Genomic Determinants of De Novo Resistance to Immune Checkpoint Blockade in Mismatch Repair–Deficient Endometrial Cancer

Doga C. Gulhan, PhD1; Elizabeth Garcia, PhD2; Elizabeth K. Lee, MD3; Neal I. Lindemann, MD2; Joyce F. Liu, MD3; Ursula A. Matulonis, MD3; Peter J. Park, PhD1; and Panagiotis A. Konstantinopoulos, MD, PhD3

INTRODUCTION

Despite the success of programmed death 1 (PD-1)/PD ligand 1 (PD-L1) inhibitors in mismatch repair–deficient (MMRD) endometrial cancer (EC), many patients exhibit de novo resistance.1,2 To identify determinants of resistance to immune checkpoint blockade (ICB) in MMRD EC, we evaluated genomic data from patients who were enrolled in an investigator-initiated clinical trial of avelumab.3 In that study, avelumab met the prespecified criteria to be considered worthy of additional investigation in MMRD EC with an objective response rate of 26.7%. Responses to avelumab were observed regardless of PD-L1 expression, the presence or absence of tumor-infiltrating lymphocytes, multiple prior lines of therapy, and somatic or germline origin of MMRD, which suggests that baseline clinical and pathologic characteristics could not predict response. Here, we report Janus kinase 1 (JAK1) and β2-microglobulin (B2M) mutations and a higher number of insertions and deletions (indels) and exposure to an MMRD-associated mutational signature—Signature 20 in the Catalogue Of Somatic Mutations In Cancer—as candidate genomic determinants of de novo resistance to ICB in MMRD EC.

PATIENTS AND METHODS

Formalin-fixed, paraffin-embedded samples were collected from patients enrolled in a phase II study of avelumab in mismatch repair–proficient (MMRP) and MMRD EC.3 Detailed information on sequencing and bioinformatic analyses is provided in the Data Supplement. The clinical trial was approved by the institutional review boards of all participating institutions and the US Food and Drug Administration (ClinicalTrials.gov identifier: NCT02912572). All procedures involving human participants were carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from patients before enrollment in the study as previously described.3

RESULTS

All Patients With MMRD EC Who Did Not Experience Response to Avelumab Harbored JAK1 or B2M Mutations

Of 15 patients in the MMRD cohort—determined by immunohistochemistry (IHC)—who initiated avelumab therapy, targeted sequencing via OncoPanel was performed on 12 tumors (as a result of tissue availability). Ten of the 12 tumors were determined to be MMRD by OncoPanel on the basis of mutational signature analysis using two independent algorithms,4,5 which was consistent with the IHC determination. The remaining 2 tumors were determined to be MMRP by OncoPanel and microsatellite stable using polymerase chain reaction—that is, both OncoPanel and polymerase chain reaction were discordant with IHC—and none of them responded to avelumab. Of note, both tumors had a low number of indel mutations—only 4 indels in the first tumor and 2 indels in the second—compared with 34.5 indel mutations, on average, in the 10 tumors with concordant IHC and OncoPanel findings. In addition, both tumors harbored TP53 mutations and extensive copy number alterations, rendering them most compatible with the copy number–high

| Patient ID | IHC Status | Histology | Stage at Diagnosis | Avelumab |
|------------|------------|-----------|--------------------|----------|
| 1          | MMRD      | Endometrioid | I                  | Responder |
| 2          | MMRD      | Endometrioid | I                  | Responder |
| 3          | MMRD      | Endometrioid | I                  | Responder |
| 4          | MMRD      | Endometrioid | IV                 | Nonresponder |
| 5          | MMRD      | Endometrioid | III                | Nonresponder |
| 6          | MMRD      | Endometrioid | III                | Nonresponder |
| 7          | MMRD      | Endometrioid | I                  | Nonresponder |
| 8          | MMRD      | Endometrioid | III                | Nonresponder |
| 9          | MMRD      | Endometrioid | I                  | Nonresponder |
| 10         | MMRD      | Endometrioid | I                  | Nonresponder |

Abbreviations: IHC, immunohistochemistry; MMRD, mismatch repair deficient.
subgroup of endometrial carcinomas which are distinct from MMRD tumors. Therefore, these 2 tumors were more likely to be MMRP and were excluded from the analysis.

