

Targeted Cancer Next-Generation Sequencing as a Primary Screening Tool for Microsatellite Instability and Lynch Syndrome in Upper Gastrointestinal Tract Cancers



Alexander G. Christakis¹, David J. Papke¹, Jonathan A. Nowak¹, Matthew B. Yurgelun², Agoston T. Agoston¹, Neal I. Lindeman¹, Laura E. MacConaill³, Lynette M. Sholl¹, and Fei Dong¹

Abstract

Background: No consensus guideline has been established for microsatellite instability testing in upper gastrointestinal tract cancers. This study aims to determine whether targeted cancer next-generation sequencing can accurately detect microsatellite instability in upper gastrointestinal tract cancers and screen for patients with Lynch syndrome.

Methods: In a cohort of 645 upper gastrointestinal tract cancers, targeted next-generation sequencing assessed microsatellite instability by identifying characteristic insertion and deletion mutations. Sequencing classification was compared with mismatch repair protein IHC. Cancers with microsatellite instability by sequencing were analyzed using a testing protocol to identify patients with Lynch syndrome.

Results: Sequencing identified microsatellite instability in 3.6% (23/645) of upper gastrointestinal tract cancers, includ-

ing 28% (8/29) of small intestinal and 9% (9/97) of gastric carcinomas. In 20 cancers classified as having microsatellite instability, 19 demonstrated loss of expression of MLH1, PMS2, MSH2, or MSH6, and one cancer was indeterminate by IHC. In contrast, 52 control cancers demonstrated retained expression of all mismatch repair proteins. Using targeted sequencing as the initial screening test, 1.1% (7/645) of patients were identified to have pathogenic germline variants confirming a diagnosis of Lynch syndrome.

Conclusions: Targeted cancer next-generation sequencing is an accurate first-line test to detect microsatellite instability in upper gastrointestinal tract cancers.

Impact: This study provides a proof of concept for the use of targeted next-generation sequencing to detect microsatellite instability and screen for Lynch syndrome.

Introduction

Microsatellite instability defines a characteristic genomic phenotype of insertion and deletion mutations in DNA repeat regions (1). Microsatellite instability is caused by the genetic or epigenetic inactivation of DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6*, or *PMS2* and is a feature of some sporadic cancers and cancers associated with Lynch syndrome (2).

Microsatellite instability is most commonly observed in colorectal and endometrial cancers. Although the overall incidence of upper gastrointestinal tract cancers is lower than that of colorectal

and endometrial cancers, recent studies evaluating large cancer datasets have identified significant rates of microsatellite instability in cancers of the stomach and small intestine (3–5). Clinical guidelines recommend universal screening for Lynch syndrome in colorectal cancers (6), but no consensus guidelines currently exist for microsatellite instability testing in upper gastrointestinal tract cancers. The accurate identification of microsatellite instability in upper gastrointestinal tract cancers may directly benefit patients in two ways. Microsatellite instability serves as a biomarker to predict response to immune checkpoint inhibitor therapy in solid tumors, including upper gastrointestinal tract cancers (5, 7). Patients who have cancers with microsatellite instability may be at increased risk for Lynch syndrome, and patients and affected family members may benefit from genetic counseling and germline testing (8).

As next-generation sequencing technology becomes clinically available for cancer genotyping to identify actionable driver mutations, we hypothesize that targeted cancer sequencing panels can also detect microsatellite instability and be used as a primary screening tool for Lynch syndrome in upper gastrointestinal tract cancers.

Materials and Methods

Patients were prospectively enrolled in an institutional cohort study for cancer genotyping. All participants provided written informed consent for tumor sequencing. This study was approved

¹Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts. ²Department of Medical Oncology, Dana Farber Cancer Institute, Boston, Massachusetts. ³Center for Cancer Genome Discovery, Dana Farber Cancer Institute, Boston, Massachusetts.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

A.G. Christakis and D.J. Papke contributed equally as co-first authors of this article.

