To: submissions@nccn.org
Re: Submission Request – Prostate Cancer Panel

Submitted by:
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NCCN Guidelines Panel: Prostate Cancer Panel

On behalf of Promega Corporation, we respectfully request the NCCN Prostate Cancer Panel to review the enclosed information in support of making changes to the current guidelines for Prostate 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 prostate cancer be given equal weight and be recommended as a parallel technology with mismatch repair deficiency (MMR) protein expression analysis by immunohistochemistry (IHC) for prostate 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:
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 prostate 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)[11]. 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, 2008) [12,13]. 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; Guedes, 2017)[12,14,16,17]. Therefore, any assay, NGS or PCR, with proven performance with prostate samples should be sufficient for use in detection of MSI.

Assays for MSI and MMR protein expression measure separate but related cellular events (Baudrin, 2018)[12]. 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).[1,2]. Moreover, in practice there is substantial interobserver variation, which varies by the expertise of the pathologist (Funkhouser, 2012; Klarskov, 2010)[1,3]. 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)[4]. Contemporary MSI by PCR panels have a false negative rate of just 0.3-5% (Funkhouser, 2012; Bacher 2004; Xicola 2007)[1, 18, 19]. Several studies have noted discordant results between IHC and MSI by PCR (Bartley, 2012; Goodfellow, 2015; Bruegl, 2017)[5,6,7]. Due to the complementary nature of these technologies and the potential impact of misdiagnosis, there is growing recognition in the field that these tests should be performed together for maximal sensitivity when identifying patients for hereditary cancer risk and immunotherapy eligibility (Goodfellow, 2015)[6].

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)[8]. In addition, 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[9,10,15].

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

NCCN Guidelines version 1.2019- March 6, 2019 Updates (Prostate Cancer)

Section	Page#	current update	Promega proposal	Evidence/Publication
PROS-9	5, footnote "dd"	DNA analysis for MSI and IHC for MMR are different assays measuring the same biological effect.	DNA analysis of MSI and IHC for MMR proteins **measure different biological effects caused by deficient mismatch repair function.**	Bartley, 2012.

PROS-9	5, footnote "dd"	If MSI is used, testing using an NGS assay validated for prostate cancer is preferred. If MSI-H or dMMR is found, refer to genetic counseling to assess for the possibility of Lynch syndrome. MSI-H or dMMR indicate eligibility for pembrolizumab in later lines of treatment for castration-resistant prostate cancer (CRPC) (see PROS-17 and PROS-18). Hempelmann JA, Lockwood CM, Konnick EQ, et al. Microsatellite instability in prostate cancer by PCR or NGS. J Immunother Cancer 2018; 6:29.	**When performing MSI analysis, an assay that has been validated for prostate cancer is preferred.**	Salipante 2014; Baudrin, 2018; Zhang, 2008; Rodrigues, 2018; Latham, 2019; Guedes, 2017
PROS-9 (Table, row 1)	18, footnote "dd"	DNA analysis for MSI and IHC for MMR are different assays measuring the same biological effect.	DNA analysis of MSI and IHC for MMR proteins **measure different biological effects caused by deficient mismatch repair function.**	Bartley, 2012.
PROS-9 (Table, row 1)	18, footnote "dd"	If MSI is used, testing using an NGS assay validated for prostate cancer is preferred. If MSI-H or dMMR is found, refer to genetic counseling to assess for the possibility of Lynch	**When performing MSI analysis, an assay that has been validated for prostate cancer is preferred.**	Salipante 2014; Baudrin, 2018; Zhang, 2008; Rodrigues, 2018; Latham, 2019; Guedes, 2017

		syndrome. MSI-H or dMMR indicate eligibility for pembrolizumab in later lines of treatment for castration-resistant prostate cancer (CRPC) (see PROS-17 and PROS-18). Hempelmann JA, Lockwood CM, Konnick EQ, et al. Microsatellite instability in prostate cancer by PCR or NGS. J Immunother Cancer 2018; 6:29.		

