To: submissions@nccn.org
Re: Submission Request – Colon/Rectal/Anal Cancers Panel

Submitted by:
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Date of request: July 29, 2019
NCCN Guidelines Panel: Colon/Rectal/Anal Cancers Panel

On behalf of Promega Corporation, we respectfully request the NCCN Colon/Rectal/Anal Cancers Panel to review the enclosed information in support of making changes to the current guidelines for Colon and Rectal cancer diagnosis using PCR-based Microsatellite Instability (MSI) assays.

Specific Changes:
We ask the panel to equally emphasize any assay for the detection of microsatellite instability (MSI) that has been validated in colon/rectal/anal cancers be given equal weight and be recommended as a parallel technology with mismatch repair deficiency (MMR) protein expression analysis by immunohistochemistry (IHC) for Colon and Rectal cancer patients.

FDA Clearance:
The recommendation to assess MSI status is not associated with any specific FDA-cleared product/s. Laboratory developed tests (LDT) and Site Specific IVDs to assess MSI status are currently widely available for clinical use to inform patient treatment options.

Rationale:
Assays for MSI and MMR protein expression measure separate but related cellular events (Richman 2015)[1]. Mutations or epigenetic silencing events at MMR genes result in inactivation or loss of MMR proteins. The resulting loss of mismatch repair function then allows detectable errors to accumulate at microsatellite regions in DNA. Immunohistochemistry testing for MMR protein expression can miss up to 12% of dMMR cases, which is thought to be due to retained expression and immunoreactivity in non-functional proteins or defects in MMR genes other than the four major genes available for IHC testing (Funkhouser, 2012; Dudley, 2016; Shia, 2008).[2,3,4]. Moreover in practice there is substantial interobserver variation due to nuanced techniques and interpretation involved (Funkhouser, 2012; Klarskov, 2010; Engel, 2011)[2,5,30]. Current NCCN guidelines for Genetic/Familial Assessment state that IHC testing for MMR has a 5-10% false negative rate (page LS-A, 1 of 5 and 3 of 5)[6]. Contemporary MSI by PCR panels with 4 or more mononucleotide repeat markers have a false negative rate of just 0.3-5% (Shia, 2005; Murphy 2006; Pagin 2013; Cicek, 2011; Goel, 2010; Southey, 2005)[7,8,9,10,11,12]. Several studies

have noted discordant results between IHC and MSI by PCR (Cicek, 2011; Bartley 2012)[10,13], including clinical trial studies evaluating immunotherapy in metastatic colorectal patients where discrepancies between local assessment and central laboratory results were observed (Cohen, 2018: Overman, 2017)[14,15]. A recent report on immunotherapy in metastatic colorectal cancer highlighted that almost 10% of patients who had been enrolled in immunotherapy trials had experienced failure based on false positive dMMR or MSI PCR results assessed by local laboratories (Cohen, 2018)[14]. This led a consensus panel of the European Society of Medical Oncology to recommend use of both IHC and MSI PCR to assess eligibility for treatment with immune checkpoint inhibitors of metastatic colorectal cancer and other cancers of the Lynch Syndrome spectrum in a recent recommendation on MSI testing (Luchini, 2019 pg 5 Table 2)[17].

Approval of PD-1 checkpoint inhibitors nivolumab and pembrolizumab for treatment of MSI-H/dMMR metastatic colorectal cancer indicates the impact of false negative or false positive results produced by one test method can adversely affect treatment decisions. Due to the importance of DNA mismatch repair status in hereditary cancer risk screening, adjuvant therapy decisions, and immunotherapy eligibility there is growing recognition that these tests should be performed together for maximal sensitivity (Funkhouser, 2012; Sepulveda, 2017; Cohen, 2018; Overman, 2017 Hedge, 2014; Luchini, 2019; Cohen, 2016)[2,18,14,15,16,17,29].

There is no peer reviewed data generated in a statistically significant cohort of primary tumor samples to suggest that NGS is impactfully superior to PCR for the detection of MSI in colorectal cancer samples. While it can be argued that surveying more loci could in theory provide more sensitivity, in practice most microsatellite loci are minimally informative (Salipante, 2014)[19]. MSI analysis by NGS requires more time to generate results due to the more complex bioinformatics analysis required and exhibits similar sensitivity compared to standard capillary electrophoresis procedures (Baudrin, 2018; Zhang, 2018)[20,21]. Additionally, cut offs between MSI-H and MSS are difficult to determine and vary from assay to assay posing problems of definitive calling of MSI status (Baudrin, 2018; Rodrigues, 2018; Latham, 2019)[20,22,23].

