OPEN ACCESS

Mismatch repair deficiency, next-generation sequencing-based microsatellite instability, and tumor mutational burden as predictive biomarkers for immune checkpoint inhibitor effectiveness in frontline treatment of advanced stage endometrial cancer

Breana L Hill,[1] Ryon P Graf,[2] Kunal Shah,[3] Natalie Danziger,[4] Douglas I Lin,[4] Julia Quintanilha,[5] Gerald Li,[5] James Haberberger,[6] Jeffrey S Ross,[4] Alessandro D Santin,[7] Brian Slomovitz,[8] Julia A Elvin,[4] Ramez N Eskander[1]

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/ijgc-2022-004026).

For numbered affiliations see end of article.

Correspondence to
Dr Breana L Hill, Department of Obstetrics, Gynecology and Reproductive Sciences, Division of Gynecologic Oncology, University of California San Diego Moores Cancer Center, La Jolla, CA 92093-0987, USA; blhill@health.ucsd.edu

BLH and RPG contributed equally.

Received 26 September 2022
Accepted 23 January 2023
Published Online First 7 February 2023

To cite: Hill BL, Graf RP, Shah K, *et al. Int J Gynecol Cancer* 2023;**33**:504–513.

ABSTRACT

Objective Molecular profiling is developing to inform treatment in endometrial cancer. Using real world evidence, we sought to evaluate frontline immune checkpoint inhibitor vs chemotherapy effectiveness in advanced endometrial cancer, stratified by Tumor Mutational Burden (TMB) ≥10 mut/MB and microsatellite instability (MSI).

Methods Patients with advanced endometrial cancer in the US-based de-identified Flatiron Health-Foundation Medicine Clinico-Genomic Database were included. Data originated from patients treated between January 2011- March 2022 at 280 US clinics. Next-generation sequencing assays were performed via FoundationOne or FoundationOneCDx. Longitudinal clinical data were derived from electronic health records. Immune checkpoint inhibitor treatment included pembrolizumab, dostarlimab, and nivolumab monotherapies. Time to next treatment, time to treatment discontinuation, and overall survival were assessed with the log-rank test and Cox proportional hazard models with adjusted hazard ratios (aHR) for known prognostic factors. We used the Likelihood ratio test to compare biomarker performance.

Results A total of 343 patients received chemotherapy and 28 received immune checkpoint inhibitor monotherapy as frontline treatment. Patients who received monotherapy were more likely to be stage III at diagnosis (immune checkpoint inhibitor: 54.6% vs chemotherapy: 15.0%; p<0.001) and more likely to test MSI-high via next-generation sequencing (immune checkpoint inhibitor: 53.6% vs chemotherapy: 19.2%; p<0.001). In MSI-high cancers, single-agent immune checkpoint inhibitor had a more favorable time to next treatment (aHR: 0.18, p=0.001) and overall survival (aHR 0.29, p=0.045). Additional analyses on 70 unique tumor specimens revealed mismatch repair deficiency (dMMR) via immunohistochemistry and MSI-high via next-generation sequencing concordance (91%), with nominal improvement of MSI over dMMR to predict time to treatment discontinuation (p=0.030), time to next treatment (p=0.032), and overall survival (p=0.22). MSI status was concordant with tumor mutational burden ≥10 in 94.3% of cases.

Conclusion Immune checkpoint inhibitors may have improved efficacy over chemotherapy in frontline treatment for advanced endometrial cancer defined by MSI-high using next-generation sequencing as a nominally better predictor of outcomes than dMMR with immunohistochemistry. This provides the biologic rationale of active phase III trials.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Biomarker-directed, FDA-approved immune checkpoint inhibitors exist as later-line treatment options in advanced endometrial cancer.

WHAT THIS STUDY ADDS

⇒ While limited by small sample size and its retrospective nature, this study found that single-agent frontline immune checkpoint inhibitor therapy use was associated with more favorable time to next treatment and overall survival compared with cytotoxic chemotherapy in MSI-high/tumor mutational burden-high advanced endometrial cancer in a retrospective data set with well-validated next-generation sequencing assays and survival endpoints. MSI-high by next-generation sequencing and dMMR by immunohistochemistry were highly concordant, and next-generation sequencing was a nominally better predictor of immune checkpoint inhibitor outcomes than immunohistochemistry.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These findings support the rationale of active biomarker-selected phase III trials, which are examining the utility of immune checkpoint inhibitors in the frontline setting.

INTRODUCTION

Endometrial cancer is the most common gynecologic malignancy in the United States and its incidence is on the rise worldwide.[1] In 2022, there were an estimated 65 950 new cases and 12 550 deaths from the disease in the United States alone.[1 2] While most women have early-stage disease associated with excellent outcomes, those with advanced disease reflect an unmet need with an estimated 5 year survival for stage IVA and stage IVB as 17% and 15%, respectively.[3] Additionally, recent data support disproportionately worse outcomes for minority patients that may be partially explained by molecular aberrations contributing to a more aggressive tumor biology.[4]

Molecular profiling and subtyping of endometrial cancer is becoming integral to informing treatment and prognostication. In 2013, the Cancer Genome Atlas (TCGA) described four molecular classes of endometrial cancer based on comprehensive molecular profiling.[3] These included polymerase e (POLE)-mutant/ultramutated, microsatellite instability (MSI), copy-number low, and copy-number high. Each class is associated with implications for histologic type and prognosis. In advanced endometrial cancer, 67–91% of patients have at least one actionable genomic alteration.[3] Among these molecular subtypes are two that are characterized by aberrations resulting in high tumor mutational burden: one with MSI and another by the presence of POLE mutations.[5]

MSI-high is found in approximately 30% of primary endometrial carcinomas and in an estimated 13–30% of recurrences, with rates significantly higher in endometrioid-type carcinomas compared with other histotypes.[6] Conversely, POLE mutations are found in 10% of all endometrioid-types and are defined by presentation at a younger age, earlier stage, and have good prognosis.[7] Currently, there are several methods to detect vulnerability to immune checkpoint inhibition in endometrial cancer: identifying deficiencies in mismatch repair protein expression by immunohistochemistry, measuring size of dinucleotide repeats at five selected loci by PCR, and by quantifying the amount of instability across hundreds of repeat loci or detecting an MMR COSMIC signature by next-generation sequencing-based comprehensive genomic profiling. dMMR and PCR methods have been widely utilized in the past but have limitations: dMMR by immunohistochemistry interpretation is subject to interobserver variability due to underrecognized patterns and/or background tissue staining and sampling limitations that miss tumors with subclonal loss, among others.[8 9] PCR methods require larger tumor specimen blocks than next-generation sequencing and may be inconclusive in patients with lower or difficult to assess tumor burdens.[10] In contrast, the relatively newer next-generation sequencing-based MSI-high detection methods have not been compared with traditional methods for clinical validity or utility for predicting the efficacy of immune checkpoint inhibitors in MSI-high endometrial cancer.

For frontline treatment of advanced or recurrent endometrial cancer, the combination of carboplatin and paclitaxel continues to be the standard of care, regardless of biomarker-defined molecular subtype.[11] Several studies have reported the promising efficacy of single-agent anti-PD1 and anti-PDL1 immunotherapy, predominantly in the second-line or later setting.[12–15] However, these agents have not been approved in the frontline treatment setting due to the lack of randomized trial data comparing outcomes against chemotherapy.

