Submitted by: Alex Aleshin, MD, MBA

Company/Organization: Natera, Inc.

Address: 201 Industrial Road, San Carlos, CA, 94070

Phone: 650-249- 9090

Email: aaleshin@natera.com

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NCCN Guidelines Panel: Colon Cancer

Dear Panel Members,

On behalf of Natera, I respectfully request the NCCN® Colon Cancer Guideline Panel consider the requested updates and enclosed references, pertaining to high-risk factor for recurrence in T3N0, T4N0, and T1-3N1 in COL-3.

Specific Changes: We respectfully request that the presence of post-surgical tumor-informed ctDNA be added as a high-risk factor for recurrence in T3N0, T4N0, and T1-3N1 in COL-3. Proposed modification of footnote in COL-3: "High-risk factors for recurrence (exclusive of those cancers that are MSI-H: poorly differentiated/undifferentiated histology, lymphatic/vascular invasion, bowel obstruction, <12 lymph nodes examined, perineural invasion, localized perforation, or close, indeterminate, or positive margins. *Presence of postsurgical tumor-informed ctDNA is associated with a high risk of recurrence.* In high-risk stage II patients, there are no data that correlate risk features and selection of chemotherapy."

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 literature that illustrates the presence of post-surgical ctDNA (MRD detection) 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 (n=130) with Stage I-III CRC who showed a ctDNA positive status at postoperative day 30 were 7 times more likely to relapse than ctDNA-negative patients (hazard ratio [HR], 7.2; 95% CI, 2.7-19.0; $P < 0.001$). 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 14 (95% CI: 6.1 – 30; $p < 0.001$) wherein ctDNA was detected in 9.2% (14/152) of patients, post-surgery. Of these patients, 78.5% (11/14) eventually relapsed **(2).** 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.

A clinical experience study with the tumor-informed 16-plex PCR, sequencing assay showed assessment of MRD rates across patients with early and advanced stage CRC, wherein post-surgical MRD rate was observed to be significantly higher in patients with Stage II, T4N0 (28.6%;4/14) compared to Stage II T3N0 (5.6%; 3/53) and a similar trend was observed with post-surgical Stage III, high-risk T4, N1-2, T-Any, N2 MRD rate (39.4%;15/38), compared with Stage III, low-risk T1-3N1 (9.3%; 3/32). This further emphasizes the clinical utility of ctDNA (personalized, tumor-informed) analysis for accurately detecting ctDNA at post-surgical MRD timepoint **(3)**.

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/colon cancer recurrence with ctDNA, post-surgery only

Reference	**Cancer type and stage**	**No. of patients enrolled**	**ctDNA detection technique**	**Recurrence Free Survival: Hazard Ratio, p-value**	**Percentage of ctDNA-positive patients at MRD who eventually relapsed**
Reinert T, et al 2019, JAMA Onc.	CRC, I-III	130	Tumor-informed 16-plex PCR, sequencing assay	7.2, p<0.001	70% (7/10) Sensitivity: 88% Specificity: 98%
Tarazona N, et al. 2020, ASCO	CRC, I-III	198	Tumor-informed 16-plex PCR, sequencing assay	14, p<0.001	78.5% (11/14)
Tarazona N, et al. 2019, Annals of Onc.	Colon localized	150	NGS panel	6.96, P=0.0001	57.1% (8/14)
Tie J, et al. 2019, JAMA Onc.	Colon, III	100	Safe-SeqS	3.8, P<0.001	42% (10/24)

Murray DH, et al. 2018, JCRCO	CRC II-IV	479	qPCR assay-identifying methylated *BCAT1* and *IKZF1* DNA	3.8, P=0.004	(30%) 7/23
Diehn M, et al. 2017, JCO	CRC, II-III	145	NGS-based AVENIO ctDNA surveillance kit	10.3, P<0.00001	92% (11/12)
Scholer et al. 2017, CCR	CRC, I-III	118	ddPCR	37.7, P<0.001	100% (14/14)
Overman MJ, et al. 2017, JCO	CRC	54	NGS panel (Guardant)	3.1, P=0.002	58% (31/54)
Reinert T, et al. 2016, Gut	CRC, I-IV	118	ddPCR	-	100% (6/6)
Tie J, et al. 2016, Sci Transl Med	Colon, II	250	Safe-SeqS	18, p<0.001	78.6% (11/14)

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. Kasi PM, Dayyani F, Morris V, et al. Tumor-Informed Assessment of Molecular Residual Disease and its Incorporation into Practice for Patients with Early and Advanced Stage Colorectal Cancer (CRC-MRD Consortia). Poster 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]**

4. Tarazona N, Gimeno-Valiente F, Gambardella V, Zuniga S, P Rentero-Garrido, M Huerta, S Roselló, C Martinez-Ciarpaglini, J A Carbonell-Asins, F Carrasco, A Ferrer-Martínez, G Bruixola, T Fleitas, J Martín, R Tébar-Martínez, D Moro, J Castillo, A Espí, D Roda, A Cervantes. Targeted next generation sequencing of circulating tumor DNA for tracking minimal residual disease in localized colon cancer. *Annals of Oncology*. 2019; 30: 1804–1812.

5. Tie J, Cohen JD, Wang Y, Christie M, Simons K, Lee M, Wong R, Kosmider S, Ananda S, McKendrick J, Lee B, Cho JH, Faragher I, Jones IT, Ptak J, Schaeffer MJ, Silliman N, Dobbyn L, Li L, Tomasetti C, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating tumor DNA analyses as markers of recurrence risk and benefit of adjuvant therapy for stage III colon cancer. *JAMA Oncology*. 2019;5(12):1710-1717.

6. Murray DH, Symonds EL, Young GP, Byrne S, Rabbitt P, Roy A, Cornthwaite K, Karapetis CS, Pedersen SK. Relationship between post-surgery detection of methylated circulating tumor DNA with risk of residual disease and recurrence-free survival. *J Cancer Res Clin Oncol*. 2018;144(9):1741-1750.

7. Diehn M, Alizadeh AA, Adam HP, et al. Early prediction of clinical outcomes in resected stage II and III colorectal cancer (CRC) through deep sequencing of circulating tumor DNA (ctDNA). *Journal of Clinical Oncology*. 2017;35(15_suppl):3591-3591

8. 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.

9. **Overman MJ**, Vauthey JH, Aloia TA, et al. Circulating tumor DNA (ctDNA) utilizing a high-sensitivity panel to detect minimal residual disease post liver hepatectomy and predict disease recurrence. Journal of Clinical Oncology. 2017;35(15_suppl):3522-3522.

10. 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.

11. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, Silliman N, Tacey M, Wong HL, Christie M, Kosmider S, Skinner I, Wong R, Steel M, Tran B, Desai J, Jones I, Haydon A, Hayes T, Price TJ, Strausberg RL, Diaz LA Jr, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med. 2016;8(346):346ra92.

Sincerely,