Maria I. Carlo, MD
Semanti Mukherjee, PhD
Kenneth Offit, MD, MPH

Author Affiliations: Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York (Carlo, Offit); Sloan Kettering Institute, New York, New York (Mukherjee, Offit).

Corresponding Author: Maria I. Carlo, MD, Department of Medicine, Memorial Sloan Kettering Cancer Center, 353 E 68th St, New York, NY 10065 (carlom@mskcc.org).

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2. Mandelker D, Zhang L, Kemel Y, et al. Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal dna vs guideline-based germline testing. *JAMA*. 2017;318(9):825-835. doi:10.1001/jama.2017.11137

Analyzing Frequently Mutated Genes and the Association With Tumor Mutation Load

To the Editor In their recent article, Li et al[1] found that somatic mutations in *MUC16* were associated with high tumor mutation load (TML) and improved survival in patients with gastric cancer (GC). The authors concluded that their findings "may be immediately applicable for guiding immunotherapy treatment for patients with GC," giving the impression that *MUC16* mutations are special with regard to high TML and improved survival. However, *MUC16* mutations are more likely to be the result of high TML rather than the cause and do not have a predictive value for prognosis beyond that of other frequently mutated genes.

Any gene in the genome has a higher chance of being randomly mutated in tumors with high TML. The *MUC16* gene, which is the second longest gene and covers approximately 65 kb, has a reasonable chance of being mutated in tumors with high TML. In fact, when reanalyzing the Cancer Genome Atlas GC cohort used by Li et al,[1] 210 of the 212 genes mutated in more than 10% of patients with GC are significantly associated with higher TML (all with $P < 10^{-6}$, Wilcoxon signed rank test). This suggests that mutations in highly mutable genes are indicators, not drivers, of high TML.

This problem of determining significant associations occurs across many disciplines, and we recently adopted an approach,[2] first applied in the field of numerical ecology,[3] to perform association analyses while controlling for TML. Empirical *P* values are calculated by randomizing the mutations in a cohort while maintaining both the total number of mutations in each patient and each gene. Genes with mutations simply owing to random chance will have the same association with TML as in the random permutations, and hence will not be statistically significant. Applying this approach to the data used by Li et al, *MUC16* mutations are not significantly associated with high TML (empirical $P = .32$) or improved survival (23 of 100 randomized sets had log rank *P* values < .007 and 32 had lower Wilcoxon test *P* values; empirical $P = .23$).

Furthermore, when associating genes with survival using a naive approach (without controlling for TML), 25 of the 212 most frequently mutated genes (those mutated in >10% of patients reported on by Li et al[1]) are nominally associated with prolonged survival (log-rank comparison of patients with vs without a mutation in the gene; $P < .05$ for all 25 genes). Indeed, 6 genes (eg, *CDH23* and *ANK1*) were even more significant than *MUC16*, demonstrating that the association with survival is not unique to *MUC16*.

Given that almost all frequently mutated genes are associated with high TML and that the associations of *MUC16* mutations and improved survival with TML are not significant when controlling for the overall mutation load, we believe that *MUC16* is merely one of many long genes that can reflect high TML. There is no reason to assume from these analyses that *MUC16* has a special functional association with survival or a unique prognostic predictive value. Any further analyses regarding the functional role of mutations in *MUC16* or any other gene (eg, association with gene expression changes) in GC should test whether the mutations in the gene reflect high TML or selection owing to a functional role.

Yosef E. Maruvka, PhD
Nicholas J. Haradhvala, BA
Gad Getz, PhD

Author Affiliations: Cancer Center, Massachusetts General Hospital, Boston.

Corresponding Author: Gad Getz, PhD, Cancer Center, Massachusetts General Hospital, 55 Fruit St, Boston, MA 02114 (gadgetz@broadinstitute.org).

