**Guideline on the Evaluation of Molecular Markers for Colorectal Cancer Expert Panel**
**Draft Recommendations Summary for Open Comment Period**

***Which colorectal cancer molecular marker tests should be performed?***

1. Recommendation: *RAS* mutational testing of colorectal carcinoma tissue should be performed for patients who are being considered for anti-EGFR therapy. Mutational analysis must include *KRAS* and *NRAS* codons 12, 13 of exon 2; 59, 61 of exon 3; and 117 and 146 of exon 4 ("expanded" or "extended" *RAS*).
2. Recommendation: *BRAF* V600 mutational analysis in conjunction with deficient mismatch repair/microsatellite instability (dMMR/MSI) testing must be performed in colorectal carcinoma tissue of patients with metastatic colorectal carcinoma for prognostic stratification. *[BRAF mutation has limited prognostic value in MSI-H colorectal carcinoma, in contrast to non-MSI-H colorectal carcinoma.]*
3. Recommendation: Deficient mismatch repair/microsatellite instability (dMMR/MSI) testing must be performed in all colorectal cancers for prognostic stratification and identification of Lynch syndrome patients. *[BRAF mutation testing is not needed for Lynch syndrome if there is no MSI-H with loss of MLH1]*
4. No Recommendation: There is insufficient evidence to recommend *BRAF* V600 mutational status as a predictive molecular marker for response to anti-EGFR inhibitors.
5. No Recommendation: There is insufficient evidence to recommend *PIK3CA* mutational analysis of colorectal carcinoma tissue for therapy selection outside of a clinical trial.
    - *Note:* Retrospective studies have suggested improved survival with post-operative aspirin use in patients whose colorectal carcinoma harbors a *PIK3CA* mutation.
6. No Recommendation: There is insufficient evidence to recommend *PTEN* analysis [expression by immunohistochemistry (IHC) or deletion by fluorescence *in situ* hybridization (FISH)] in colorectal carcinoma tissue for patients who are being considered for therapy selection outside of a clinical trial.

***What is the appropriate sample for colorectal cancer molecular marker testing?***

7. Expert Consensus Opinion: Molecular marker testing (*KRAS*, extended *RAS*, *BRAF*, and dMMR/MSI) of the primary colorectal carcinoma tissue is acceptable. If metastatic colorectal carcinoma tissue is available, it is also an acceptable specimen *[preferable in patients with metastatic disease].*
8. Expert Consensus Opinion: Formalin fixed paraffin embedded tissue is an acceptable specimen for molecular marker mutational testing in colorectal carcinoma. Use of other specimens (e.g. cytology specimens) will require additional adequate validation, as would any changes in tissue processing protocols.

***How should colorectal cancer molecular marker testing be performed?***

**A. Validation**

9. Strong Recommendation: Laboratories must use validated colorectal carcinoma molecular marker testing methods with sufficient performance characteristics for the intended clinical use. Colorectal carcinoma molecular marker testing validation should follow accepted standards for clinical molecular diagnostics tests.
10. Strong Recommendation: Performance of molecular marker testing for colorectal carcinoma must be validated in accordance with best laboratory practices.
11. Strong Recommendation: Performance of IHC testing for colorectal carcinoma molecular markers (e.g. dMMR/MSI testing) must be validated in accordance with best laboratory practices.

**B. Pre-analytical**

12. Expert Consensus Opinion: Laboratories must provide clinically appropriate turnaround times and optimal utilization of tissue specimens by using appropriate techniques (e.g. multiplexed assays) for clinically relevant molecular and immunohistochemical markers of colorectal cancer.
13. Recommendation: Colorectal carcinoma molecular and IHC markers for therapy selection, based on the clinical scenario, must be ordered in a timely fashion in accordance with institutionally accepted practices to be established by each laboratory, in collaboration with the patient's clinical team.
14. Expert Consensus Opinion: Colorectal carcinoma tissue should be prioritized for *RAS*, *BRAF*, and dMMR/MSI testing. The laboratory must anticipate the need for molecular testing in routine diagnostics (e. g. retention of unstained sections, implementing tissue conserving techniques such as limiting the number of tissue specimens per block), particularly for minimal tissue specimens.
15. Expert Consensus Opinion: Colorectal carcinoma molecular marker test orders may be initiated by members of the patient's medical team, including pathologists, in accordance with institutionally accepted practices.
16. Expert Consensus Opinion: If send out testing is required by the laboratory, colorectal carcinoma specimens should be processed and sent to reference molecular laboratories in a timely manner (90% of specimens should be sent out within 3 working days).

**C. Analytical**

17. Expert Consensus Opinion: Pathologists must determine the adequacy of specimens, including cytology specimens, for colorectal carcinoma molecular marker testing by assessing the quality of the tissue and estimating the percentage of viable carcinoma cells to determine if microdissection or other enrichment is necessary.
18. Expert Consensus Opinion: Laboratories should use colorectal carcinoma molecular marker testing methods that are able to detect mutations in specimens with at least 5% mutant allele frequency, taking into account the analytical sensitivity of the assay (level of detection/LOD) and tumor enrichment (e.g. microdissection). It is recommended that the operational minimal neoplastic carcinoma cell content tested should be set at least 2 times the assay's LOD.

**D. Post-analytical**

19. Expert Consensus Opinion: Prognostic and predictive colorectal carcinoma molecular marker results should be available as promptly as feasible, within 10 working days from the initial receipt of the specimen in the pathology laboratory. 90% of test results should be reported within 10 working days.
20. Expert Consensus Opinion: Colorectal carcinoma molecular marker testing reports should include a results and interpretation section readily understandable by oncologists and pathologists. Appropriate Human Genome Variation Society (HVGS) and Human Genome Organisation (HUGO) nomenclature must be used in conjunction with any historical genetic designations.
21. Strong Recommendation: Laboratories must incorporate colorectal carcinoma molecular marker testing methods into their overall laboratory quality improvement program, establishing appropriate quality improvement monitors as needed to assure consistent performance in all steps of the testing and reporting process. In particular, laboratories performing colorectal carcinoma molecular marker testing must participate in formal proficiency testing programs, if available, or an alternative proficiency assurance activity.