MSI analysis by PCR using mononucleotide loci can be performed with less than a section of tissue and is extremely cost effective, making it amenable to being performed alongside IHC as an initial screening tool (Muller, 2004; Gould, 2014)[24,28]. MSI by PCR is an established, reimbursable test which should be considered for routine MSI analysis where large NGS panels are unnecessary, have long turnaround time, and are cost prohibitive outside of a research-hospital setting.

Promega's MSI Analysis System has been used as the reagent basis for LDTs in clinical laboratories and research organizations worldwide for over 15 years. This assay has been used as a gold standard to determine MSI status in numerous clinical trials as well as drug and companion diagnostic submissions for FDA approval (Le et al., 2017; Le et al., 2015)[25,26].

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We believe the evidence provided below supports our request for changes in the following areas of the Colon and Rectal Cancer Guidelines and Evidence Blocks (proposed changes are highlighted in **bold**):

NCCN Guidelines version 2.2019- May 15, 2019 (Colon Cancer)

Section	Page#	Current update	Promega proposal	Evidence/Publication
COL-B 4 of 6	23	Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing: Bullet 1• Universal MMR* or MSI* testing is recommended in all patients with a personal history of colon or rectal cancer.	Bullet 1• Universal MMR* **and** MSI* testing is recommended in all patients with a personal history of colon or rectal cancer.	Funkhouser 2012; Bartley, 2012; Gould, 2014
COL-B 4 of 6	23	Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing: Bullet 5 - Testing for MSI may be accomplished with a validated NGS panel, especially in patients with metastatic disease who require genotyping of RAS and BRAF.	Bullet 5 - Testing for MSI may be accomplished **by PCR or** a validated NGS panel, especially in patients with metastatic disease who require genotyping of RAS and BRAF.	Salipante, 2014; Baudrin, 2018; Zhang, 2018; Rodrigues, 2018; Latham, 2019
COL-B 4 of 6	23	Footnote: *IHC for MMR and DNA analysis for MSI are different assays measuring the same biological effect. See references on COL-B 5 of 6	IHC for MMR and DNA analysis for MSI **measure different biological effects caused by deficient mismatch repair function**	Richman, 2015; Bartley, 2012
MS-5	54	The NCCN Colon/Rectal Cancer Panel endorses universal MMR or MSI testing of all patients with a personal history of colon or rectal cancer to	The NCCN Colon/Rectal Cancer Panel endorses **universal MMR and MSI**	Funkhouser, 2012; Sepulveda, 2017; Gould, 2014

		identify individuals with Lynch syndrome.	testing of all patients with a personal history of colon or rectal cancer to identify individuals with Lynch syndrome.	
MS-16	65	The panel recommends universal MMR or MSI testing for all patients with a personal history of colon or rectal cancer to identify individuals with Lynch syndrome	The panel recommends universal **MSI by PCR and MMR by IHC** testing for all patients with a personal history of colon or rectal cancer to identify indivciduals with Lynch syndrome	Funkhouser, 2012; Sepulveda, 2017; Gould 2014

NCCN Evidence Blocks version 2.2019-June 17, 2019 – Colon Cancer:

Section	Page#	current update	Promega proposal	Evidence/Publication
COL-B 4 of 6	25	Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing: Bullet 1• Universal MMR* or MSI* testing is recommended in all patients with a personal history of colon or rectal cancer.	Universal MMR* **and** MSI* testing is recommended in all patients with a personal history of colon or rectal cancer.	Funkhouser 2012, Bartley 2012; Gould, 2014
COL-B 4 of 6	25	Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing: Bullet 5 - Testing for MSI may be accomplished with a validated NGS panel, especially in patients with metastatic disease who require genotyping of RAS and BRAF.	Bullet 5 - Testing for MSI may be accomplished **by PCR or** a validated NGS panel, especially in patients with metastatic disease who require genotyping of RAS and BRAF.	Salipante, 2014; Baudrin, 2018; Zhang, 2008; Rodrigues, 2018; Latham, 2019; Zhang, 2018;