Corresponding Author: Fei Dong, Brigham and Women's Hospital, 75 Francis St, Amory 3, Boston, MA 02115. Phone: 617-525-7813; Fax: 617-264-5118; E-mail: fdong1@bwh.harvard.edu

Cancer Epidemiol Biomarkers Prev 2019;28:1246–51

doi: 10.1158/1055-9965.EPI-18-1250

©2019 American Association for Cancer Research.

by the Institutional Review Board of the Dana Farber Cancer Institute and the Partners Human Research Committee.

Targeted next-generation sequencing on tumor tissue was performed as described previously (9). DNA was isolated from formalin-fixed, paraffin-embedded (FFPE) tissue with at least 20% tumor nuclei. Indexed sequencing libraries were enriched for exons of 275 genes (102 specimens) or 298 genes (543 specimens), encompassing 757,787 base pairs or 831,033 base pairs of targeted genome, respectively, using solution-based hybrid capture (Agilent SureSelect; Agilent Technologies). Massively parallel sequencing was performed using Illumina HiSeq2500 (Illumina, Inc.). Data analysis was performed using a custom pipeline, including Indelocator (GATK; Broad Institute) for calling insertion and deletion variants.

Microsatellite instability as detected by next-generation sequencing was defined as greater than 3 microsatellite indel events per megabase (Mb) pair in the targeted genome, with events defined as single nucleotide insertion or deletion variants in homopolymeric DNA repeats of four or more nucleotides. Cancers with total microsatellite indel events below this threshold were classified as microsatellite stable. This criterion achieved 96% sensitivity and 99% specificity compared with mismatch repair protein IHC in colorectal adenocarcinomas (10).

IHC for MLH1, PMS2, MSH2, and MSH6 protein expression was performed as a case-control study. We tested all samples classified to have microsatellite instability with available pathology material. For each case sample predicted to have microsatellite instability, at least two control samples classified as microsatellite stable, matched by diagnosis, were tested. In addition, IHC was performed on all samples with greater than 2 but fewer than 3 microsatellite indel events per megabase if

tissue was available. IHC and *MLH1* promoter methylation analyses were performed per standard laboratory protocol as described previously (11).

Subsequent germline sequencing was performed using the same targeted next-generation sequencing assay on DNA isolated from nonneoplastic, FFPE tissue. The assay included coverage of coding regions of mismatch repair genes *MLH1*, *PMS2*, *MSH2*, and *MSH6*.

Results

Patient characteristics

A total of 645 upper gastrointestinal tract carcinomas were sequenced, including 230 from esophagus or gastroesophageal junction, 199 from pancreas, 97 from stomach, 60 from bile duct, 29 from small intestine, 19 from gallbladder, and 11 from ampulla of Vater. A total of 426 patients (66%) were male, and 219 (34%) were female. The median age at the time of testing was 65.3 years (mean 64.2 years, range 19.0–93.6 years).

Detection of microsatellite instability in upper gastrointestinal tract cancers by targeted sequencing

Microsatellite instability was identified by next-generation sequencing in 23 of 645 (3.6%) upper gastrointestinal tract cancers. Cancers classified to have microsatellite instability had a median of 9.2 microsatellite indel events per megabase (mean 10.9, range 3.4–22.4). A total of 555 of 622 (89.2%) cancers classified as microsatellite stable had 0 microsatellite indel events. Fifty-five of 622 (8.8%) cancers classified as microsatellite stable had between 0 and 2 microsatellite indel events, and 12 of 622 (1.9%) had between 2 and 3 microsatellite indel events (Fig. 1A).

