

Clinical Utility of Prospective Molecular Characterization in Advanced Endometrial Cancer



Tara E. Soumerai^{1,2}, Mark T.A. Donoghue³, Chaitanya Bandlamudi³, Preethi Srinivasan⁴, Matthew T. Chang^{1,5,6}, Dmitriy Zamarin², Karen A. Cadoo^{2,7}, Rachel N. Grisham^{2,7}, Roisin E. O'Cearbhaill^{2,7}, William P. Tew^{2,7}, Jason A. Konner^{2,7}, Martee L. Hensley^{2,7}, Vicky Makker^{2,7}, Paul Sabbatini^{2,7}, David R. Spriggs^{2,7}, Tiffany A. Troso-Sandoval^{2,7}, Alexandra Snyder Charen², Claire Friedman^{2,7}, Mila Gorsky^{2,7}, Sarah J. Schweber^{2,7}, Sumit Middha⁸, Rajmohan Murali⁸, Sarah Chiang⁸, Kay J. Park⁸, Robert A. Soslow⁸, Marc Ladanyi⁸, Bob T. Li^{2,7}, Jennifer Mueller^{7,9}, Britta Weigelt⁸, Ahmet Zehir⁸, Michael F. Berger^{3,8}, Nadeem R. Abu-Rustum^{7,9}, Carol Aghajanian^{2,7}, Deborah F. DeLair⁸, David B. Solit^{1,2,3,7}, Barry S. Taylor^{1,3,5}, and David M. Hyman^{2,7}

Abstract

Purpose: Advanced-stage endometrial cancers have limited treatment options and poor prognosis, highlighting the need to understand genetic drivers of therapeutic vulnerabilities and/or prognostic predictors. We examined whether prospective molecular characterization of recurrent and metastatic disease can reveal grade and histology-specific differences, facilitating enrollment onto clinical trials.

Experimental Design: We integrated prospective clinical sequencing and IHC data with detailed clinical and treatment histories for 197 tumors, profiled by MSK-IMPACT from 189 patients treated at Memorial Sloan Kettering Cancer Center.

Results: Patients had advanced disease and high-grade histologies, with poor progression-free survival on first-line therapy (PFS₁). When matched for histology and grade, the genomic landscape was similar to that of primary untreated disease profiled by TCGA. Using multiple complementary genomic and mutational signature-based methods, we identified patients with microsatellite instability (MSI), even when

standard MMR protein IHC staining failed. Tumor and matched normal DNA sequencing identified rare pathogenic germline mutations in *BRCA2* and *MLH1*. Clustering the pattern of DNA copy-number alterations revealed a novel subset characterized by heterozygous losses across the genome and significantly worse outcomes compared with other clusters (median PFS₁ 9.6 months vs. 17.0 and 17.4 months; *P* = 0.006). Of the 68% of patients harboring potentially actionable mutations, 27% were enrolled to matched clinical trials, of which 47% of these achieved clinical benefit.

Conclusions: Prospective clinical sequencing of advanced endometrial cancer can help refine prognosis and aid treatment decision making by simultaneously detecting microsatellite status, germline predisposition syndromes, and potentially actionable mutations. A small overall proportion of all patients tested received investigational, genomically matched therapy as part of clinical trials. *Clin Cancer Res*; 24(23): 5939–47. ©2018 AACR.

Introduction

Endometrial cancer is a collection of unique histologic subtypes that, in aggregate, constitute the most common gynecologic malignancy. An estimated 63,000 new cases will be diagnosed in the United States in 2018, resulting in more than 11,000 deaths (1). Prognosis within this diverse group of cancers is based largely on histologic grade and clinical stage.

Previous comprehensive profiling of endometrial carcinomas has identified four distinct molecular subtypes, each with its own prognostic significance: *POLE*-mutant/ultramutated, microsatellite instability (MSI) high/hypermethylated, copy number low, and copy number high (2). Similarly, recent genomic analysis of uterine carcinosarcoma has identified important potential therapeutic targets, including the PI3K pathway, cell-cycle inhibition, and epigenetic regulation (3). The Cancer Genome Atlas (TCGA)

¹Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, New York. ²Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York. ³Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, New York. ⁴Tri-Institutional PhD Program in Computational Biology and Medicine, Weill-Cornell Medical College, Cornell University, Memorial Sloan Kettering Cancer Center, New York, New York. ⁵Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, New York. ⁶Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, California. ⁷Department of Medicine, Weill Cornell Medical College, Cornell University, New York, New York. ⁸Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York.

⁹Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, New York.

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T.E. Soumerai, M.T.A. Donoghue, B.S. Taylor, and D.M. Hyman contributed equally to this article.