Of the remaining 10 patients (Table 1) with tumors determined to be MMRD using both IHC and OncoPanel, 3 exhibited an objective response to avelumab (responders), whereas 7 did not (nonresponders). All 7 nonresponders harbored either \( JAK1 \) (6 tumors) or \( B2M \) mutations (1 tumor), while only 1 of the 3 responders harbored a \( JAK1 \) mutation (Fisher exact test, two-sided \( P = .067 \); Fig 1A). In addition, of the 7 nonresponders, 4 harbored two mutations of \( JAK1 \) (3 tumors) or \( B2M \) (1 tumor), possibly reflecting biallelic inactivation of these genes. Conversely, none of the 3 responders exhibited two mutations in either gene (Fisher exact test, two-sided \( P = .2 \)).

Type of mutation, allelic fraction, and position on \( JAK1 \) and \( B2M \) genes is shown in Figure 1A. All \( JAK1 \) mutations were frameshift (deletions or insertions), with the exception of two missense mutations that occurred together with frameshift mutations: a missense mutation Q750R on the pseudokinase domain (exon 16) and a missense mutation L1071P toward the end of the kinase domain (exon 23). Frameshift \( JAK1 \) mutations involved the hotspot position K860/P861 (deletions in 5 tumors and insertion in 1 tumor) and the hotspot position P430/L431 (insertions in 2 tumors). The sole tumor with mutation in \( B2M \) was a nonresponder that harbored two \( B2M \) mutations previously reported in The Cancer Genome Atlas (TCGA): a \( B2M \) c.68-2A>G splice-site mutation (19 of 10,953 patients in TCGA across all
tumor types) and a p.M1? mutation changing the start codon.

Frequency of JAK1 and B2M Mutations Was Higher Compared With TCGA Data

As shown in Figure 1B, the frequency of JAK1 and B2M mutations in our data set was higher compared with the frequency of these mutations among the MMRD ECs included in TCGA. For example, in our trial, 60% (6 of 10 MMRD patients) harbored JAK1 frameshift mutations involving the hotspot K860/P861 position compared with only 14.8% of patients with MMRD cancers in the TCGA EC data set (Fig 1B). Overall, 70% of patients (7 of 10) exhibited frameshift JAK1 mutations in our trial compared with only 23.8% of patients with MMRD cancers in the TCGA EC data set (Fig 1C). This difference may reflect the fact that patients with MMRD tumors included in the TCGA EC data set were all newly diagnosed EC cases, regardless of whether their tumors eventually recurred. On the contrary, our data set—that is, patients enrolled in the avelumab study for recurrent endometrial cancer—consisted solely of patients whose tumors recurred. Furthermore, in another data set (MSK-IMP), which included 29 patients with recurrent MMRD ECs (defined by IHC), the overall incidence of frameshift JAK1 mutations was 51.7%—significantly higher than that in the TCGA data set, but comparable with that in our data set (Fig 1C). A similar trend was also observed with B2M mutations (Fig 1C).

Number of Total Indel Mutations and Exposure to Mutational Signatures of MMRD

As shown in Figure 2A, nonresponders had a significantly higher number of total indels compared with responders (two-sided t test; \( P = .03 \); bootstrapping \( P = .05 \)). Nonresponders had a significantly higher number of total deletion mutations compared with responders (\( P = .03 \)), but the number of total insertion mutations was not different (Fig 2A). There was no difference in the total

---

**FIG 2.** Comparison of indel and mutation counts. (A) Number of indels, deletions, insertions, and tumor mutational burden, defined as the number of nonsynonymous mutations per Mb for responders (R) and nonresponders (NR) in our data set. (B) Same as panel A comparing patients with endometrial cancer (EC) in The Cancer Genome Atlas (TCGA) data set with and without frameshift JAK1 mutations. \( P \) values are calculated with double-sided \( t \) test and are shown in panels whenever they are \( < .05 \). fs, frameshift.
number of nonsynonymous mutations, also defined as tumor mutational burden (TMB), between nonresponders and responders (Fig 2A). To assess whether a higher number of total indels correlated with the presence of JAK1 mutations in MMRD EC, we evaluated this association in the TCGA data set. Indeed, the presence of JAK1 mutations in the MMRD tumors of the TCGA EC data set was associated with a significantly higher number of total indel mutations, number of deletions, and number of insertions, but not higher TMB (Fig 2B), which suggests that a higher number of indels, but not TMB, may be tracking the presence of JAK1 mutations in MMRD EC.