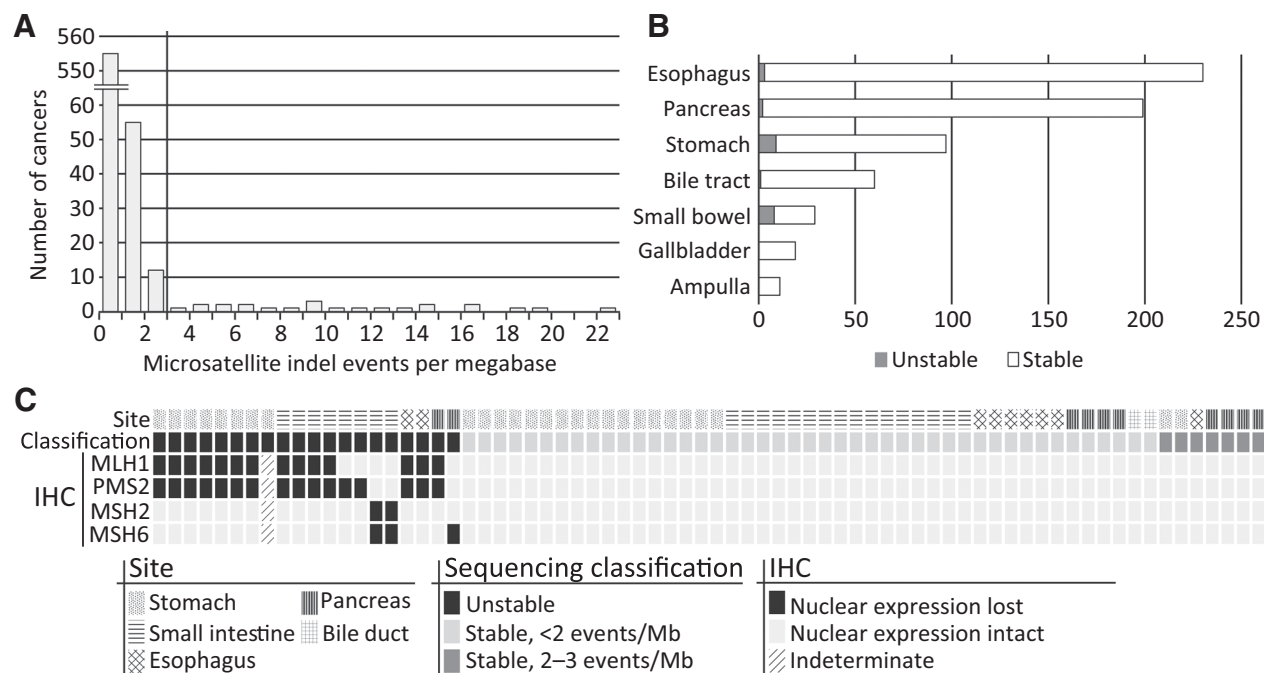
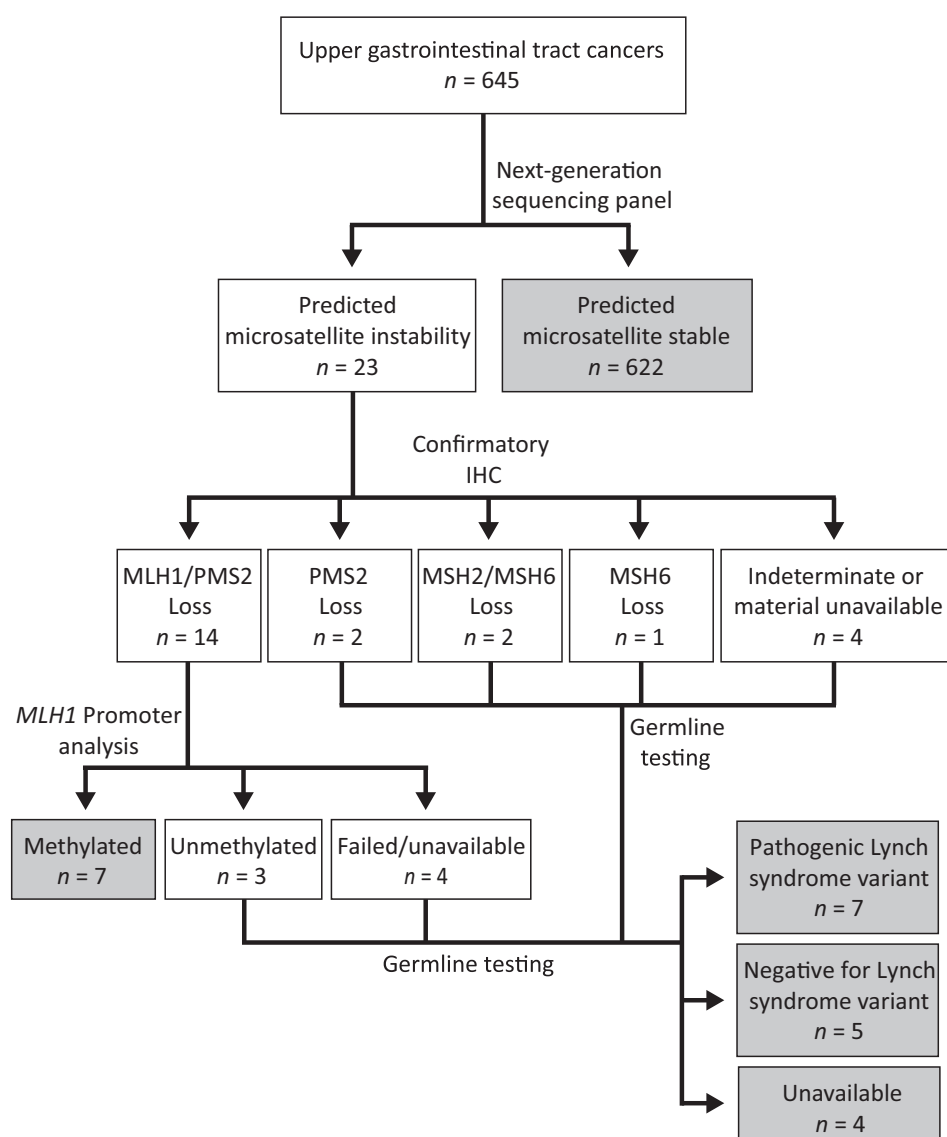


