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NCCN Guidelines Panel: Rectal Cancer

Dear Panel Members,

On behalf of Natera, I respectfully request the NCCN® Rectal Cancer Guideline Panel consider the requested updates and enclosed references, pertaining to postsurgical surveillance for stage II, III rectal cancer as an additional bullet in REC-11.

Specific Changes: We respectfully request that tumor-informed ctDNA assay be added as a component of postsurgical surveillance for stage II, III rectal cancer as an additional bullet in REC-11. Proposed bullet: "Longitudinal tumor-informed ctDNA assay every 3 months for 2 years, then every 6 months for a total of 5 years." Additionally, in REC-11, we recommend changing "Serial CEA elevation or documented recurrence" to "Serial CEA elevation, detection of tumor-informed ctDNA, or documented recurrence." In REC-12, recommend changing "Serial CEA elevation" to "Serial CEA elevation or detection of tumor-informed ctDNA by serial analysis"

FDA Clearance: A personalized and a tumor-informed 16-plex PCR, next generation sequencing assay measuring circulating tumor DNA assay is a laboratory developed test (LDT) performed in the central laboratory of Natera, Inc., which is ISO 13485-certified and is regulated under the Clinical Laboratory Improvement Amendments (CLIA) and the College of American Pathologists (CAP).

On May 3, 2019, FDA granted a breakthrough device designation for this personalized and tumor-informed 16-plex PCR, next generation sequencing assay in patients with localized and advanced colorectal cancer.

On August 22, 2019 Medicare published a proposed Local Coverage determination for this personalized and tumor-informed 16-plex PCR, next generation sequencing assay for patients with stage II/III CRC.

Rationale: Circulating tumor DNA (ctDNA) provides a direct measurement of residual molecular or micro-metastatic disease that originated directly from the tumor. Below we provide supporting evidence from the literature that illustrates the presence of post-surgical ctDNA (MRD timepoint) to be associated with high-risk of recurrence.

A recent study with retrospective ctDNA analysis using a personalized and tumor-informed 16-plex PCR, next generation sequencing assay showed patients showed patients (n=130) with Stage I-III CRC who showed a ctDNA positive status during surveillance after definitive therapy to be 43 times more likely to relapse than ctDNA negative patients (hazard ratio [HR], 43.5; 95% CI, 9.8-193.5; $P < 0.001$). In addition, the study also showed disease recurrence up to 16.5 months ahead of radiological imaging (average 8.7 months) with serial ctDNA analysis (sensitivity - 88% and specificity 98%) **(1)**. A follow-up study by the same group enrolled 198 patients with Stage I-III CRC and showed an HR of 47.5 (95% CI: 17.3 – 130.3; $p < 0.001$) in post-definitive treatment setting **(2).** ctDNA-positivity during longitudinal follow-up was associated with significantly worse disease-free survival. Overall, these two studies determined that, in a multivariable analysis, the tumor-informed 16-plex PCR, sequencing assay's MRD status was the only factor significantly associated with relapse-free survival, after adjusting for all other standard clinicopathological factors.

Table below summarizes key studies that show the presence of postsurgical ctDNA as determined by different methodologies to be associated with reduced recurrence-free survival. In most of the studies listed below, ctDNA status was independently associated with relapse after adjusting for known clinicopathologic risk factors.

Table: Detection of colorectal/rectal cancer recurrence with ctDNA, during surveillance (serial testing, post-definitive therapy