Using electronic health record data from diverse practices across the United States, we sought to compare the outcomes of patients treated with immune checkpoint inhibitors in the frontline setting with those treated with standard chemotherapy, stratified by both next-generation sequencing-based MSI assessment and tumor mutational burden level. Further, while dMMR and MSI are often concordant, they are not synonymous,[16] and understanding the implications of discordance on response to immune checkpoint inhibition was explored.[15 16]

METHODS

Patient Selection

This study comprised patients with confirmed diagnosis of advanced endometrial carcinoma or carcinosarcomas included in the US-wide Flatiron Health (FH)-Foundation Medicine (FMI) de-identified clinico-genomic database between January 2011 and March 2022. All underwent genomic testing using Foundation Medicine comprehensive genomic profiling assays. De-identified clinical data originated from approximately 280 US cancer clinics (800 sites of care).[17] Retrospective longitudinal clinical data were derived from electronic health records, comprising patient-level structured and unstructured data, curated via technology-enabled abstraction of clinical notes and radiology/pathology reports, which were linked to genomic data derived from Foundation Medicine testing by de-identified, deterministic matching.[18] Clinical data included demographics, clinical and laboratory features, timing of treatment exposure, and survival. MMR status was also abstracted from electronic health records. Specific assay details or expression loss details are unfortunately unavailable. Regimens considered single-agent immune checkpoint inhibitor treatment included pembrolizumab, dostarlimab, and nivolumab monotherapies. Any regimen containing platinum chemotherapy without an immune checkpoint inhibitor was considered chemotherapy. The reason for choice of therapy was unknown at the time of data collection.

Analyses were conducted in three cohorts (Figure 1). Frontline Comparative Effectiveness Cohort: Patients with advanced endometrial cancer who received either a single-agent immune checkpoint inhibitor or chemotherapy regimen as the frontline. Patients were excluded if they had no MSI call, clear cell histology, record gap, or did not receive platinum-based chemotherapy or an immune checkpoint inhibitor. dMMR and MSI Comparison Cohort: Patients who received an immune checkpoint inhibitor in any line of therapy and had records of dMMR, MSI, and tumor mutational burden testing. Patients with clear cell histology, no dMMR or MSI assessment, record gap, had follow-up of less than 1 week, or did not receive an immune checkpoint inhibitor were excluded from this cohort. Tumor mutational burden and MSI Comparison Cohort: Any patient with evaluable MSI and tumor mutational burden. Those without tissue biopsy or MSI call were excluded. Those with multiple lines of therapy with multiple tissue biopsies were counted once. Institutional Review Board approval was obtained before study conduct and included a waiver of informed consent.

Comprehensive Genomic Profiling

Hybrid capture-based next-generation sequencing assays were performed on patient tumor specimens in Clinical Laboratory Improvement Amendments–certified, College of American Pathologists (CAP)-accredited laboratory (Foundation Medicine, Cambridge, MA). Samples were evaluated for alterations as previously described.[19] Tumor mutational burden was determined on up

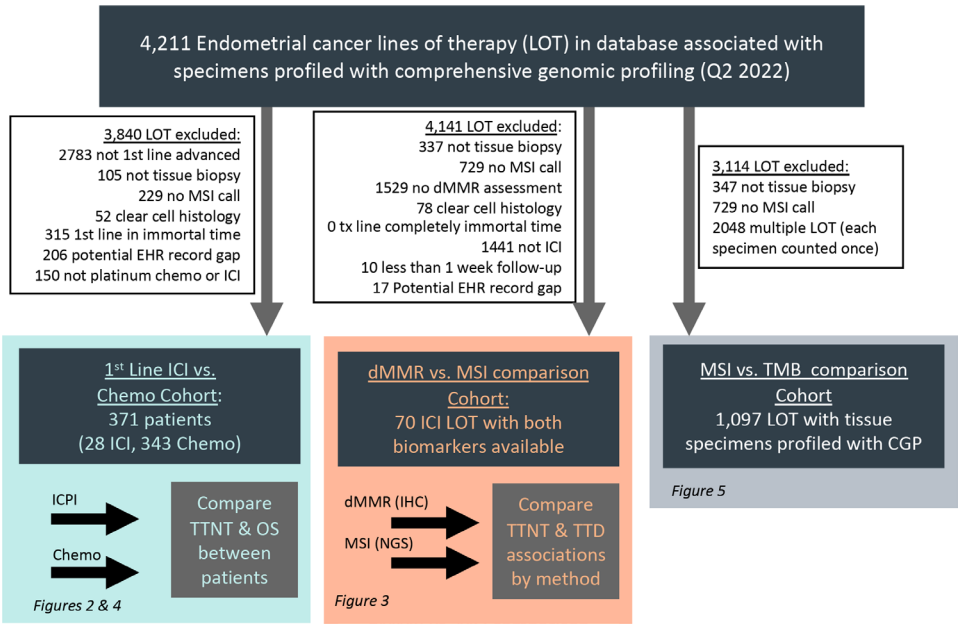

Figure 1 Cohort selection diagrams and analysis overviews. (MSI=microsatellite instability; EHR=electronic health records; ICI=immune checkpoint inhibitor; dMMR=mismatch repair deficiency; TMB=tumor mutational burden; TTNT=time to next treatment; OS=overall survival; TTD=time to treatment discontinuation; CGP=comprehensive genomic profiling)

to 1.1 Mb of sequenced DNA.[20] MSI status was determined by pre-specified analytical criteria, as previously described.[21 22]

Outcomes

Time to next treatment and time to treatment discontinuation are time-to-event proxies for drug clinical effectiveness.[23] Time to next treatment was calculated from treatment start date until the start of next treatment line. Patients not yet reaching next treatment line were right censored at date of last clinical visit, laboratory result, or medication order.[17] Patients who died before treatment switch were right censored. Time to treatment discontinuation was calculated from treatment start date until cessation of use of the life-prolonging treatment for any reason or cause. Patients not yet discontinuing treatment were right censored at date of last prescription or infusion. Overall survival was calculated from start of treatment to death from any cause, and patients without a record of mortality were right censored at the date of their last clinic visit or structured activity.[17] Because patients cannot enter the database until a comprehensive genomic profiling report is delivered, overall survival risk intervals were left truncated to the date of report to account for immortal time.[24 25] Flatiron Health database mortality information is a composite derived from three sources: the electronic health record, Social Security Death Index, and a commercial death dataset from obituaries and funeral homes. The mortality information has been externally validated in comparison to the National Death Index with >90% accuracy.[26]

Statistical Analysis and Interpretations

A prospectively declared statistical analysis plan was developed and executed. Consistent with ISPOR guidelines,[27] the inclusion criteria, exclusion criteria, potential biases, primary outcome measures, exploratory outcome measures, handling of missing data, and all methods described below were specified before analysis execution unless otherwise noted. The pre-specified analysis included comparing the effectiveness of immune checkpoint inhibitors vs chemotherapy in patients treated in the frontline setting stratified by MSI status, by tumor mutational burden <10 mut/Mb and tumor mutational burden ≥10 mut/Mb, and the effectiveness of patients receiving an immune checkpoint inhibitor by dMMR status vs MSI status. We hypothesized that patients with MSI-high or tumor mutational burden ≥10 would have comparable or improved outcomes with frontline immune checkpoint inhibitors over chemotherapy, but not when MSS or tumor mutational burden was <10. We also hypothesized that MSI assessed by next-generation sequencing would have better predictive ability to MMR assessed by immunohistochemistry.