Figure 1.

Detection of microsatellite instability by next-generation sequencing. **A**, Number of microsatellite insertion or deletion events per megabase in upper gastrointestinal tract cancers. Cancers with greater than three events per megabase were classified as having microsatellite instability. **B**, Frequency of microsatellite instability in upper gastrointestinal tract cancers, by site. **C**, Validation of microsatellite instability classification by sequencing compared with IHC.

**Figure 2.**

Study protocol for the evaluation of upper gastrointestinal tract cancers to identify patients with Lynch syndrome. Targeted next-generation sequencing was performed on 645 upper gastrointestinal tract cancers. Confirmatory IHC was performed on cancers classified as having microsatellite instability, and *MLH1* promoter methylation analysis was tested for cancers with loss of *MLH1* and *PMS2* expression. Germline testing was performed on cases suspicious for Lynch syndrome-associated cancers, where nonneoplastic tissue was available.

Microsatellite instability was seen in 8 of 29 small intestinal (28%), 9 of 97 gastric (9%), 1 of 60 biliary tract (2%), 3 of 230 esophageal or gastroesophageal junction (1.3%), 2 of 199 pancreatic (1.0%), 0 of 19 gallbladder, and 0 of 11 ampullary cancers (Fig. 1B).

Validation of results with mismatch repair protein IHC

Twenty of 23 cases classified to have microsatellite instability had available tumor tissue for IHC. Nineteen of 20 (95%) showed loss of expression of at least one mismatch repair protein including 14 with loss of *MLH1* and *PMS2*, 2 with loss of *PMS2*, 2 with loss of *MSH2* and *MSH6*, and 1 with loss of *MSH6*. The final case showed an unusual pattern, with nuclear staining of 5% of tumor nuclei for all four mismatch repair proteins, and was interpreted as indeterminate.

