To: submissions@nccn.org
Re: Submission Request – Esophageal/Gastric Cancers Panel

Submitted by:
Name: Dr. Randall Dimond, Ph.D., Vice President & Chief Scientific Officer
Company: Promega Corporation
Address: 2800 Woods Hollow Rd., Madison, WI 53711
Phone: 608-277-2517
Email: randy.dimond@promega.com
Date of request: September 23, 2019
NCCN Guidelines Panel: Esophageal/Gastric Cancers Panel

On behalf of Promega Corporation, we respectfully request the NCCN Esophageal/Gastric Cancers Panel to review the enclosed information in support of making changes to the current guidelines for Gastric and Esophageal 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 gastric and esophageal cancers be given equal weight and be recommended as a parallel technology with mismatch repair deficiency (MMR) protein expression analysis by immunohistochemistry (IHC) for Gastric and Esophageal 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)[2,5,6]. 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)[7]. 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; Suraweera, 2002)[8,9,10,11,12,13,14]. Several

studies have noted discordant results between IHC and MSI by PCR (Cicek, 2011; Bartley, 2012; Smyth, 2017; Martinez-Ciarpaglini, 2019)[11,15,16,17]. In clinical trial studies evaluating immunotherapy in metastatic colorectal patients discrepancies between local assessment and central laboratory results were observed (Cohen, 2018: Overman, 2017)[18,19]. 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)[18]. 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, including gastric-oesophageal tumors, in a recent recommendation on MSI testing (Luchini, 2019 pg 5 Table 2)[20].

Approval of PD-1 checkpoint inhibitors nivolumab and pembrolizumab for treatment of MSI-H/dMMR solid tumors 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)[2,21,18,19,22,20,].

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 gastroesophageal 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)[29]. 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)[30,31]. 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)[30,32,33].

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)[23,24]. MSI by PCR is an established, reimbursable test which should be considered for routine MSI analysis.

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].

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

NCCN Guidelines version 2.2019- May 29, 2019 (Esophageal and Esophagogastric Junction Cancers)

Section	Page#	Current update	Promega proposal	Evidence/Publication
ESOPH-1	7	MSI-H/dMMR testing if metastatic disease is documented/suspected	**MSI by PCR and** dMMR testing if metastatic disease is documented/suspected	Funkhouser 2012; Bartley, 2012; Gould, 2014; Luchini, 2019
ESOPH-19	25	Perform HER2, PD-L1, MSI/MMR testing (if not done previously) if metastatic adenocarcinoma is suspected	Perform HER2, PD-L1, **MSI by PCR and MMR** testing (if not done previously) if metastatic adenocarcinoma is suspected	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019
ESOPH-B 4 of 5	34	MMR or MSI testing should be considered on locally advanced, recurrent, or metastatic esophageal and EGJ cancers, in patients who are candidates for treatment with PD-1 inhibitors. The testing is performed on formalin-fixed, paraffin-embedded (FFPE) tissue and results are interpreted as MSI-high (MSI-H) or mismatch protein repair-deficient (dMMR) in accordance with CAP DNA	**MSI by PCR and MMR** testing should be considered on locally advanced, recurrent, or metastatic esophageal and EGJ cancers, in patients who are candidates for treatment with PD-1 inhibitors.	Luchini, 2019; Sepulveda, 2017; Cohen, 2018

		Mismatch Repair Biomarker Reporting Guidelines. MMR or MSI testing should be performed only in CLIA-approved laboratories.		
ESOPH-B 4 of 5	34	[k]IHC for MMR and polymerase chain reaction (PCR) for MSI are different assays measuring the same biological effect	[k]Polymerase chain reaction (PCR) for MSI and IHC for MMR proteins **measure different biological effects caused by deficient mismatch repair function**.	Bartley, 2012.
MS-14	87	MSI is assessed by polymerase chain reaction (PCR) to measure gene expression levels of microsatellite markers (ie, BAT25, BAT26, MONO27, NR21, NR24). It should be noted that IHC for MMR and PCR for MSI are different assays measuring the same biological effect.	MSI is assessed by polymerase chain reaction (PCR) to measure **changes in length** of microsatellite markers (ie, BAT25, BAT26, MONO27, NR21, NR24) **caused by failure of the mismatch repair machinery**. It should be noted that IHC for MMR and **DNA analysis for MSI by PCR measure different biological effects caused by deficient mismatch repair function**	Bartley, 2012; Richman, 2015
MS-38	111	HER2, MSI-H/dMMR, and PD-L1 testing is recommended at the time of diagnosis if metastatic disease is documented or suspected.	HER2, **MSI by PCR, dMMR**, and PD-L1 testing is recommended at the time of diagnosis if metastatic disease is documented or suspected.	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019
MS-42	115	If not done previously, HER2, MSI-H/dMMR, and PD-L1 testing should be performed in patients with suspected metastatic disease.	If not done previously, HER2**, MSI by PCR, dMMR**, and PD-L1 testing should be performed in patients with suspected metastatic disease.	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019