COL-B 4 of 6	25	Footnote: *IHC for MMR and DNA analysis for MSI are different assays measuring the same biological effect. See references on COL-B 5 of 6	IHC for MMR and DNA analysis for MSI **measure different biological effects caused by deficient mismatch repair function**	Richman, 2015; Bartley, 2012
MS-5	59	The NCCN Colon/Rectal Cancer Panel endorses universal MMR or MSI testing of all patients with a personal history of colon or rectal cancer to identify individuals with Lynch syndrome.	The NCCN Colon/Rectal Cancer Panel endorses universal MMR **and** MSI testing of all patients with a personal history of colon or rectal cancer to identify individuals with Lynch syndrome.	Funkhouser, 2012; Sepulveda, 2017; Gould, 2014
MS-16	70	The panel recommends universal MMR or MSI testing for all patients with a personal history of colon or rectal cancer to identify individuals with Lynch syndrome...	The panel recommends universal **MSI by PCR and MMR by IHC** testing for all patients with a personal history of colon or rectal cancer...	Funkhouser, 2012; Sepulveda, 2017; Gould 2014

NCCN Guidelines version 2.2019-May 15, 2019 – Rectal Cancer:

Section	Page#	current update	Promega proposal	Evidence/Publication
REC-B 5 of 7	30	Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing: Bullet 1• Universal MMR* or MSI* testing is recommended in all patients with a personal history of colon or rectal cancer.	Universal MMR* **and** MSI* testing is recommended in all patients with a personal history of colon or rectal cancer.	Funkhouser, 2012; Bartley, 2012; Gould, 2014
REC-B 5 of 7	30	Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing: Bullet 5 - Testing for MSI may be accomplished with a validated NGS panel, especially in patients with metastatic disease who require genotyping of RAS and BRAF.	Bullet 5 - Testing for MSI may be accomplished **by PCR** or a validated NGS panel, especially in patients with metastatic disease who require genotyping of RAS and BRAF.	Salipante, 2014; Baudrin, 2018; Zhang, 2008; Rodrigues, 2018; Latham, 2019; Zhang, 2018
REC-B 5 of 7	30	Footnote: *IHC for MMR and DNA analysis for MSI are different assays measuring the same biological effect.	IHC for MMR and DNA analysis for MSI **measure different biological effects caused by deficient mismatch repair function**	Richman, 2015; Bartley, 2012
MS-5	60	The NCCN Colon/Rectal Cancer Panel endorses universal MMR or MSI testing of all patients with a personal history of colon or rectal cancer to identify individuals with Lynch syndrome.	The NCCN Colon/Rectal Cancer Panel endorses universal MMR **and MSI by PCR** testing of all patients with a personal history of colon or rectal cancer to	Funkhouser, 2012; Sepulveda, 2017; Gould, 2014

			identify individuals with Lynch syndrome.	

NCCN Evidence Blocks version 2.2019-June 17, 2019 – Rectal Cancer:

Section	Page#	current update	Promega proposal	Evidence/Publication
REC-B 5 of 7	33	Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing: Bullet 1• Universal MMR* or MSI* testing is recommended in all patients with a personal history of colon or rectal cancer.	Universal MMR* **and** MSI* testing is recommended in all patients with a personal history of colon or rectal cancer.	Funkhouser 2012; Bartley, 2012; Gould, 2014
REC-B 5 of 7	33	Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing: Bullet 5 - Testing for MSI may be accomplished with a validated NGS panel, especially in patients with metastatic disease who require genotyping of RAS and BRAF.	Bullet 5 - Testing for MSI may be accomplished **by PCR or** a validated NGS panel, especially in patients with metastatic disease who require genotyping of RAS and BRAF.	Salipante, 2014; Baudrin, 2018; Zhang, 2008; Rodrigues, 2018; Latham, 2019
REC-B 5 of 7	33	Footnote: *IHC for MMR and DNA analysis for MSI are different assays measuring the same biological effect.	IHC for MMR and DNA analysis for MSI **measure different biological effects caused by deficient mismatch repair function**	Bartley, 2012
MS-5	66	The NCCN Colon/Rectal Cancer	The NCCN Colon/Rectal Cancer Panel endorses	Funkhouser, 2012; Sepulveda, 2017; Gould, 2014

		Panel endorses universal MMR or MSI testing of all patients with a personal history of colon or rectal cancer to identify individuals with Lynch syndrome.	universal MMR **and** MSI testing of all patients with a personal history of colon or rectal cancer to identify individuals with Lynch syndrome.	

The following scholarly research publications are submitted in support of the proposed changes above.