In addition, we assessed whether the presence of mutational signatures of MMRD, namely Signatures 6, 14, 15, 20, 21, and 26 in the Sanger COSMIC catalog, was associated with response of MMRD ECs to avelumab. As shown in Figure 3A and the Data Supplement, only Mutational Signature 20 was enriched in nonresponders compared with responders (two-sided t test $P = .009$; bootstrapping $P = .014$). The fraction of Signature 20 (Fig 3B) was also higher in nonresponders (fold change in mean value, 3.7; two-sided $t$ test $P = .08$; bootstrapping $P = .09$). Unlike total indel count, Mutational Signature 20 did not correlate with the presence of JAK1 mutations in MMRD ECs in the TCGA data set (Fig 3A), which suggests that the presence of Mutational Signature 20 may be tracking alternative mechanisms of resistance. Finally, as shown in Figure 3B, the mutational signature composition of the samples in our trial was different from that of the TCGA, with a higher fraction of T>C mutations (Signatures 20, 21, and 26) being enriched ($P < .0001$) in our trial.

**DISCUSSION**

Mutations in genes that are involved in the interferon signaling and antigen-presentation pathways are well-characterized mechanisms of resistance to ICB.\(^{11-13}\)
Abrogation of interferon-gamma signaling via loss-of-function mutations in JAK1 and JAK2 has been previously shown to allow escape from interferon-induced inhibition of growth and thus confer resistance to PD-1/ PD-L1 and cytotoxic T-lymphocyte–associated protein 4 blockade.1,11 Dysregulation of antigen-processing machinery via mutations in B2M—a gene involved in proper major histocompatibility complex class I folding and transport to the cell surface that is required for CD8 T-cell recognition—is another well-recognized mechanism of resistance to ICB.12 To our knowledge, this is the first study to report JAK1 and B2M mutations in association with response to ICB in MMRD EC and the first to report these as a mechanism of de novo—as opposed to acquired—resistance.

It is important to underscore that, outside of the context of response to ICB, frameshift JAK1 mutations have been previously reported to occur de novo in EC and have been functionally characterized to abrogate interferon-γ signaling as well as contribute to tumor immune evasion in this disease.14-16 In this regard, it was not surprising to find that the incidence of JAK1 mutations in our data set, which included only patients whose tumors eventually recurred, was significantly higher than that in the TCGA EC data set, which included all comers with newly diagnosed EC, suggesting that these mutations may be enriched in MMRD ECs which eventually recur.

Finally, exploratory analysis demonstrated a significantly higher number of total indels in avelumab nonresponders. It is well established that indel mutations contribute to the generation of neoantigens, which increase tumor immunogenicity and the likelihood of response to ICB.17 However, more indels also increase the likelihood that important genes, such as JAK1 and B2M, that are necessary for effective antitumor immune response may become truncated and thereby contribute to resistance to ICB. Taken together, whereas the presence of a higher number of indels in MMRD tumors compared with MMRP tumors explains their higher immunogenicity and response to ICB, a higher number of indels among MMRD tumors may drive the presence of JAK1 mutations and resistance to ICB.

**AFFILIATIONS**
1Harvard Medical School, Boston, MA
2Brigham and Women’s Hospital, Boston, MA
3Dana-Farber Cancer Institute, Boston, MA

**CORRESPONDING AUTHOR**
Panagiotis A. Konstantinopoulos, MD, PhD, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston, MA 02115; e-mail: panagiotis_konstantinopoulos@dfci.harvard.edu.

**SUPPORT**
Supported by the Ludwig Center at Harvard and by a fund in memory of Bina Sareen. Also supported by NIH U10CA180868 (PI Wolmark) and UM1 CA186709 (PI Shapiro and Kufe), both to P.A.K.

**AUTHOR CONTRIBUTIONS**
Conception and design: Neal I. Lindemann, Ursula A. Matulonis, Peter J. Park, Panagiotis A. Konstantinopoulos
Administrative support: Panagiotis A. Konstantinopoulos
Provision of study materials or patients: Joyce F. Liu, Ursula A. Matulonis, Panagiotis A. Konstantinopoulos
Collection and assembly of data: Neal I. Lindemann, Joyce F. Liu, Ursula A. Matulonis
Data analysis and interpretation: Doga C. Gulhan, Elizabeth Garcia, Elizabeth K. Lee, Neal I. Lindemann, Panagiotis A. Konstantinopoulos
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**
The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO’s conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Doga C. Gulhan
Patents, Royalties, Other Intellectual Property: A provisional patent application is being drafted for an algorithm developed by the author for which a coversheet provisional has been filed on September 24, 2018, titled “Computational method to identify mutational signatures from sequencing data” (Inst)