NCCN Evidence Blocks version 2.2019-June 21, 2019 – Esophageal and Esophagogastric Junction Cancers:

Section	Page#	current update	Promega proposal	Evidence/Publication
ESOPH-1	5	MSI-H/dMMR testing if metastatic disease is documented/suspected	**MSI by PCR and** dMMR testing if metastatic disease is documented/suspected	Funkhouser 2012; Bartley, 2012; Gould, 2014; Luchini, 2019
ESOPH-19	23	Perform HER2, PD-L1, MSI/MMR testing (if not done previously) if metastatic adenocarcinoma is suspected	Perform HER2, PD-L1, **MSI by PCR, and MMR** testing (if not done previously) if metastatic adenocarcinoma is suspected	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019
ESOPH-B 4 of 5	32	MMR or MSI testing should be considered on locally advanced, recurrent, or metastatic esophageal and EGJ cancers, in patients who are candidates for treatment with PD-1 inhibitors. The testing is performed on formalin-fixed, paraffin-embedded (FFPE) tissue and results are interpreted as MSI-high (MSI-H) or mismatch protein repair-deficient (dMMR) in accordance with CAP DNA Mismatch Repair Biomarker Reporting Guidelines. MMR or MSI testing should be performed only in CLIA-approved laboratories.	**MSI by PCR and MMR** testing should be considered on locally advanced, recurrent, or metastatic esophageal and EGJ cancers, in patients who are candidates for treatment with PD-1 inhibitors.	Luchini, 2019; Sepulveda, 2017; Cohen, 2018
ESOPH-B 4 of 5	32	[k]IHC for MMR and polymerase chain reaction (PCR) for MSI are different assays measuring the same biological effect	[k]Polymerase chain reaction (PCR) for MSI and IHC for MMR proteins **measure different biological**	Bartley, 2012.

			effects caused by deficient mismatch repair function.	
MS-14	89	MSI is assessed by polymerase chain reaction (PCR) to measure gene expression levels of microsatellite markers (ie, BAT25, BAT26, MONO27, NR21, NR24). It should be noted that IHC for MMR and PCR for MSI are different assays measuring the same biological effect.	MSI is assessed by polymerase chain reaction (PCR) to measure changes in length of microsatellite markers (ie, BAT25, BAT26, MONO27, NR21, NR24) caused by failure of the mismatch repair machinery. It should be noted that IHC for MMR and **DNA analysis for MSI by PCR measure different biological effects caused by deficient mismatch repair function**	Bartley, 2012; Richman, 2015
MS-38	113	HER2, MSI-H/dMMR, and PD-L1 testing is recommended at the time of diagnosis if metastatic disease is documented or suspected.	HER2, **MSI by PCR and dMMR**, and PD-L1 testing is recommended at the time of diagnosis if metastatic disease is documented or suspected.	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019
MS-42	117	If not done previously, HER2, MSI-H/dMMR, and PD-L1 testing should be performed in patients with suspected metastatic disease.	If not done previously, HER2**, MSI by PCR, dMMR**, and PD-L1 testing should be performed in patients with suspected metastatic disease.	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019

NCCN Guidelines version 2.2019-June 3, 2019 – Gastric Cancer:

Section	Page#	current update	Promega proposal	Evidence/Publication
GAST-1	6	MSI-H/dMMR testing if metastatic disease is documented/suspected	**MSI by PCR and** dMMR testing if metastatic disease is documented/suspected	Funkhouser 2012; Bartley, 2012; Gould, 2014; Luchini, 2019
GAST-9	14	Perform HER2, PD-L1, MSI/MMR testing (if not done previously) if metastatic adenocarcinoma is documented or suspected	Perform HER2, PD-L1, **MSI by PCR and MMR** testing (if not done previously) if metastatic adenocarcinoma is suspected	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019
GAST-B 4 of 5	21	MMR or MSI testing should be considered on locally advanced, recurrent, or metastatic gastric carcinoma, in patients who are candidates for treatment with PD-1 inhibitors. The testing is performed on formalin-fixed, paraffin-embedded (FFPE) tissue and results are interpreted as MSI-high (MSI-H) or mismatch protein repair-deficient (dMMR) in accordance with CAP DNA Mismatch Repair Biomarker Reporting Guidelines. MMR or MSI testing should be performed only in CLIA-approved laboratories.	**MSI by PCR and MMR** testing should be considered on locally advanced, recurrent, or metastatic esophageal and EGJ cancers, in patients who are candidates for treatment with PD-1 inhibitors.	Luchini, 2019; Sepulveda, 2017; Cohen, 2018
GAST-B 4 of 5	21	[d]IHC for MMR and polymerase chain reaction (PCR) for MSI	[d]Polymerase chain reaction (PCR) for MSI and IHC for MMR	Bartley, 2012.

		are different assays measuring the same biological effect	proteins **measure different biological effects caused by deficient mismatch repair function**.	
MS-10	69	MSI is assessed by polymerase chain reaction (PCR) to measure gene expression levels of microsatellite markers (ie, BAT25, BAT26, MONO27, NR21, NR24). It should be noted that IHC for MMR and PCR for MSI are different assays measuring the same biological effect.	MSI is assessed by polymerase chain reaction (PCR) to measure **changes in length** of microsatellite markers (ie, BAT25, BAT26, MONO27, NR21, NR24) **caused by failure of the mismatch repair machinery**. It should be noted that IHC for MMR and **DNA analysis for MSI by PCR measure different biological effects caused by deficient mismatch repair function**	Bartley, 2012; Richman, 2015
MS-29	88	HER2, MSI-H/dMMR, and PD-L1 testing is recommended at the time of diagnosis if metastatic disease is documented or suspected.	HER2, **MSI by PCR and dMMR**, and PD-L1 testing is recommended at the time of diagnosis if metastatic disease is documented or suspected.	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019
MS-32	91	If not done previously, HER2, MSI-H/dMMR, and PD-L1 testing should be performed in patients with suspected metastatic adenocarcinoma.	If not done previously, HER2**, MSI by PCR, dMMR**, and PD-L1 testing should be performed in patients with suspected metastatic disease.	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019

NCCN Evidence Blocks version 2.2019-June 4, 2019 – Gastric Cancer:

Section	Page#	current update	Promega proposal	Evidence/Publication
GAST-1	5	MSI-H/dMMR testing if metastatic disease is documented/suspected	**MSI by PCR and** dMMR testing if metastatic disease is documented/suspected	Funkhouser 2012; Bartley, 2012; Gould, 2014; Luchini, 2019
GAST-9	13	Perform HER2, PD-L1, MSI/MMR testing (if not done previously) if metastatic adenocarcinoma is documented or suspected	Perform HER2, PD-L1, **MSI by PCR and MMR** testing (if not done previously) if metastatic adenocarcinoma is suspected	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019
GAST-B 4 of 5	20	MMR or MSI testing should be considered on locally advanced, recurrent, or metastatic gastric carcinoma, in patients who are candidates for treatment with PD-1 inhibitors. The testing is performed on formalin-fixed, paraffin-embedded (FFPE) tissue and results are interpreted as MSI-high (MSI-H) or mismatch protein repair-deficient (dMMR) in accordance with CAP DNA Mismatch Repair Biomarker Reporting Guidelines. MMR or MSI testing should be performed only in CLIA-approved laboratories.	**MSI by PCR and MMR** testing should be considered on locally advanced, recurrent, or metastatic esophageal and EGJ cancers, in patients who are candidates for treatment with PD-1 inhibitors.	Luchini, 2019; Sepulveda, 2017; Cohen, 2018