Differences in time-to-event outcomes were assessed with the log-rank test and Cox proportional hazard models. Chi-square tests and Wilcoxon Rank Sum tests were used to assess differences between categorical and continuous variables, respectively. Multiple comparison adjustments were not performed; p-values are reported to quantify the strength of association for biomarker and each outcome, not for null hypothesis significance testing. Interpretations are adopted broadly considering consistency of multiple outcome measures in concert (time to treatment discontinuation, time to next treatment, overall survival) across defined cohorts with no outcome measure or cohort standing on its own.[17] The default interpretation is that a biomarker correlating with overall survival but not time to next treatment and time to treatment discontinuation within a cohort is likely a confounding artifact. A biomarker correlating with time to next treatment and time to treatment discontinuation but not overall survival is not more than nominally remarkable. This is because chemotherapy use in the frontline setting often has a maximum number of cycles. Time to treatment discontinuation was not used for comparisons involving chemotherapy.[17] Missing values were handled by simple imputation with expected values determined using random forests with the R package 'missForest.' In subsequent analyses, imputed values were treated identically to measured values.[17]

Table 1 Patient characteristics in front-line treatment groups

	MSI-H on chemo (n=66)	MSI-H on ICI (n=15)	MSS on chemo (n=277)	MSS on ICI (n=13)	Total (n=371)	P value
Age at Therapy Start						0.557
Median (Q1, Q3)	67.0 (60.0, 73.0)	68.0 (61.0, 72.5)	67.0 (61.0, 72.0)	71.0 (62.0, 73.0)	67.0 (60.0, 72.5)	
Practice Type						0.694
Academic	18 (27.3%)	6 (40.0%)	73 (26.4%)	3 (23.1%)	100 (27.0%)	
Community	48 (72.7%)	9 (60.0%)	204 (73.6%)	10 (76.9%)	271 (73.0%)	
Stage at Diagnosis						< 0.001
I	31 (47.0%)	7 (46.7%)	92 (33.2%)	4 (30.8%)	134 (36.1%)	
II	3 (4.5%)	1 (6.7%)	12 (4.3%)	0 (0.0%)	16 (4.3%)	
III	4 (6.1%)	7 (46.7%)	45 (16.2%)	8 (61.5%)	64 (17.3%)	
IV	22 (33.3%)	0 (0.0%)	117 (42.2%)	1 (7.7%)	140 (37.7%)	
Unknown/not documented	6 (9.1%)	0 (0.0%)	11 (4.0%)	0 (0.0%)	17 (4.6%)	
Distant Recurrence						0.285
No	5 (7.6%)	0 (0.0%)	22 (7.9%)	0 (0.0%)	27 (7.3%)	
Unknown	31 (47.0%)	4 (26.7%)	139 (50.2%)	6 (46.2%)	180 (48.5%)	
Yes	30 (45.5%)	11 (73.3%)	116 (41.9%)	7 (53.8%)	164 (44.2%)	
Histology						< 0.001
Carcinosarcoma	7 (10.6%)	1 (6.7%)	48 (17.3%)	4 (30.8%)	60 (16.2%	
Endometrial cancer, NOS	9 (13.6%)	1 (6.7%)	30 (10.8%)	0 (0.0%)	40 (10.8%)	
Endometrioid carcinoma	48 (72.7%)	11 (73.3%)	108 (39.0%)	3 (23.1%)	170 (45.8%	
Serous carcinoma	2 (3.0%)	2 (13.3%)	91 (32.9%)	6 (46.2%)	101 (27.2%)	
Race						0.392
Black or African American	≤6 (≤9%)	≤6 (≤40%)	44 (15.9%)	≤6 (≤46%)	51 (13.7%	
Other Race	11 (16.7%)	≤6 (≤40%)	47 (17.0%)	≤6 (≤46%)	61 (16.4%	
Unknown/not documented	≤6 (≤9%)	≤6 (≤40%)	25 (9.0%)	≤6 (≤46%)	32 (8.6%)	
White	44 (66.7%)	13 (86.7%)	161 (58.1%)	9 (69.2%)	227 (61.2%	
ECOG Score						0.743
0	18 (27.3%)	5 (33.3%)	101 (36.5%)	5 (38.5%)	129 (34.8%	
1	23 (34.8%)	5 (33.3%)	102 (36.8%)	3 (23.1%)	133 (35.8%	
2+	9 (13.6%)	1 (6.7%)	26 (9.4%)	1 (7.7%)	37 (10.0%	
Unknown	16 (24.2%)	4 (26.7%)	48 (17.3%)	4 (30.8%)	72 (19.4%	
dMMR by IHC						< 0.001
Loss	29 (100.0%)	12 (100.0%)	4 (2.9%)	1 (10.0%)	46 (24.6%	
Normal	0 (0.0%)	0 (0.0%)	132 (97.1%)	9 (90.0%)	141 (75.4%	
Missing Observations	37	3	141	3	184	
Opioid Use Pre-Therapy						0.606
No	42 (63.6%)	10 (66.7%)	197 (71.1%)	8 (61.5%)	257 (69.3%)	
Yes	24 (36.4%)	5 (33.3%)	80 (28.9%)	5 (38.5%)	114 (30.7%)	
TMB						< 0.001
Median (Q1, Q3)	21.3 (16.5, 31.1)	22.5 (19.4, 27.5)	2.5 (1.3, 5.0)	1.3 (0.9, 2.5)	3.8 (1.3, 10.0)	
MSI by NGS						< 0.001
MSI-H	66 (100.0%)	15 (100.0%)	0 (0.0%)	0 (0.0%)	81 (21.8%)	
MSS	0 (0.0%)	0 (0.0%)	277 (100.0%)	13 (100.0%)	290 (78.2%)	
PDL1						0.005
TPS 0	11 (16.7%)	2 (13.3%)	44 (15.9%)	1 (7.7%)	58 (15.6%)	
TPS 1–19	3 (4.5%)	1 (6.7%)	16 (5.8%)	2 (15.4%)	22 (5.9%)	
TPS 20+	0 (0.0%)	0 (0.0%)	3 (1.1%)	2 (15.4%)	5 (1.3%)	

Continued

Table 1 Continued

	MSI-H on chemo (n=66)	MSI-H on ICI (n=15)	MSS on chemo (n=277)	MSS on ICI (n=13)	Total (n=371)	P value
unknown	52 (78.8%)	12 (80.0%)	214 (77.3%)	8 (61.5%)	286 (77.1%)	
POLE Mutation						0.49
Negative	66 (100.0%)	15 (100.0%)	270 (97.5%)	13 (100.0%)	364 (98.1%)	
Positive	0 (0.0%)	0 (0.0%)	7 (2.5%)	0 (0.0%)	7 (1.9%)	
POLD1 Mutation						< 0.001
Negative	66 (100.0%)	15 (100.0%)	277 (100.0%)	13 (100.0%)	371 (100.0%)	
TP53 Mutation						< 0.001
Negative	51 (77.3%)	12 (80.0%)	104 (37.5%)	2 (15.4%)	169 (45.6%	
Positive	15 (22.7%)	3 (20.0%)	173 (62.5%)	11 (84.6%)	202 (54.4%)	
CTNNB1 mutation						0.252
Negative	48 (72.7%)	10 (66.7%)	220 (79.4%)	12 (92.3%)	290 (78.2%)	
Positive	18 (27.3%)	5 (33.3%)	57 (20.6%)	1 (7.7%)	81 (21.8%)	
BMI						0.238
Median (Q1, Q3)	29.4 (22.1, 35.8)	26.2 (21.0, 32.1)	30.4 (25.3, 36.8)	29.2 (24.7, 37.9)	30.2 (24.7, 36.6)	
Missing Observations	1	2	13	0	16	
Subsequent second -line treatment						< 0.001
Chemo	4 (6.1%)	1 (6.7%)	60 (21.7%)	3 (23.1%)	68 (18.3%	
Hormonal tx without chemo	3 (5.0%)	2 (14.3%)	32 (12.1%)	0 (0.0%)	37 (10.6%)	
ICI	29 (43.9%)	0 (0.0%)	51 (18.4%)	2 (15.4%)	82 (221%)	
Other	33 (50.0%)	14 (93.3%)	166 (59.9%)	8 (61.5%)	221 (59.6%)	