1. Richman S. Deficient mismatch repair: Read all about it (review). International Journal of Oncology, 2015; 47: 1189-1202. DOI: 10.3892/ijo.2015.3119
2. Funkhouser WK, Lubin IM, Monzon FA, Zehnbauer BA, Evans JP, et al. Relevance, Pathogenesis, and Testing Algorithm for Mismatch Repair-Defective Colorectal Carcinomas. Journal of Molecular Diagnostics 2012; 14(2): 91-103. DOI: 10.1016/j.jmoldx.2011.11.001.
3. Dudley JC, Lin M-T, Le D-T and Eshleman JR. Microsatellite Instability as a Biomarker for PD-1 Blockade. Clin. Cancer Res, 2016, 22:813-829.
4. Shia, J. Immunohistochemistry versus Microsatellite Instability Testing for Screening Colorectal Cancer Patients at Risk for Hereditary Nonpolyposis Colorectal Cancer Syndrome. Journal of Molecular Diagnostics, 2008; 10(4): 293-300. DOI: 10.2353/jmoldx.2008.080031
5. Klarskov L, Ladelund S, Holck S, Roenlund K, Lindebjerg J et al. Interobserver variability in the evaluation of mismatch repair protein immunostaining. Human Pathology, 2010; 41(10): 1387-1396. DOI: 10.1016/j.humpath.2010.03.003
6. National Comprehensive Cancer Network. "Genetic/Familial High-Risk Assessment: Colorectal". Version 1.2019 – July 3, 2019. https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf
7. Shia J, Klimstra, DS, Nafa K, Offit K et al. Value of Immunohistochemical Detection of DNA Mismatch Repair Proteins in Predicting Germline Mutation in Hereditary Colorectal Neoplasms. American Journal of Surgical Pathology, 2005; 29(1): 96-104
8. Murphy KM, Zhang S, Geiger T, Hafez MJ, Bacher J et al. Comparison of the Microsatellite Instability Analysis System and the Bethesda Panel for the Determination of Microsatellite Instability in Colorectal Cancers. Journal of Molecular Diagnostics, 2006; 8(3): 305-311. DOI: 10.2353/jmoldx.2006.050092

9. Pagin A, Zerimech F, Leclerc J, Wacrenier A et al. Evaluation of a new panel of six monocleotide repeat markers for the detection of DNA mismatch repair-deficient tumours. British Journal of Cancer, 2013: 108: 2079-2087. doi: 10.1038/bjc.2013.213

10. Cicek MS, Lindor NM, Gallinger S, Bapat B et al. Quality Assessment and Correlation of Microsatellite Instability and Immunohistochemical Markers among Population- and Clinic-Based Colorectal Tumors. Journal of Molecular Diagnostics, 2011; 13(3): 271-281. DOI: 10.1016/j.jmoldx.2010.12.004

11. Goel A, Nagasaka T, Hamelin R and Boland CR. An Optimized Pentaplex PCR for Detecting DNA Mismatch Repair-Deficient Colorectal Cancers. PLOS One, 2010; 5(2): e9393. doi:10.1371/journal.pone.0009393.s001

12. Southey MC, Jenkins MA, Mead L, Whitty J et al. Use of Molecular Tumor Characteristics to Prioritize Mismatch Repair Gene Testing in Early-Onset Colorectal Cancer. Journal of Clinical Oncology, 2005; 23(27): 6524-6532. DOI: 10.1200/JCO.2005.04.671

13. Bartley AN, Luthra R, Saraiya DS, Urbauer DL, and Broaddus RR. Identification of Cancer Patients with Lynch Syndrome: Clinically Significant Discordances and Problems in Tissue-Based Mismatch Repair Testing. Cancer Prevention Research, 2012; 5(2): 320-327. Epub 2011 Nov 14. DOI: 10.1158/1940-6207. PubMed PMID: 22086678.

14. Cohen R, Hain E, Buhard, O, Guilloux A et al. Association of Primary Resistance to Immune Checkpoint Inhibitors in Metastatic Colorectal Cancer With Misdiagnosis of Microsatellite Instability or Mismatch Repair Deficiency Status. JAMA Oncology, 2018; E1-E5. doi:10.1001/jamaoncol.2018.4942

15. Overman MJ, McDermott R, Leach JL, Lonardi S et al. Nivolumab in patients with metastatic DNA mismatch repair deficient/microsatellite instability-high colorectal cancer (CheckMate 142): results of an open-label, multicenter, phase 2 study. Lancet Oncology, 2017; 18(9): 1182-1191. doi:10.1016/S1470-2045(17)30422-9.