Joyce F. Liu
Consulting or Advisory Role: Tesaro, Mersana, Clovis Oncology, Genentech, GlaxoSmithKline
Research Funding: GlaxoSmithKline, AstraZeneca (Inst), Boston Biomedical (Inst), Atara Biotherapeutics (Inst), Acetylon (Inst), Bristol-Myers Squibb (Inst), Agenus (Inst), CytoMx Therapeutics (Inst), Regeneron (Inst), Tesaro (Inst), Clovis Oncology (Inst), Surface Oncology (Inst), Zol Oncology (Inst), Vigeo Therapeutics (Inst), Aravive (Inst), Arch Oncology (Inst)
Travel, Accommodations, Expenses: AstraZeneca, Merck
Uncompensated Relationships: Merck, AstraZeneca

Ursula A. Matulonis
Honoraria: Advaxis
Consulting or Advisory Role: Merck, Immunogen, Novartis
Research Funding: Merck, Novartis, Tesaro, Syndax, Immunogen, Mersana, Leap Therapeutics, Fujifilm, SQZ Biotechnologies
Travel, Accommodations, Expenses: AstraZeneca

Peter J. Park
Honoraria: Pfizer
Consulting or Advisory Role: Neuroinflammation Newco
Patents, Royalties, Other Intellectual Property: Patent for mutational signature-based detection of homologous recombination deficiency
REFERENCES

1. Aghajanian C, Sill MW, Darcy KM, et al: Phase II trial of bevacizumab in recurrent or persistent endometrial cancer: A Gynecologic Oncology Group study. J Clin Oncol 29:2259-2265, 2011
2. Alvarez EA, Brady WE, Walker JL, et al: Phase II trial of combination bevacizumab and temsirolimus in the treatment of recurrent or persistent endometrial carcinoma: A Gynecologic Oncology Group study. Gynecol Oncol 129:22-27, 2013
3. Konstantinopoulos PA, Luo W, Liu JF, et al: Phase II study of avelumab in patients with mismatch repair deficient and mismatch repair proficient recurrent/persistent endometrial cancer. J Clin Oncol 37:2786-2794, 2019
4. Nowak JA, Yurgelun MB, Bruce JL, et al: Detection of mismatch repair deficiency and microsatellite instability in colorectal adenocarcinoma by targeted next-generation sequencing. J Mol Diagn 19:84-91, 2017
5. Gulhan DC, Lee JJ, Melloni GEM, et al: Detecting the mutational signature of homologous recombination deficiency in clinical samples. Nat Genet 51:912-919, 2019
6. Soumerai TE, Donoghue MTA, Bandlamudi C, et al: Clinical utility of prospective molecular characterization in advanced endometrial cancer. Clin Cancer Res 24:5939-5947, 2018
7. Alexandrov LB, Nik-Zainal S, Wedge DC, et al: Signatures of mutational processes in human cancer. Nature 500:415-421, 2013 [Erratum: Nature 502:258, 2013]
8. Haradhvala NJ, Kim J, Maruvka YE, et al: Distinct mutational signatures characterize concurrent loss of polymerase proofreading and mismatch repair. Nat Commun 9:1746, 2018
9. Meier B, Volkova NV, Hong Y, et al: Mutational signatures of DNA mismatch repair deficiency in C. elegans and human cancers. Genome Res 28:666-675, 2018
10. Zou X, Owusu M, Harris R, et al: Validating the concept of mutational signatures with isogenic cell models. Nat Commun 9:1744, 2018
11. Gao J, Shi LZ, Zhao H, et al: Loss of IFN-gamma pathway genes in tumor cells as a mechanism of resistance to anti-CTLA-4 therapy. Cell 167:397-404.e9, 2016
12. Shin DS, Zaretsky JM, Escuin-Ordinas H, et al: Primary resistance to PD-1 blockade mediated by JAK1/2 mutations. Cancer Discov 7:188-201, 2017
13. Zaretsky JM, Garcia-Diaz A, Shin DS, et al: Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med 375:819-829, 2016
14. Albacker LA, Wu J, Smith P, et al: Loss of function JAK1 mutations occur at high frequency in cancers with microsatellite instability and are suggestive of immune evasion. PLoS One 12:e0176181, 2017
15. Ren Y, Zhang Y, Liu RZ, et al: JAK1 truncating mutations in gynecologic cancer define new role of cancer-associated protein tyrosine kinase aberrations. Sci Rep 3:3042, 2013
16. Stelloo E, Versluis MA, Nijman HW, et al: Microsatellite instability derived JAK1 frameshift mutations are associated with tumor immune evasion in endometrioid endometrial cancer. Oncotarget 7:39885-39893, 2016
17. Howitt BE, Shukla SA, Sholl LM, et al: Association of polymerase e-mutated and microsatellite-ablend endometrial cancers with neoantigen load, number of tumor-infiltrating lymphocytes, and expression of PD-1 and PD-L1. JAMA Oncol 1:1319-1323, 2015