BMI, Body Mass Index; chemo, chemotherapy; dMMR, mismatch repair deficiency; ECOG, Eastern Cooperative Oncology Group; ICI, Immune Checkpoint Inhibitor; IHC, Immunohistochemistry; MSI-H, microsatellite instability-high; MSS, microsatellite stable; NOS, Not otherwise specified; PDL1, Programmed cell-death ligand 1; POLD, polymerase d; POLE, polymerase e; Q1, first quarter; Q3, third quarter; TMB, Tumor Mutational Burden; TPS, Tumor Proportion Score.

To adjust for differences in risk of death among patients treated with immune checkpoint inhibitors compared with chemotherapy, pre-therapy prognostic features associated with risk of death (Eastern Cooperative Oncology Group (ECOG) score, age, academic vs community treatment setting, body mass index, stage at diagnosis, opioid use, TP53 and CTNNB1 mutation status, alkaline phosphatase levels, albumin levels, hemoglobin levels) among patients treated with chemotherapy were combined into a multivariable model (online supplemental Figure 1), as previously described.[28 29] The resulting sum of coefficients (online supplemental Figure 3) was applied to the group receiving chemotherapy, as and separately to the "held out" group of patients receiving an immune checkpoint inhibitor. This risk score was used for adjustment in all Cox proportional hazard models and reported out as an adjusted hazard ratio (aHR).

Comparison of predictive biomarker performance was evaluated using the likelihood ratio test.[30] R version 3.6.3 software was used for all statistical analyses.

RESULTS

Characteristics of Analysis Cohorts

Frontline Comparative Effectiveness Cohort: After selection, 343 patients received frontline chemotherapy, and 28 patients received frontline immune checkpoint inhibitor monotherapy (Figure 1, Table 1). Compared with patients receiving chemotherapy, patients receiving immune checkpoint inhibitor monotherapy were less likely to be stage IV at diagnosis (4.0% vs 40.5%), more likely stage III at diagnosis (53.6% vs 14.3%), and more likely to test MSI-high (53.6% vs 19.2%, p<0.001). (Table 1).

dMMR and MSI Comparison Cohort: After selection, 70 patients had dMMR immunohistochemistry status abstracted from electronic health records, as well as MSI and tumor mutational burden testing via next-generation sequencing. Of these, 20 (29%), 25 (36%), and 25 (36%) were treated in first, second, and third+lines of therapy, and 20 (29%), 26 (37%), 9 (13%), and 15 (21%) had ECOG 0, 1, 2+, unknown respectively (online supplemental Table, Figure 1).

Tumor mutational burden and MSI Comparison Cohort: After filtering, 1097 unique tissue specimens were evaluable for tumor mutational burden and MSI (Figure 1).

Real World Patients Receiving Frontline Immune Checkpoint Inhibitor Monotherapy versus Chemotherapy Have Favorable Outcomes when MSI-H by Next-generation Sequencing but not MSS

Among patients treated with single-agent immune checkpoint inhibitor vs chemotherapy in frontline, those with MSI-high as assessed by next-generation sequencing had more favorable time to next treatment (aHR: 0.18, 95% CI: 0.06 to 0.52, p=0.001) and overall survival (aHR: 0.29, 95% CI: 0.09 to 0.97, p=0.045)

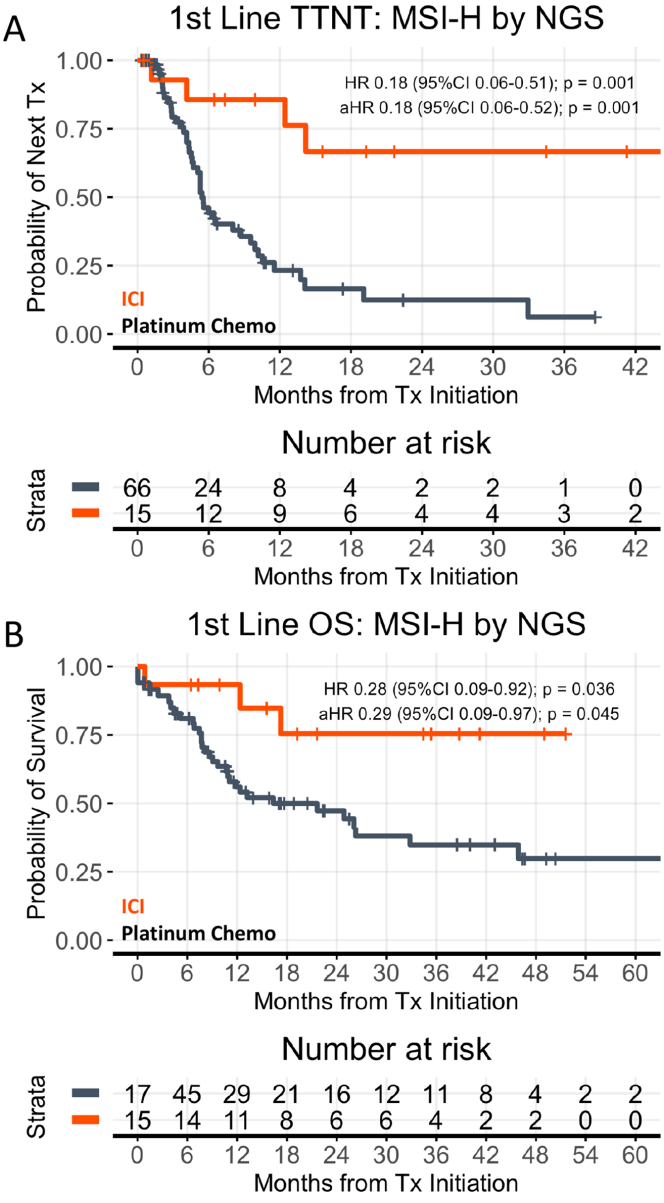

Figure 2 Outcomes of front-line treatment. (A)Time to next treatment (TTNT) and (B)Overal survival (OS) is shown by drug class. Overall survival estimates are left truncated (see Methods) with at-risk tables adjusted accordingly.