16. Hegde M, Ferber M, Mao R, Samowitz W et al. ACMG technical standards and guidelines for genetic testing for inherited colorectal cancer (Lynch Syndrome, familial adenomatous polyposis, and MYH-associated polyposis). American College of Medical Genetics and Genomics Standards and Guidelines. Genetics in Medicine, 2013; 16(1): 101-116. doi:10.1038/gim.2013.166

17. Luchini C, Bibeau F, Ligtenberg MJL, Singh N et al. ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach. Annals of Oncology, 2019; 0:1-12. DOI:10.1093/annoc/mdz116

18. Sepulveda AR, Hamilton SR, Allegra CJ, Grody W et al. Molecualr Biomarkers for the Evaluation of Colorectal Cancer. Guidelines from ASCP, CAP, AMP and ASCO. Archives of Pathology and Laboratory Medicine, 2017; 141: 625-657. doi: 10.5858/arpa.2016-0554-CP

19. Salipante, SJ, Scroggins SM, Hampel, HL et al. Microsatellite Instability Detection by Next Generation Sequencing. Clinical Chemistry, 2014; 60 (9): 1192-1199. DOI: 10.1373/clinchem.2014.223677
20. Baudrin, LG, Deleuze, JF and How-Kit, A. Molecular and Computational Methods for the Detection of Microsatellite Instability in Cancer. Frontiers in Oncology, 2018. DOI: doi.org/10.3389/fonc.2018.00621
21. Zhang L, Peng Y and Peng G. Mismatch repair-based stratification for immune checkpoint blockade therapy. American Journal of Cancer Research, 2018; 8(10):1977-1988.
22. Rodrigues DN, Rescigno P, Liu D, Yuan W, Carreira S et al. Immunogenomic analyses associate immunological alterations with mismatch repair defects in prostate cancer. Journal of Clinical Investigation, 2018; 128 (10): 4441-4453. DOI: 10.1172/JCI121924.
23. Latham *et al.* Microsatellite Instability is Associated with the Presence of Lynch Syndrome Pan-Cancer. J Clin Oncology (2019) 37:286-95
24. Muller A, Giuffre G, Edmonston TB, Mathiak M, Roggendorf B et al. Challenges and Pitfalls in HNPCC Screening by Microsatellite Analysis and Immunohistochemistry. Journal of Molecular Diagnostics, 2004; 6(4): 308-315. DOI: 10.1016/S1525-1578(10)60526-0. PubMed PMID: 15507669.
25. Le DT, Jennifer N. Durham JN, Kellie N. Smith KN, Hao Wang H, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science, 2017; 357 (6349): 409-413. Epub 2017 Jun 8. DOI: 10.1126/science.aan6733. PubMed PMID: 28596308.
26. Le DT, Uram JM, Wang H, Bartlett BR et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. New England Journal of Medicine, 2015; 372: 2509-20. DOI: 10.1056/NEJMoa1500596
27. Zhang, L. Immunohistochemistry versus Microsatellite Instability Testing for Screening Colorectal Cancer Patients at Risk for Hereditary Nonpolyposis Colorectal Cancer Syndrome. Journal of Molecular Diagnostics, 2008; 10(4): 301-307. doi: 10.2353/jmoldx.2008.080062. PubMed PMID: 18556776.
28. Gould M, El-Serag HB, Musher B, Franco LM et al. Cost-effectiveness and Diagnostic effectiveness of Analyses of Multiple Algorithms for the Diagnosis of Lynch Syndrome. Digestive Diseases and Sciences, 2014; 59(12): 2913-26. doi:10.1007/s10620-014-3248-6
29. Cohen SA, Laurino M, Bowen DJ, Upton MP et al. Initiation of universal tumor screening for Lynch syndrome in colorectal cancer patients as a model for the implementation of genetic information into clinical oncology practice. Cancer, 2016; 122(3): 393-401. doi:10.1002/cncr.29758
30. Engel KB and Moore HM. Effects of Preanalytical Variables on the Detection of Proteins by Immunohistochemistry in Formalin-Fixed, Paraffin-Embedded Tissue. Archives of Pathology and Laboratory Medicine, 2011; 135: 537-543.

Promega

Thank you for your consideration.

Sincerely,

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