In contrast, all 45 controls classified as microsatellite stable by sequencing showed intact staining for *MLH1*, *PMS2*, *MSH2*, and *MSH6* (Fig. 1C). In addition, all 7 cases with between 2

and 3 microsatellite indel events per megabase (classified as microsatellite stable but near the threshold) and with available tissue showed intact staining for *MLH1*, *PMS2*, *MSH2*, and *MSH6*.

Identification of Lynch syndrome in patients with upper gastrointestinal tract cancers

For cases with microsatellite instability and available matched normal tissue, we followed a testing algorithm commonly applied to colorectal cancers to identify patients with Lynch syndrome (Fig. 2). For cancers with loss of *MLH1* and *PMS2* expression, *MLH1* promoter methylation analysis was performed. Of 10 neoplasms with *MLH1* and *PMS2* loss and successful promoter methylation analysis, *MLH1* promoter methylation was identified in 7.

Nonneoplastic tissue for germline testing was available for 12 of 16 specimens with microsatellite instability and without *MLH1* promoter methylation. Pathogenic Lynch syndrome variants were

Table 1. A total of 23 upper gastrointestinal tract carcinomas classified as mismatch repair deficient by targeted panel sequencing, validation with IHC, and identification of pathogenic germline variants to diagnose Lynch syndrome

Site	Microsatellite events per Mb		IHC			MLH1 Promoter		Germline variant	Germline VAF or log ₂ ratio	Cancer VAF or log ₂ ratio	Somatic variant	Cancer VAF or log ₂ ratio
	MLH1	PMS2	MSH2	MSH6	MLH1							
Stomach	12.6	Loss	Intact	Intact	Methylated	Not indicated						
Stomach	9.2	Loss	Intact	Intact	Methylated	Not indicated						
Stomach	6.9	Loss	Intact	Intact	Methylated	Not indicated						
Stomach	3.4	Loss	Intact	Intact	Methylated	Not indicated						
Stomach	10.3	Loss	Intact	Intact	Unmethylated	Wild-type					MLH1 deletion	-0.7
Stomach	4.6	Loss	Intact	Intact	Unmethylated	MLH1 exon 13 del		-0.7	-1.6			
Stomach	19.5	Loss	Intact	Intact	Failed	Wild-type						
Stomach	9.2	Indeterminate	Indeterminate	Indeterminate	Unmethylated	Wild-type						
Stomach	13.7	Not available	Not available	Not available	Not available	Not available						
Small intestine	18.3	Loss	Intact	Intact	Methylated	Not indicated						
Small intestine	16.0	Loss	Intact	Intact	Methylated	Not indicated						
Small intestine	5.7	Loss	Intact	Intact	Unmethylated	MLH1 c.2194_2197dup		45%	43%			
Small intestine	11.5	Intact	Intact	Intact	Not indicated	(p.H733Gfs*14)		51%	45%		PMS2 c.23+1G>T	22%
Small intestine	8.0	Intact	Intact	Intact	Not indicated	PMS2 c.1500dup		58%	45%			
Small intestine	22.4	Intact	Intact	Loss	Not indicated	(p.V501Rfs*4)					MSH2 c.2581C>T (p.Q861*)	77%
Small intestine	7.9	Intact	Intact	Loss	Not indicated	MSH2 c.1786_1788del (p.N596del)		48%	43%			
Small intestine	6.9	Loss	Intact	Intact	Not available	Not available						
Pancreas	5.7	Intact	Intact	Loss	Not indicated	MSH2 c.1906G>C (p.A636P)		48%	54%		MSH2 c.622G>T (p.G208*)	8%
Pancreas	9.2	Loss	Intact	Intact	Not available	Not available						
Esophagus	16.0	Loss	Intact	Intact	Methylated	Not indicated						
Esophagus	14.9	Loss	Intact	Intact	Failed	Wild-type						
Esophagus	14.9	Not available	Not available	Not available	Not available	MLH1 c.2059C>T (p.R687W)		47%	51%		MLH1 c.298C>T (p.R100*)	40%
Bile duct	4.6	Not available	Not available	Not available	Not available	Not available						

NOTE: Log₂ ratio, relative copy number, where 0 represents copy number neutral and -1 represents one copy loss in a specimen where 100% of cells are affected. Abbreviation: VAF, variant allele fraction. NOTE: Pathogenic variants identified by germline or somatic sequencing are highlighted in bold.