(Figure 2). Analyses unadjusted for imbalances show similar results. Stratifying patients by tumor mutational burden ≥10 instead of MSI had similar associations (Figure 3).

dMMR by Immunohistochemistry and MSI by Next-generation Sequencing are Highly Concordant, but Favor MSI for the Limited Number of Discordant Cases

Among patients evaluated for immune checkpoint inhibitor effectiveness (any line of therapy), 58 of 64 (91%) had concordant calls (Figure 4). While highly concordant, the addition of MSI to a Cox model evaluating only dMMR resulted in nominal improvement to predict time to treatment discontinuation (p=0.030), time to next treatment (p=0.032) and while not statistically significant, overall survival (p=0.22). However, if the model instead started with MSI, with the addition of MMR, similar improvement was seen for time to treatment discontinuation (p=0.68), time to next treatment (p=0.88)

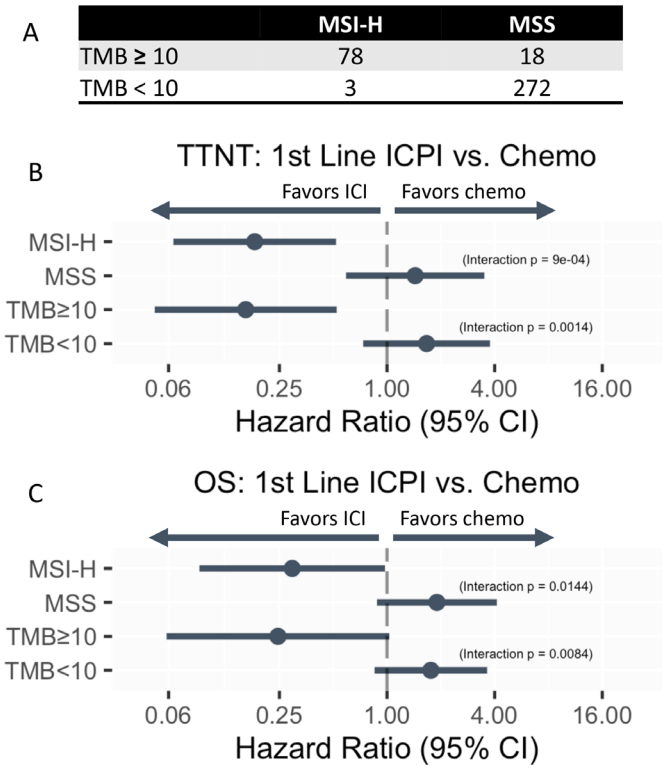

Figure 3 Micosatellite instability-high (MSI-high) concordance with tumor mutational burden (TMB≥10). (A). Comparison of adjusted* subgroups and interaction test p-values for (B)Time to next treatment (TTNT) and (C) Overall survival (OS). *Adjusted for Age at treatment start, Eastern Cooperative Oncology Group (ECOG), Academic vs Community setting, Body Mass Index, Stage at diagnosis, opioid use pre-therapy, tumor protein 53 (TP53) mutation status, and catenin beta 1 (CTNNB1) mutation status. Chemo, chemotherapy; ICPI, immune checkpoint inhibitor; MSS, microsatellite stability.

and overall survival (p=0.99). Limited benefit from an immune checkpoint inhibitor was observed for the five dMMR/MSS, while the one patient who was pMMR/MSI-high was continuing fourth-line immune checkpoint inhibitor after 37.8 months (Figure 4).

In Frontline Cohort, MSI-high Status is Highly Concordant with Tumor Mutational Burden ≥ 10, with Similar Outcomes and Strength of Interactions

Among the 371 patients evaluated for frontline drug effectiveness, 350 (94.3%) had concordant calls between MSI status and tumor mutational burden ≥10 status (Figure 3A). Using p-values as a proxy for strength of association, interaction tests by MSI status and tumor mutational burden ≥10 status were similar for time to next treatment (p=0.0009 and 0.0014) and overall survival (p=0.0144 and p=0.0084) (Figure 3B,C). However, addition of tumor mutational burden to a Cox model already containing MSI did not result in improvement of the model to predict time to treatment discontinuation, time to next treatment, or overall survival (data not shown). Only one patient who received an immune checkpoint inhibitor had a discordant call, being MSI-high and tumor mutational burden of 3.75 mut/Mb, had time to next treatment of 1.2 months, and overall

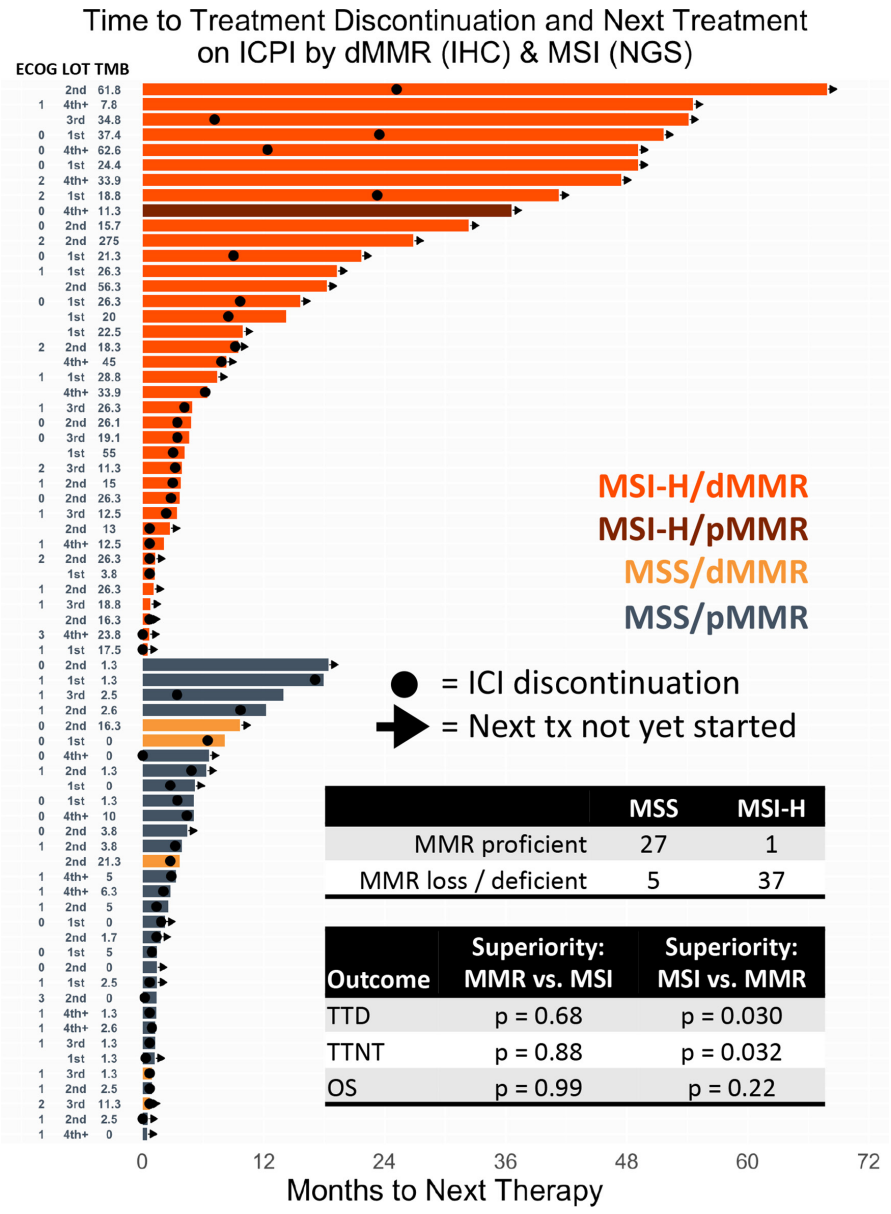

Figure 4 Mismatch repair deficiency (dMMR) by immunohistochemistry (IHC) and microsatellite instability (MSI) by next-generation sequencing (NGS). Concordance. Swimmer plot shows time to next treatment for all immune checkpoint inhibitor (ICPI,ICI) lines of therapy with more than 1 week of follow-up. TTD, time to treatment discontinuation; TTNT, time to next treatment; Tx. therapy; OS, overall survival.

survival of 17.3 months. Full interaction models are provided in online supplemental Figure 4.