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1. Li X, Pasche B, Zhang W, Chen K. Association of *MUC16* mutation with tumor mutation load and outcomes in patients with gastric cancer. *JAMA Oncol*. 2018; 4(12):1691-1698. doi:10.1001/jamaoncol.2018.2805

2. Kim J, Mouw KW, Polak P, et al. Somatic ERCC2 mutations are associated with a distinct genomic signature in urothelial tumors. *Nat Genet*. 2016;48(6): 600-606. doi:10.1038/ng.3557

3. Strona G, Nappo D, Boccacci F, Fattorini S, San-Miguel-Ayanz J. A fast and unbiased procedure to randomize ecological binary matrices with fixed row and column totals. *Nat Commun*. 2014;5:4114. doi:10.1038/ncomms5114

In Reply Maruvka et al analyzed the associations of *MUC16* mutation with tumor mutation load (TML) and survival outcome in the Cancer Genome Atlas gastric cohort by using the Curveball algorithm[1] to randomize the mutation matrix without altering the row and column totals. In our view, their analysis underestimated the variability of genetic mutation in the cohort. Their interpretations of the conclusions of our study[2] may have resulted from some misunderstanding.

We agree that most frequently mutated genes are associated with high TML. A higher TML in the tumor genome is largely reflected by frequent mutation in genes. However, the association of *MUC16* mutation with TML does not indicate that *MUC16* mutation is the driver of high TML. We did not make this claim in our article.

Maruvka et al used the Curveball algorithm to permutate the mutation matrix by fixing the total number of mutations in each patient and each gene. The Curveball algorithm, as de-

scribed by Strona and colleagues,[1] was developed to randomize presence-absence matrices in numerical ecology by fixing row and column totals. Herein we demonstrate that the variability of the mutation matrix is likely to be underestimated when both margin totals are fixed. Let A and B denote 2 binary mutation matrices with identical dimension ordered by TML. The dissimilarity d of A and B was measured as 1 − sum(A and B) / sum($A|B$). For the binary mutation matrix of the Cancer Genome Atlas data set, we performed random permutation with the Curveball algorithm 1000 times and calculated the dissimilarity d between each permutated matrix and the original binary mutation matrix; the final dissimilarity vector was denoted as $d_{Curveball}$. In addition, we randomly sampled with replacement 2 matrices from the original binary mutation and calculated their dissimilarity. This procedure was also repeated 1000 times; we denoted the dissimilarity vector as $d_{sampling}$. The average value of $d_{Curveball}$ was significantly smaller than $d_{sampling}$ (t test, 0.83 vs 0.88; $P < .001$), which suggests that randomizing the binary mutation matrix with the row and column totals fixed cannot fully capture the variability of the original mutation matrix. Furthermore, the TML of a tumor also varies over the course of disease development and progression. Thus, application of the Curveball algorithm to examine the associations of gene mutation with TML and clinical phenotypes is rather conservative.

In our study,[1] the association of *MUC16* mutations with TML and improved survival in patients with gastric cancer was addressed for confounding factors in multivariate models including DNA mismatch repair signatures that can be used as surrogate variables for high TML. These associations were validated in the independent Asian cohort. The *CDH23* and *ANK1* genes mentioned by Maruvka et al were not associated with prognosis in the multivariate Cox analysis of the Asian cohort (hazard ratio [HR], 2.15; 95% CI, 0.55-8.30; $P = .27$ and HR, 2.15; 95% CI, 0.59-7.76; $P = .24$, respectively).

Different algorithms often give rise to different results. The association of *MUC16* mutations with high TML and improved survival obtained from traditional multivariate logistic and Cox models in our study should not be negated when the results were not significant based on the Curveball algorithm, especially when the application of this algorithm in such a circumstance still needs to be discussed. We would like to note that 2 recent studies reported the association of *MUC16* mutations with favorable prognosis in gastric cancer[3] and endometrial cancer by enhancing cytotoxic T-lymphocyte infiltration.[4]

Xiangchun Li, PhD
Wei Zhang, PhD
Kexin Chen, MD, PhD

Author Affiliations: Tianjin Medical University Cancer Institute and Hospital, Tianjin, China (Li); Comprehensive Cancer Center of Wake Forest Baptist Medical Center, Winston Salem, North Carolina (Zhang); Department of Epidemiology and Biostatistics, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China (Chen).