Christakis et al.

identified in 7 patients, representing 1.1% of upper gastrointestinal tract cancers in our cohort, including 4 small intestinal, 1 esophageal, 1 stomach, and 1 pancreatic cancers (Table 1; Supplementary Data). Patients with Lynch syndrome had a median age of 69.6 years (range 49.8–74.6 years). Upper gastrointestinal tract cancer was the first clinical manifestation of Lynch syndrome in 6 of 7 patients. Colonic adenocarcinoma was the first Lynch syndrome-associated cancer in the other patient.

Somatic and second hit mutations in upper gastrointestinal tract cancers with microsatellite instability

We evaluated the tumor sequencing data to identify somatic second hit mutations in mismatch repair genes in patients with Lynch syndrome. In 3 of 7 patients with Lynch syndrome, a second loss-of-function mutation was identified in the tumor specimen. These mutations included a splice site variant and two nonsense variants. In 1 patient with an isolated *MLH1* exon 13 germline deletion, the tumor specimen showed exon 13 deletion involving both copies of *MLH1*, and the second hit in this cancer was most likely due to loss of heterozygosity of the *MLH1* gene locus. In the other 3 patients with Lynch syndrome, a second somatic mutation was not identified, and the observed variant allele fractions in tumor specimens did not support LOH. This result might be due to limitations of the study, including possibly limited ability to detect loss of heterozygosity in relatively low tumor purity conditions or the presence of sequence or structural alterations in noncoding regions of the gene, which were not assessed by this assay. In two upper gastrointestinal tract cancers with microsatellite instability, the full testing algorithm was completed, and germline testing did not identify a pathogenic Lynch syndrome variant. Tumor testing showed somatic inactivation of mismatch repair genes by mutation or focal gene deletion (Table 1).

Discussion

Next-generation sequencing has emerged as an effective diagnostic tool in cancer care and is being rapidly adopted into clinical practice (12). In addition to the identification of oncogenic driver mutations, sequencing can identify patterns of passenger mutations associated with microsatellite instability. Multiple algorithms have been developed to identify microsatellite instability from sequencing assays (13–15), including from targeted panel sequencing of tumor only specimens (10, 16). Recently, tumor sequencing has been suggested as a replacement for traditional Lynch syndrome screening methods in colorectal cancer (17).

Compared with colorectal cancer, Lynch syndrome screening in the upper gastrointestinal tract faces practical and diagnostic challenges. Many upper gastrointestinal tract cancers are diagnosed from fine needle aspiration cytologic preparations or small biopsies, and patients are frequently treated with neoadjuvant therapy before surgical resection. These diagnostic and treatment patterns limit the availability of diagnostic tumor tissue and make accurate interpretation by IHC methods more difficult. In this setting, targeted cancer sequencing is an appealing alternative for microsatellite instability evaluation. The next-generation sequencing assay used in this study has been validated to be performed on as little as 50 ng of tumor-enriched input DNA.

Our findings demonstrate accurate microsatellite instability assessment in upper gastrointestinal tract cancers by sequencing, and our protocol identifies microsatellite instability in 3.6% and pathogenic germline Lynch syndrome variants in 1.1% of upper gastrointestinal tract cancers in this cohort. Although the frequency of microsatellite instability is lower than that of colorectal cancers, the overall rates of microsatellite instability and Lynch syndrome in upper gastrointestinal tract cancers are clinically significant. Microsatellite instability is a biomarker for response of solid tumors to immune checkpoint inhibitor therapy (5, 18), and the identification of microsatellite instability provides a treatment option for patients who have failed other systemic therapies. In addition, the ability to screen for microsatellite instability has implications for a subset of patients with upper gastrointestinal tract cancers and Lynch syndrome. Our study has identified 7 patients with Lynch syndrome in an unselected cohort. These patients are of similar age compared with patients with sporadic upper gastrointestinal tract cancers. Notably, three of seven pathogenic Lynch syndrome variants are missense variants, and two variants involve *PMS2*. Genetic alterations involving *PMS2* have been associated with a moderately increased risk of colorectal and endometrial cancers, and the significance of *PMS2*-associated Lynch syndrome in cancer risk at other sites is controversial (19).