NCCN Evidence Blocks version 4.2018 – Prostate Cancer:

Section	Page#	current update	Promega proposal	Evidence/Publication
PROS-3	6 (Regional Risk group row)	Consider tumor testing for homologous recombination gene mutations and for microsatellite instability (MSI) or mismatch repair deficiency (dMMR)	Consider tumor testing for homologous recombination gene mutations and for microsatellite instability (MSI) **and** mismatch repair deficiency (dMMR)	Bartley, 2012; Bruegl, 2017; Goodfellow, 2015.
PROS-3	6 (Metastic Risk group row)	Consider tumor testing for homologous recombination gene mutations and for microsatellite instability (MSI) or mismatch repair deficiency (dMMR)	Consider tumor testing for homologous recombination gene mutations and for microsatellite instability (MSI) **and** mismatch repair deficiency (dMMR)	Bartley, 2012; Bruegl, 2017; Goodfellow 2015.

PROS-3	7	DNA analysis for MSI and IHC for MMR are different assays measuring the same biological effect. If MSI-H or dMMR is found, refer to genetic counseling to assess for the possibility of Lynch syndrome. MSI or dMMR indicate eligibility for pembrolizumab in later lines of treatment for CRPC	DNA analysis of MSI and IHC for MMR proteins **measure different biological effects caused by deficient mismatch repair function**.	Bartley, 2012.
PROS-15	20	Consider tumor testing for MSI-H or dMMR[m]. [m]DNA analysis for MSI and IHC for MMR are different assays measuring the same biological effect. If MSI-H or dMMR is found, refer to genetic counseling to assess for the possibility of Lynch syndrome. MSI-H or dMMR indicate eligibility for pembrolizumab in later lines of treatment for CRPC (see PROS-16 and PROS-17)	Consider tumor testing for MSI-H **and** dMMR[m]. [m]DNA analysis of MSI and IHC for MMR proteins **measure different biological effects caused by deficient mismatch repair function**.	Bartley, 2012; Bruegl, 2017; Goodfellow, 2015.

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

1. 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.

2. 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.
3. 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
4. National Comprehensive Cancer Network. "Guideline Name" (Version 2.2019). http://www.nccn.org/professionals/physician_gls/pdf/bone.pdf. Accessed April 10, 2019
5. 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.
6. 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.
7. 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.
8. 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.
9. Le TD, 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.
10. 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
11. 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
12. 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

13. 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.
14. 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.
15. Graff JN, Alumkal JJ, Drake, CG, Thomas GV et al. Early evidence of anti-PD-1 activity in enzalutamide-resistant prostate cancer. Oncotarget, 2016; 7(33): 52810-52817. DOI: 10.18632/oncotarget.10547
16. Latham A, Srinivasan P, Kemel Y, Shia J et al. Microsatellite Instability Is Associated with the Presence of Lynch Syndrome Pan-Cancer. Journal of Clinical Oncology, 2019; 37(4): 286-295. DOI: 10.1200/JCO.18.00283
17. Guedes LB, Antonarakis, ES, Schweizer, MT, Mirkheshti, N et al. MSH2 Loss in Primary Prostrate Cancer. Clinical Cancer Research, 2017; 23(22): 6863-6874. DOI: 10.1158/1078-0432
18. Bacher JW, Flanagan LA, Smalley RL, Nassif NA et al. Development of a fluorescent multiplex assay for detection of MSI-H tumors. Disease Markers, 2004; 20:237-250.
19. Xicola, RM, Llor X, Pons E, Castells A et al. Performance of Different Microsatellite Marker Panels for Detection of Mismatch Repair-Deficient Colorectal tumors. Journal of National Cancer Institute, 2007; 99(3): 244-252. DOI: 10.1093/jnci/djk033

Thank you for your consideration.

Sincerely,

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