Reference	Cancer type and stage	No. of patients enrolled	ctDNA detection technique	Recurrencefree Survival: Hazard Ratio, p-value	Percentage of ctDNA-positive patients' post-definitive treatment, who eventually relapsed	Sensitivity (%), Specificity (%)	Lead time (months) over radiological recurrence
Reinert T, et al 2019, JAMA Onc.	CRC, I-III	130	Tumor-informed 16-plex PCR, sequencing assay	43.5, p<0.001	93.3% (14/15)	88, 98	Mean:8.7 months (0.8-16.5)
Tarazona N, et al. 2020, ASCO	CRC, I-III	198	Tumor-informed 16-plex PCR, sequencing assay	47.5, p<0.001	95.2% (20/21) Natera's internal data	79.1, 99	Median:8.15 months (0.56-16.6)
Tie J, et al. 2019, Gut	Rectal, locally advanced	159	Safe-SeqS	22, P<0.001	74% (17/23)	-	-
Symonds EL, et al. 2020, Cancer	CRC, I-IV	548	Methylated *BCAT1*/*IKZF1* ctDNA test	89.3, p<0.001	61% (25/41)	66, 97.9	Up to 11.6 months
Scholer et al. 2017, CCR	CRC, I-III	118	ddPCR	9.5, P<0.001	100% (14/14)	100,100 postoperative	Median: 9.4 (0.4-14.9) months
Reinert T, et al. 2016, Gut	CRC, I-IV	118	ddPCR	-	100% (6/6)	100, 100	Mean: 10 months (2-15)

The following articles are submitted in support of this proposed change. 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. Reinert T, Henriksen TV, Christensen E, Sharma S, Salari R, Sethi H, Knudsen M, Nordentoft I, Wu HT, Tin AS, Heilskov Rasmussen M, Vang S, Shchegrova S, Frydendahl Boll Johansen A, Srinivasan R, Assaf Z, Balcioglu M, Olson A, Dashner S, Hafez D, Navarro S, Goel S, Rabinowitz M, Billings P, Sigurjonsson S, Dyrskjøt L, Swenerton R, Aleshin A, Laurberg S, Husted Madsen A, Kannerup AS, Stribolt K, Palmelund Krag S, Iversen LH, Gotschalck Sunesen K, Lin CJ, Zimmermann BG, Lindbjerg Andersen C. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stage I to III colorectal cancer. *JAMA Oncology*. 2019;5(8):1124-1131. **[Clinical validation of personalized and tumor-informed 16-plex PCR, next generation sequencing assay]**

2. Tarazona N, Henriksen TV, Carbonell-Asins JA, et al. Circulating tumor DNA to detect minimal residual disease, response to adjuvant therapy and identify patients at high risk of recurrence in stage I-III CRC. Oral presentation presented at: *American Society of Clinical Oncology;* May 29-31, 2020; Virtual Meeting. **[Clinical validation of personalized and tumor-informed 16-plex PCR, next generation sequencing assay]**

3. Tie J, Cohen J, Wang Y, et al. Serial circulating tumour DNA analysis during multimodality treatment of locally advanced rectal cancer: a prospective biomarker study. Gut 2019;68:663–671

4. Symonds EL, Perdersen SK, Murray D, Byrne SE, Roy A, Karapetis C, Hollington P, Rabbitt P, Jones FS, LaPointe L, Segelov E, Young GP. Circulating epigenetic biomarkers for detection of recurrent colorectal cancer. Cancer 2020;126:1460-1469.

5. Schøler LV, Reinert T, Ørntoft MW, Kassentoft CG, Árnadóttir SS, Vang S, Nordentoft I, Knudsen M, Lamy P, Andreasen D, Mortensen FV, Knudsen AR, Stribolt K, Sivesgaard K, Mouritzen P, Nielsen HJ, Laurberg S, Ørntoft TF, Andersen CL. Clinical Implications of Monitoring Circulating Tumor DNA in Patients with Colorectal Cancer. Clin Cancer Res. 2017;23(18):5437-5445.

6. Reinert T, Schøler LV, Thomsen R, Tobiasen H, Vang S, Nordentoft I, Lamy P, Kannerup AS, Mortensen FV, Stribolt K, Hamilton-Dutoit S, Nielsen HJ, Laurberg S, Pallisgaard N, Pedersen JS, Ørntoft TF, Andersen CL. Analysis of circulating tumour DNA to monitor disease burden following colorectal cancer surgery. Gut. 2016 Apr;65(4):625-34.

Sincerely,