RAS mutation status should be performed using an FDA-approved test for this use. [8,9] An NGS-based FDA-approved companion diagnostic for panitumumab is available which detects 56 mutations in *KRAS* exons 2, 3, and 4 and *NRAS* exons 2, 3, and 4. Current PCR-based FDA-approved tests detect 7 mutations in *KRAS* exon 2 only.

Additional Changes: Discussion

In the Discussion section of the guidelines, there is discussion of *KRAS* or *NRAS* testing, including data from clinical studies, and a method for determining *KRAS*/*NRAS* mutation status. We recommend similar changes, noting that, "An FDA-approved NGS companion diagnostic for panitumumab is available that can simultaneously test for mutations in *KRAS* exons 2, 3, and 4, and *NRAS* exons 2, 3, and 4."

The following articles are submitted in support of the above recommendations.

1. Praxis(TM) Extended RAS Panel [package insert]. San Diego, CA: Illumina; 2017.
2. Allegra CJ, Rumble RB, Schilsky RL. Extended RAS Gene Mutation Testing in Metastatic Colorectal Carcinoma to Predict Response to Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Therapy: American Society of Clinical Oncology Provisional Clinical Opinion Update 2015 Summary. Journal of oncology practice. 2016;12(2):180-1. Epub 2015/10/08. doi: 10.1200/JOP.2015.007898. PubMed PMID: 26443838.
3. Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. The New England journal of medicine. 2013;369(11):1023-34. Epub 2013/09/13. doi: 10.1056/NEJMoa1305275. PubMed PMID: 24024839.
4. Sorich MJ, Wiese MD, Rowland A, Kichenadasse G, McKinnon RA, Karapetis CS. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. Annals of oncology : official journal of the European Society for Medical Oncology. 2015;26(1):13-21. Epub 2014/08/15. doi: 10.1093/annonc/mdu378. PubMed PMID: 25115304.
5. Sepulveda AR, Hamilton SR, Allegra CJ, Grody W, Cushman-Vokoun AM, Funkhouser WK, et al. Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. The Journal of molecular diagnostics : JMD. 2017;19(2):187-225. Epub 2017/02/12. doi: 10.1016/j.jmoldx.2016.11.001. PubMed PMID: 28185757.
6. Van Cutsem E, Lenz HJ, Kohne CH, Heinemann V, Tejpar S, Melezinek I, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015;33(7):692-700. Epub 2015/01/22. doi: 10.1200/JCO.2014.59.4812. PubMed PMID: 25605843.
7. Bokemeyer C, Kohne CH, Ciardiello F, Lenz HJ, Heinemann V, Klinkhardt U, et al. FOLFOX4 plus cetuximab treatment and RAS mutations in colorectal cancer. European journal of cancer (Oxford, England : 1990). 2015;51(10):1243-52. Epub 2015/05/06. doi: 10.1016/j.ejca.2015.04.007. PubMed PMID: 25937522.
8. Vectibix(R) (panitumumab) [package insert]. Thousand Oaks, CA: Amgen Inc.; 2015.
9. Erbitux(R) (cetuximab) [package insert]. Indianapolis, IN: Eli Lilly and Company; 2016.
10. Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28(31):4697-705. Epub 2010/10/06. doi: 10.1200/JCO.2009.27.4860. PubMed PMID: 20921465.
11. cobas(R) KRAS Mutation Test [package insert]. Indianapolis, IN: Roche Diagnostics; 2015.
12. therascreen(R) KRAS RGQ PCR Kit [package insert]. Manchester, UK: QIAGEN; 2012.

Thank you for your consideration,

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