GAST-B 4 of 5	20	[d]IHC for MMR and polymerase chain reaction (PCR) for MSI are different assays measuring the same biological effect	[d]Polymerase chain reaction (PCR) for MSI and IHC for MMR proteins **measure different biological effects caused by deficient mismatch repair function**.	Bartley, 2012.
MS-10	71	MSI is assessed by polymerase chain reaction (PCR) to measure gene expression levels of microsatellite markers (ie, BAT25, BAT26, MONO27, NR21, NR24). It should be noted that IHC for MMR and PCR for MSI are different assays measuring the same biological effect.	MSI is assessed by polymerase chain reaction (PCR) to measure **changes in length** of microsatellite markers (ie, BAT25, BAT26, MONO27, NR21, NR24) **caused by failure of the mismatch repair machinery**. It should be noted that IHC for MMR and **DNA analysis for MSI by PCR measure different biological effects caused by deficient mismatch repair function**	Bartley, 2012; Richman, 2015
MS-29	90	HER2, MSI-H/dMMR, and PD-L1 testing is recommended at the time of diagnosis if metastatic disease is documented or suspected.	HER2, **MSI by PCR and dMMR**, and PD-L1 testing is recommended at the time of diagnosis if metastatic disease is documented or suspected.	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019
MS-32	93	If not done previously, HER2, MSI-H/dMMR, and PD-L1 testing should be performed in patients with suspected metastatic adenocarcinoma.	If not done previously, HER2**, MSI by PCR, dMMR**, and PD-L1 testing should be performed in patients with suspected metastatic disease.	Bartley, 2012; Bruegl, 2017; Goodfellow 2015; Luchini, 2019

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. 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.
7. National Comprehensive Cancer Network. “Genetic/Familial High-Risk Assessment: Colorectal”. Version 2.2019 – August 8, 2019. https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf
8. 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
9. 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
10. 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
11. 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

12. 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
13. 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
14. Suraweera N, Duval A, Reperant M, Vaury C et al. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. Gastroenterology, 2002; 123(6): 1804-1811.
15. 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.
16. Smyth EC, Wotherspoon A and Peckitt C. Mismatch Repair Deficiency, Microsatellite Instability and Survival. JAMA Oncology 2017; 3(9): 1197-1203. doi:10.1001/jamaoncol.2016.6762
17. Martinez-Ciarpaglini C, Fleitas-Kanonnikoff T, Gambardella V et al. Assessing molecular subtypes of gastric cancer: microsatellite unstable and Epstein-Barr virus subtypes. Methods for detection and clinical and pathological implications. ESMO Open Cancer Horizons, 2019; 4(3): 1-8. doi:10.1136/esmoopen-2018-000470
18. 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
19. 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.
20. 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
21. 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

22. 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
23. 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.\
24. 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
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. Bruegl AS, Ring KL, Daniels M, Fellman BM, Urbauer DL and Broaddus RR. Clinical Challenges Associated with Universal Screening for Lynch Syndrome–Associated Endometrial Cancer. Cancer Prevention Research, 2017; 10(2); 108-115. Epub 2016 Dec 13. DOI: 10.1158/1940-6207. PubMed PMID: 27965287.
28. Goodfellow PJ, Billingsley CC, Lankes HA, Ali S, Cohn DE, Broaddus RJ, Ramirez NR, et al. Combined Microsatellite Instability, *MLH1* Methylation Analysis, and Immunohistochemistry for Lynch Syndrome Screening in Endometrial Cancers from GOG210: An NRG Oncology and Gynecologic Oncology Group Study. Journal of Clinical Oncology 2015; 33(36): 4301-4308. DOI: 10.1200/JCO.2015.63.9518. PubMed PMID: 266552419.
29. 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
30. 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
31. 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.

32. 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.
33. Latham *et al.* Microsatellite Instability is Associated with the Presence of Lynch Syndrome Pan-Cancer. J Clin Oncology (2019) 37:286-95

Thank you for your consideration.

Sincerely,

Ashley G Anderson Jr

Ashley Anderson, MD
Chief Medical Officer
Promega Corporation

Randall Dimond, Ph.D.
Vice President & Chief Scientific Officer
Promega Corporation