MSI, POLE, and POLD1 are Independently Associated with Tumor Mutational Burden

Among evaluable specimens in the Tumor Mutational Burden Comparison Cohort, the prevalence of specimens tested with MSS and tumor mutational burden ≥10 was infrequent. A minority of specimens were MSS with exceptionally high tumor mutational burden, and all of these had either *POLE* or *POLD1* mutations (Figure 5). Multivariable evaluation of tumor mutational burden associated with MSI, *POLE*, *POLD1*, and clinicopathologic features revealed strongest associations with MSI, *POLE*, and *POLD1* (all p<0.001, online supplemental Figure 2). The strongest clinical feature associated with tumor mutational burden was timing of specimen acquisition; specimens obtained at time of diagnosis independently had lower tumor mutational burden than those that were not (p=0.003).

DISCUSSION

Summary of Main Results

We found that patients who were MSI-high by next-generation sequencing had more favorable time to next treatment and overall survival when treated with frontline immune checkpoint inhibitor monotherapy over chemotherapy. Those who received an immune checkpoint inhibitor were more likely to have stage III disease and be dMMR/MSI-high, suggesting these characteristics likely influenced treatment selection.

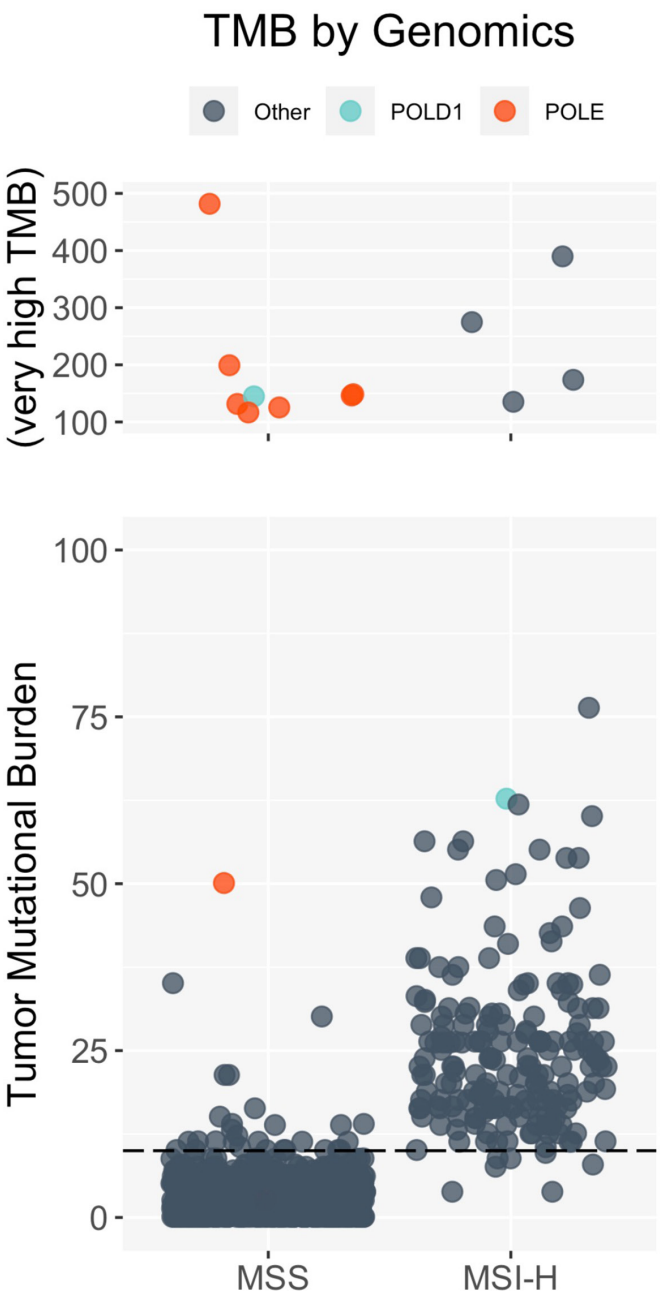

Figure 5 High TMB relationship to MSI or POLE or POLD mutations. Split graph beehive plot displays the TMB level per tissue specimen in the database with evaluable MSI status. Dashed line indicates 10 mut/Mb. ;MSI-H, microsatellite instability high; MSS, microsatellite stable; POLD, polymerase d; POLE, polymerase e; TMB=tumor mutational burden.

dMMR detected by immunohistochemistry and MSI-high measured by next-generation sequencing were highly concordant. However, results favored next-generation sequencing-based MSI-high in cases of discordance. Five of six discordances were MSS and dMMR, and one pMMR/MSI-high. Within the MSS/dMMR cohort, immune checkpoint inhibitors showed no durable efficacy. In contrast, the one patient with pMMR/MSI-high and tumor mutational burden of 11.3 mut/Mb has not received treatment following fourth-line immune checkpoint inhibitor therapy and is alive at 37.8 months.

Results in the Context of Published Literature

While the cohort is small, our observations are consistent with MSI by next-generation sequencing as having incremental improvement over dMMR by immunohistochemistry. Additionally, we hope future trials report the proportion of discordance as a subgroup analysis to better confirm this finding. MSI-high status is highly concordant with tumor mutational burden ≥10 status, with similar outcomes and improved response to immune checkpoint inhibitors when compared with chemotherapy. This supports the clinical validity of tumor mutational burden and MSI-high/dMMR as biomarkers to identify patients with advanced endometrial cancer who may benefit from immune checkpoint inhibitor therapy in future phase III clinical trials.

Although many studies have assessed the benefit of biomarker-targeted immunotherapy for treatment of advanced endometrial cancer, this study is the first to our knowledge to describe real world clinical data of biomarker driven frontline immune checkpoint inhibitor monotherapy. KEYNOTE-158, which demonstrated remarkable efficacy of pembrolizumab in dMMR patients with previously treated recurrent or metastatic endometrial cancer, resulted in the first disease site agnostic drug approval by the US FDA in May 2017.[31] Furthermore, three separate phase II trials were completed, confirming the efficacy of single agent immune checkpoint inhibitor in a biomarker selected dMMR population with recurrent disease.[13 32 33]

A recent College of American Pathologists guideline on biomarker testing cited a lack of studies comparing MMR by immunohistochemistry vs MSI (by any method) with respect to their predictive ability for immune checkpoint inhibitor response.[34] To our knowledge, our study is the first to report these analyses to predict outcomes of immune checkpoint inhibitor therapy in patients with endometrial cancer. Given the established biologic rationale to support the replacement of frontline chemotherapy, the utility of single agent immune checkpoint inhibitors in the endometrial cancer space is now being investigated.[35]

Strengths and Weaknesses

The strengths of our study include our examination of outcomes of immune checkpoint inhibitor monotherapy vs chemotherapy in the frontline treatment of advanced endometrial cancer. Additionally, our dataset provides clinical rationale supporting the actively accruing phase III MK-3475-C93/KEYNOTE-C93/GG-3064/ENGOT-en15 trial (NCT05173987). Another strength is while we recognize that tumor mutational burden calculation can vary considerably by panel size, gene content and bioinformatic filtering,[36] we used the only FDA-approved companion diagnostic test and threshold.