Corresponding Author: Kexin Chen, MD, PhD, Department of Epidemiology and Biostatistics, Tianjin Medical University Cancer Institute and Hospital, Huanhu Xi Road, Tiyuan Bei, Hexi District, Tianjin 300060, China (chenkexin@tjmuch.com).

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1. Strona G, Nappo D, Boccacci F, Fattorini S, San-Miguel-Ayanz J. A fast and unbiased procedure to randomize ecological binary matrices with fixed row and column totals. *Nat Commun*. 2014;5:4114.

2. Li X, Pasche B, Zhang W, Chen K. Association of *MUC16* mutation with tumor mutation load and outcomes in patients with gastric cancer. *JAMA Oncol*. 2018;4(12):1691-1698. doi:10.1001/jamaoncol.2018.2805

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4. Hu J, Sun J. *MUC16* mutations improve patients' prognosis by enhancing the infiltration and antitumor immunity of cytotoxic T lymphocytes in the endometrial cancer microenvironment. *Oncoimmunology*. 2018;7(10):e1487914. doi:10.1080/2162402X.2018.1487914

Omitted Disclosures of Potential Conflicts of Interest in Articles Published in *JAMA Oncology*

To the Editor I write to amend my disclosures of potential conflicts of interest in articles[1-11] published in *JAMA Oncology* in 2016 through 2018. In 1 of these articles, "Safety and Efficacy of Pembrolizumab Monotherapy in Patients With Previously Treated Advanced Gastric and Gastroesophageal Junction Cancer: Phase 2 Clinical KEYNOTE-059 Trial,"[8] which represented a clinical trial of a novel therapeutic in patients with gastric cancer, I reported relevant disclosures related to consulting work with the biotechnology and pharmaceutical industry focused on cancer drug development as follows: "Dr Fuchs has been a consultant for Eli Lilly, Entrinsic Health, Pfizer, Merck, Sanofi, Roche, Genentech, Merrimack Pharmaceuticals, Dicerna, Bayer, Agios, Gilead Sciences, Five Prime Therapeutics, and Taiho." However, that disclosure did not include my relationships at the time with Celgene and KEW. The other studies assessed the role of obesity, diet, lifestyle habits, and genetic mutations in cancer risk and outcome,[1-7,9-11] and for those articles I reported that I had no potential conflicts of interest to disclosure. With respect to these analyses of obesity, diet, lifestyle habits, and genetic mutations and cancer, I did not consider that my consulting work in drug development represented a potential conflict of interest or a relevant financial activity to the submitted works. Additionally, I did not believe that my consulting work would be perceived by readers to have influenced or give the appearance of potentially influencing analyses of obesity, diet, lifestyle habits, and genetic mutations in cancer risk and outcome.

Nevertheless, in the interest of full transparency, I wish to disclose all relationships with companies that produce products and services related to cancer care and to add the following disclosures to each of these articles[1-11]: "Dr Fuchs has been a consultant and/or a scientific advisor for Eli Lilly, Entrinsic Health, Pfizer, Merck, Sanofi, Roche, Genentech, Merrimack Pharmaceuticals, Dicerna, Bayer, Celgene, Agios, Gilead Sciences, Five Prime Therapeutics, Taiho, and KEW." Most of those disclosures were included in the article on pembrolizumab monotherapy for gastric cancer.[8] For that article, published on March 15, 2018, the following disclosure should also be added: "Dr Fuchs has been a consultant for Celgene and KEW and also serves on the Board of Directors for CytomX Therapeutics; in