Patients with Lynch syndrome and affected family members may benefit from enhanced surveillance for the prevention for additional primary cancers, including increased frequency of colonoscopy screening, consideration of prophylactic hysterectomy for women older than 40 years, and other emerging screening strategies (20). These potential benefits are in addition to the detection of driver oncogenic alterations, which may provide druggable targets in upper gastrointestinal tract cancers or determine eligibility in clinical trials (21, 22).

Our findings support the use of targeted cancer sequencing as a first-line screening test in upper gastrointestinal tract cancers to identify microsatellite instability and patients with Lynch syndrome. In laboratories already performing panel sequencing to identify driver mutations, the adoption of a similar protocol may benefit patients with upper gastrointestinal tract cancers.

Disclosure of Potential Conflicts of Interest

M.B. Yurgelun reports receiving commercial research grant from Myriad Genetic Laboratories Inc. L.M. Sholl is a consultant/advisory board member for AstraZeneca, LOXO Oncology, and Foghorn Therapeutics. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A.G. Christakis, N.I. Lindeman, L.E. MacConaill, F. Dong

Development of methodology: A.G. Christakis, N.I. Lindeman, L.E. MacConaill, F. Dong

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.G. Christakis, D.J. Papke, N.I. Lindeman, L.E. MacConaill, F. Dong

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.G. Christakis, D.J. Papke, M.B. Yurgelun, A.T. Agoston, N.I. Lindeman, L.E. MacConaill, L.M. Sholl, F. Dong

Writing, review, and/or revision of the manuscript: A.G. Christakis, D.J. Papke, J.A. Nowak, M.B. Yurgelun, N.I. Lindeman, L.E. MacConaill, L.M. Sholl, F. Dong

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.G. Christakis, N.I. Lindeman, F. Dong

Study supervision: F. Dong

Acknowledgments

This work was supported by the Brigham and Women's Hospital Department of Pathology and the Dana Farber Cancer Institute.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked

advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 19, 2018; revised February 20, 2019; accepted April 19, 2019; published first April 26, 2019.