We took multiple steps to address the limitations of our dataset. There is no central pathologic confirmation, and some included institutions lack immunohistochemistry assessment (rendering us unable to report MMR status). Retrospective analyses are limited by variable patient follow-up and inadequately validated endpoints. However, our study required stringent completeness of patient visit records and used a well-validated overall survival endpoint. Treatment assignments were at the clinician's discretion, and while biases were considered and adjusted, unknown confounders may remain. Time-to-event measures are sensitive to imbalances in overall patient frailty and disease severity. We did not have direct measures of total disease burden at times of treatment. While correlated factors like ECOG and stage at diagnosis were included

in adjustments, these are imperfect proxies for patient frailty and disease extent. Randomized controlled trials will better adjust for these and other factors potentially biasing outcome assessments. Additionally, our dataset does not distinguish germline from somatic mutations or *MLH1* promoter hypermethylation although treatment guidance would remain the same.

Implications for Practice and Future Research

Our study provides additional rationale for immune checkpoint inhibitors as monotherapy in dMMR or next-generation sequencing-based MSI-high advanced stage endometrial cancers. While conclusions are limited by sample size and retrospective nature, we eagerly await the results of the phase III MK-3475-C93/KEYNOTE-C93/GG-3064/ENGOT-en15 trial (NCT05173987), which will randomize approximately 350 patients with dMMR recurrent or metastatic endometrial cancers to receive either frontline pembrolizumab or carboplatin with paclitaxel.[35] This was examined in KEYNOTE-177, a phase III trial of 307 patients with untreated metastatic MSI-high or dMMR colorectal cancer. At the second interim analysis, pembrolizumab was superior to chemotherapy with respect to progression-free survival, and in the final analysis, median overall survival was not reached with pembrolizumab (vs 36.7 months with chemotherapy).[37 38] Our results shared above are informative and suggest there may be benefit in immune checkpoint inhibitors replacing chemotherapy in the frontline management of advanced endometrial cancer, which may be validated by the much-anticipated randomized control trial.

Future research will also look to examine potential differential response to immune checkpoint inhibition in epigenetically driven dMMR/MSI-high, compared with patients with somatic mutations (Lynch-like) or germline alterations (Lynch syndrome). While database assessments have their limitations, the rigor applied here for cohort filtering, establishing endpoint validity, and methodology creates a substantial foundation that may complement evidence from future trials.

CONCLUSIONS

In a biomarker-selected advanced stage endometrial cancer patient population, frontline immune checkpoint inhibitors may have improved efficacy over standard of care cytotoxic chemotherapy. While limited by small sample size and retrospective nature, our study suggests that MSI testing via next-generation sequencing provides incremental value over dMMR by immunohistochemistry and additionally supports the clinical rationale of the active phase III MK-3475-C93/KEYNOTE-C93/GG-3064/ENGOT-en15 trial (NCT05173987). We eagerly await the results of this important trial, which has practice-defining implications.

Author affiliations
[1]Department of Obstetrics, Gynecology and Reproductive Sciences, Division of Gynecologic Oncology, University of California San Diego Moores Cancer Center, La Jolla, California, USA
[2]Department of Clinical Development, Medical Team, Foundation Medicine Inc, San Diego, California, USA
[3]Department of Data & Insights Delivery, Foundation Medicine Inc, Cambridge, Massachusetts, USA
[4]Department of Pathology and Diagnostic Medicine, Medical Team, Foundation Medicine Inc, Cambridge, Massachusetts, USA
[5]Department of Clinical Development, Medical Team, Foundation Medicine Inc, Cambridge, Massachusetts, USA
[6]Department of Pathology and Diagnostic Medicine, Medical Team, Foundation Medicine Inc, Morrisville, North Carolina, USA
[7]Department of Obstetrics, Gynecology, and Reproductive Sciences, Division of Gynecologic Oncology, Yale School of Medicine, New Haven, Connecticut, USA
[8]Division of Gynecologic Oncology, Mount Sinai Medical Center, Miami Beach, Florida, USA

Correction notice This article has been corrected since it was first published online, to correct author name Ramez N Eskander.

Twitter Douglas I Lin @DougLinMD

Acknowledgements We thank the patients whose data made this research possible, the clinical and laboratory staff at Foundation Medicine, and the team at Flatiron Health.

Contributors All below stated persons are considered author contributors. We intend joint first authorship between Authors Breana L. Hill and Ryon P. Graf, who contributed equally. Ryon Graf is responsible for the overall content as guarantor. Details of authorship are as follows: Breana L. Hill, MD*- Conceptualization, Validation, Writing-Original draft, Review & Editing. Ryon P. Graf; PhD*- Conceptualization, Methodology, Validation, Investigation, Writing-Original draft, Review and editing, Visualization. Kunal Shah, BS - Conceptualization, Methodology, Validation, Formal analysis, Writing-Review and editing, Visualization. Natalie Danziger, BS - Conceptualization, Methodology, Validation, Formal analysis, Writing-Original draft, Review and editing, Visualization. Julia Quintanilha, PhD - Methodology, Validation, Writing-Review and editing, Visualization. Gerald Li, PhD - Methodology, Validation, Writing-Review & Editing; Douglas I. Lin, MD, PhD; James Haberberger, BS, Jeffrey S. Ross, MD, Alessandro D. Santin, MD, and Brian Slomovitz, MD-Validation, Writing-Review & Editing. Julia A. Elvin, MD, PhD and Ramez N. Eskander, MD - Conceptualization, Validation, Writing-Review & Editing, Supervision.

Funding Foundation Medicine, a wholly owned subsidiary of Roche, is a for-profit company and producer of FDA-regulated molecular diagnostics. Roche produces atezolizumab, an ICI. Authors employed by Foundation Medicine were involved in the design and conduct of the study, analysis, interpretation of the data, preparation, review, and approval of the manuscript.

Competing interests Ryon P. Graf, PhD; Kunal Shah, BS; Natalie Danziger, BS; Douglas I. Lin, MD, PhD, Julia Quintanilha, PhD; Gerald Li, PhD; Julia Elvin, MD, PhD are employees of Foundation Medicine, a wholly owned subsidiary of Roche, and have equity interest in Roche. Roche produces atezolizumab, an ICI. Alessandro D. Santin receives grants from Puma, Immunomedics, Gilead, Synthon, Merck, Boehinger-Ingelheim, Genentech, Eisai, and Tesaro; and also personal fees from Merck, Tesaro, and Eisai. Brian Slomovitz has consultant roles with AstraZeneca, Clovis, GSK, Genentech, Lilly, Merck, Eisai, Genmab, Novartis, Addy, Immunogen. Ramez Eskander reports the following: consulting, advisory, and grant funding from AstraZeneca, Clovis Oncology, Genentech, Merck, Eisai, Genmab, Seagen, Immunogen, Mersana, Myriad. No other authors have conflicts of interest to report.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

REFERENCES

1 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020;70:7–30.
2 Siegel RL, Miller KD, Fuchs HE, *et al*. Cancer statistics, 2022. *CA Cancer J Clin* 2022;72:7–33.
3 Green AK, Feinberg J, Makker V. A review of immune checkpoint blockade therapy in endometrial cancer. *Am Soc Clin Oncol Educ Book* 2020;40:1–7.
4 Javadian P, Washington C, Mukasa S, *et al*. Histopathologic, genetic and molecular characterization of endometrial cancer racial disparity. *Cancers (Basel)* 2021;13:1900.
5 Cao W, Ma X, Fischer JV, *et al*. Immunotherapy in endometrial cancer: rationale, practice and perspectives. *Biomark Res* 2021;9:49.
6 Lin DI, Fine A, Danziger NA, *et al*. Molecular analysis of endometrial serous carcinoma reveals distinct clinicopathologic and genomic subgroups. *Gynecol Oncol* 2022;164:558–65.
7 Vermij L, Smit V, Nout R, *et al*. Incorporation of molecular characteristics into endometrial cancer management. *Histopathology* 2020;76:52–63.
8 Jaffrelot M, Farés N, Brunac AC, *et al*. An unusual phenotype occurs in 15 % of mismatch repair-deficient tumors and is associated with non-colorectal cancers and genetic syndromes. *Mod Pathol* 2022;35:427–37.
9 Ta RM, Hecht JL, Lin DI. Discordant loss of mismatch repair proteins in advanced endometrial endometrioid carcinoma compared to paired primary uterine tumors. *Gynecol Oncol* 2018;151:401–6.
10 Shimozaki K, Hayashi H, Tanishima S, *et al*. Concordance analysis of microsatellite instability status between polymerase chain reaction based testing and next generation sequencing for solid tumors. *Sci Rep* 2021;11:20003.
11 Miller DS, Filiaci VL, Mannel RS, *et al*. Carboplatin and paclitaxel for advanced endometrial cancer: final overall survival and adverse event analysis of a phase III trial (NRG oncology/GOG0209). *J Clin Oncol* 2020;38:3841–50.
12 Marabelle A, Fakih M, Lopez J, *et al*. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *Lancet Oncol* 2020;21:1353–65.
13 Marabelle A, Le DT, Ascierto PA, *et al*. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the phase II KEYNOTE-158 study. *J Clin Oncol* 2020;38:1–10.
14 Oaknin A, Tinker AV, Gilbert L, *et al*. Clinical activity and safety of the anti-programmed death 1 monoclonal antibody dostarlimab for patients with recurrent or advanced mismatch repair-deficient endometrial cancer: a nonrandomized phase 1 clinical trial. *JAMA Oncol* 2020;6:1766–72.
15 Azad NS, Gray RJ, Overman MJ, *et al*. Nivolumab is effective in mismatch repair-deficient noncolorectal cancers: results from arm Z1D-A subprotocol of the NCI-MATCH (EAY131) study. *J Clin Oncol* 2020;38:214–22.
16 Malapelle U, Parente P, Pepe F, *et al*. Evaluation of micro satellite instability and mismatch repair status in different solid tumors: a multicenter analysis in a real world setting. *Cells* 2021;10:1878.
17 Graf RP, Fisher V, Creeden J, *et al*. Real-World validation of TMB and microsatellite instability as predictive biomarkers of immune checkpoint inhibitor effectiveness in advanced gastroesophageal cancer. *Cancer Research Communications* 2022;2:1037–48.
18 Singal G, Miller PG, Agarwala V, *et al*. Association of patient characteristics and tumor genomics with clinical outcomes among patients with non-small cell lung cancer using a clinicogenomic database. *JAMA* 2019;321:1391–9.
19 Frampton GM, Fichtenholtz A, Otto GA, *et al*. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol* 2013;31:1023–31.
20 Chalmers ZR, Connelly CF, Fabrizio D, *et al*. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med* 2017;9:34.
21 Trabucco SE, Gowen K, Maund SL, *et al*. A novel next-generation sequencing approach to detecting microsatellite instability and pan-tumor characterization of 1000 microsatellite instability-high cases in 67,000 patient samples. *J Mol Diagn* 2019;21:1053–66.
22 Foundation Medicine I. In: *SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)*. 2022.
23 Khozin S, Miksad RA, Adami J, *et al*. Real-World progression, treatment, and survival outcomes during rapid adoption of immunotherapy for advanced non-small cell lung cancer. *Cancer* 2019;125:4019–32.
24 McGough SF, Incerti D, Lyalina S, *et al*. Penalized regression for left-truncated and right-censored survival data. *Stat Med* 2021;40:5487–500.
25 Brown S, Lavery JA, Shen R, *et al*. Implications of selection bias due to delayed study entry in clinical genomic studies. *JAMA Oncol* 2022;8:287–91.
26 Zhang Q, Gossai A, Monroe S, *et al*. Validation analysis of a composite real-world mortality endpoint for patients with cancer in the united states. *Health Serv Res* 2021;56:1281–7.
27 Berger ML, Mamdani M, Atkins D, *et al*. Good research practices for comparative effectiveness research: defining, reporting and interpreting nonrandomized studies of treatment effects using secondary data sources: the ISPOR good research practices for retrospective database analysis Task force report -- Part I. *Value Health* 2009;12:1044–52.
28 Arbogast PG, Ray WA. Performance of disease risk scores, propensity scores, and traditional multivariable outcome regression in the presence of multiple confounders. *Am J Epidemiol* 2011;174:613–20.
29 Graf RP, Hullings M, Barnett ES, *et al*. Clinical utility of the nuclear-localized AR-V7 biomarker in circulating tumor cells in improving physician treatment choice in castration-resistant prostate cancer. *Eur Urol* 2020;77:170–7.
30 Vickers AJ, Cronin AM, Begg CB. One statistical test is sufficient for assessing new predictive markers. *BMC Med Res Methodol* 2011;11:13.
31 O'Malley DM, Bariani GM, Cassier PA, *et al*. Pembrolizumab in patients with microsatellite instability-high advanced endometrial cancer: results from the KEYNOTE-158 study. *J Clin Oncol* 2022;40:752–61.
32 Antill YC, Kok PS, Robledo K, *et al*. Activity of durvalumab in advanced endometrial cancer (AEC) according to mismatch repair (MMR) status: the phase II PHAEDRA trial (ANZGOG1601). *JCO* 2019;37(15_suppl):5501.
33 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* 2019;37:2786–94.
34 Bartley AN, Mills AM, Konnick E, *et al*. Mismatch repair and microsatellite instability testing for immune checkpoint inhibitor therapy: guideline from the College of American pathologists in collaboration with the association for molecular pathology and fight colorectal cancer. *Arch Pathol Lab Med* 2022;146:1194–210.
35 Slomovitz BM, Cibula D, Simsek T, *et al*. KEYNOTE-C93/GOG-3064/ENGOT-en15: a phase 3, randomized, open-label study of first-line pembrolizumab versus platinum-doublet chemotherapy in mismatch repair deficient advanced or recurrent endometrial carcinoma. *J Clin Oncol* 2022;40(16_suppl):TPS5623.
36 Vega DM, Yee LM, McShane LM, *et al*. Aligning tumor mutational burden (TMB) quantification across diagnostic platforms: phase II of the friends of cancer research TMB harmonization project. *Ann Oncol* 2021;32:1626–36.
37 Andre T, Amonkar M, Norquist JM, *et al*. Health-related quality of life in patients with microsatellite instability-high or mismatch repair deficient metastatic colorectal cancer treated with first-line pembrolizumab versus chemotherapy (KEYNOTE-177): an open-label, randomised, phase 3 trial. *Lancet Oncol* 2021;22:665–77.
38 André T, Shiu K-K, Kim TW, *et al*. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer. *N Engl J Med* 2020;383:2207–18.