References

1. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993;260:816–9.
2. Lynch HT, Snyder CL, Shaw TG, Heinen CD, Hitchins MP. Milestones of Lynch syndrome: 1895–2015. *Nat Rev Cancer* 2015;15:181–94.
3. Hause RJ, Pritchard CC, Shendure J, Salipante SJ. Classification and characterization of microsatellite instability across 18 cancer types. *Nat Med* 2016;22:1342–50.
4. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen HZ, et al. Landscape of microsatellite instability across 39 cancer types. *JCO Precis Oncol* 2017;2017:1–15.
5. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017;357:409–13.
6. Giardiello FM, Allen JJ, Axilbund JE, Boland CR, Burke CA, Burt RW, et al. Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the U.S. Multi-Society Task Force on colorectal cancer. *Gastroenterology* 2014;147:502–26.
7. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015;372:2509–20.
8. Engel C, Loeffler M, Steinke V, Rahner N, Holinski-Feder E, Dietmaier W, et al. Risks of less common cancers in proven mutation carriers with Lynch syndrome. *J Clin Oncol* 2012;30:4409–15.
9. Garcia EP, Minkovsky A, Jia Y, Ducar MD, Shivdasani P, Gong X, et al. Validation of OncoPanel: a targeted next-generation sequencing assay for the detection of somatic variants in cancer. *Arch Pathol Lab Med* 2017;141:751–8.
10. Papke DJ, Nowak JA, Yurgelun MB, Frieden A, Srivastava A, Lindeman NI, et al. Validation of a targeted next-generation sequencing approach to detect mismatch repair deficiency in colorectal adenocarcinoma. *Mod Pathol* 2018;31:1882–90.
11. Nowak JA, Yurgelun MB, Bruce JL, Rojas-Rudilla V, Hall DL, Shivdasani P, et al. Detection of mismatch repair deficiency and microsatellite instability in colorectal adenocarcinoma by targeted next-generation sequencing. *J Mol Diagn* 2017;19:84–91.
12. Nagarajan R, Bartley AN, Bridge JA, Jennings LJ, Kamel-Reid S, Kim A, et al. A window into clinical next-generation sequencing-based oncology testing practices. *Arch Pathol Lab Med* 2017;141:1679–85.
13. Niu B, Ye K, Zhang Q, Lu C, Xie M, McLellan MD, et al. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics* 2014;30:1015–6.
14. Huang MN, McPherson JR, Cutcutache I, Teh BT, Tan P, Rozen SG. MSIseq: software for assessing microsatellite instability from catalogs of somatic mutations. *Sci Rep* 2015;5:13321.
15. Kautto EA, Bonneville R, Miya J, Yu L, Krook MA, Reeser JW, et al. Performance evaluation for rapid detection of pan-cancer microsatellite instability with MANTIS. *Oncotarget* 2017;8:7452–63.
16. Salipante SJ, Scroggins SM, Hampel HL, Turner EH, Pritchard CC. Microsatellite instability detection by next generation sequencing. *Clin Chem* 2014;60:1192–9.
17. Hampel H, Pearlman R, Beightol M, Zhao W, Jones D, Frankel WL, et al. Assessment of tumor sequencing as a replacement for Lynch syndrome screening and current molecular tests for patients with colorectal cancer. *JAMA Oncol* 2018;4:806–13.
18. Lemery S, Keegan P, Pazdur R. First FDA approval agnostic of cancer site - when a biomarker defines the indication. *N Engl J Med* 2017;377:1409–12.
19. Ten Broeke SW, van der Klift HM, Tops CMJ, Aretz S, Bernstein I, Buchanan DD, et al. Cancer risks for PMS2-associated Lynch syndrome. *J Clin Oncol* 2018;36:2961–8.
20. Vasen HFA, Blanco I, Aktan-Collan K, Gopie JP, Alonso A, Aretz S, et al. Revised guidelines for the clinical management of Lynch syndrome (HNPCC): recommendations by a group of European experts. *Gut* 2013;62:812–23.
21. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010;376:687–97.
22. Schwaederle M, Zhao M, Lee JJ, Lazar V, Leyland-Jones B, Schilsky RL, et al. Association of biomarker-based treatment strategies with response rates and progression-free survival in refractory malignant neoplasms: a meta-analysis. *JAMA Oncol* 2016;2:1452–9.

Cancer Epidemiology, Biomarkers & Prevention

Targeted Cancer Next-Generation Sequencing as a Primary Screening Tool for Microsatellite Instability and Lynch Syndrome in Upper Gastrointestinal Tract Cancers

Alexander G. Christakis, David J. Papke, Jonathan A. Nowak, et al.

Cancer Epidemiol Biomarkers Prev 2019;28:1246-1251. Published OnlineFirst April 26, 2019.

Updated version Access the most recent version of this article at:
doi:[10.1158/1055-9965.EPI-18-1250](https://doi.org/10.1158/1055-9965.EPI-18-1250)

Supplementary Material Access the most recent supplemental material at:
<http://cebp.aacrjournals.org/content/suppl/2019/04/26/1055-9965.EPI-18-1250.DC1>

Cited articles This article cites 22 articles, 5 of which you can access for free at:
<http://cebp.aacrjournals.org/content/28/7/1246.full#ref-list-1>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cebp.aacrjournals.org/content/28/7/1246>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.