Corresponding Author: David M. Hyman, Memorial Sloan Kettering Cancer Center, 300 E 66th Street, New York, NY 10065. Phone: 646-888-4544; Fax: 646-888-4268; E-mail: hymand@mskcc.org

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Translational Relevance

Advanced endometrial cancers carry a poor prognosis, and there are few treatment options. However, these tumors harbor multiple, potentially actionable genomic alterations, including microsatellite instability. Prospective tumor and matched normal molecular characterization of advanced endometrial cancers provides the opportunity to identify microsatellite instability, pathogenic germline mutations, and other somatic mutations with potential clinical relevance, beyond the standard variables of tumor grade and histology. Matching therapies to these alterations may result in clinical benefit. The application of prospective molecular characterization in the setting of advanced endometrial cancer can be used to inform practice, potentially expanding therapeutic options, and enhancing the future practice of cancer medicine.

studies focused on primary tumor samples, the majority collected from patients who did not develop recurrent disease (75% of 373 patients with endometrial carcinoma; 46% of 57 patients with uterine carcinosarcoma). Even less is known about the genomic landscape of rare endometrial cancer subtypes, undifferentiated, mixed, and clear cell carcinomas, that in addition to the other high-grade histologies account for the majority of disease-related mortality. To our knowledge, no study has evaluated the outcomes of broad-prospective molecular characterization of tumors and matched normal specimens in patients with active advanced endometrial cancer.

We investigated whether such prospective molecular characterization of patients with recurrent and metastatic endometrial cancer could reveal grade- and histology-specific differences, and guide enrollment onto therapeutic clinical trials.

Materials and Methods

Written informed consent was obtained from all participating patients. The study (ClinicalTrials.gov, NCT01775072) was conducted in accordance with International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws, and approved by an Institutional Review Board.

Patients

Patients were consented to this study under an Institutional Review Board–approved protocol (ClinicalTrials.gov, NCT01775072). Detailed disease-specific clinical annotation was collected. All cases underwent pathologic review at Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY) by an expert gynecologic pathologist. Cases for which histologic classification remained challenging after morphologic and IHC characterization were presented at a Gynecologic Pathology Case Conference and a consensus diagnosis rendered. All reported mismatch repair IHC was initially performed or repeated at MSKCC.

Genomic sequencing

Next-generation sequencing (NGS) was performed in the Clinical Laboratory Improvement Amendments (CLIA)–certified MSKCC Molecular Diagnostics Service Laboratory on DNA extracted from tumor and matched normal from blood. This was

accomplished utilizing MSK-IMPACT, an exon capture assay targeting all coding exons of 341 ($n = 70$ samples) or 410 ($n = 127$ samples) key cancer-associated genes, as described previously (4, 5). DNA was sequenced to an average of 735-fold sequence coverage. Prior validation established good uniformity of coverage across covered exons, and all variants were reviewed by a molecular pathologist prior to signing out results into the medical record (4). All patient-level clinical and genomic data used in this analysis are available via cBioPortal for Cancer Genomics (www.cbioportal.org).

Allele-specific copy-number analysis

We performed FACETS analysis to determine allele-specific and absolute DNA copy-number genome wide in all patients (FACETS version 0.5.6, $cval = 100$; ref. 6). We used these data to analytically estimate tumor purity and ploidy. All samples were utilized for most genomic analyses, while samples with a purity estimate $<20\%$ were excluded from further DNA copy number focused analyses (81.5% of patient samples retained, $n = 154/189$). Prior to further analysis, total copy-number log ratios were corrected for ploidy and purity. Recurrent copy-number alterations (CNAs) were identified using CNTools and were hierarchically clustered using Manhattan distance and Ward linkage method (7). Tumors with whole-genome doubling were those in which $>50\%$ of the autosomal genome had a major copy number ≥ 2 . Cancer cell fractions were calculated using a binomial distribution; maximum likelihood estimation was normalized to produce posterior probabilities (8).

MSI

The presence of MSI was assessed genomically using MSIsensor (version 0.2; ref. 9). MSIsensor assigns a numeric score based on percentage of unstable microsatellite sites, divided by total number of microsatellite sites tested from aligned sequencing data. On the basis of prior clinical validation of MSIsensor (10), MSI status was defined on the basis of scores: <3 , microsatellite stable (MSS); >3 and <10 , MS indeterminate; and ≥ 10 , MSI-high (MSI-H).

Mutational signature decomposition

Mutational signature decomposition analysis was performed for all samples: (i) mismatch repair deficiency (MMR-D) identified by IHC; or (ii) ≥ 10 single nucleotide somatic mutations (11). From the somatic mutations in an individual tumor sample, contributions were inferred on the basis of mutational signatures, which are probability distributions over the nucleotide change and flanking 5' and 3' nucleotide context of each mutation. If more than one signature was present a weighted combination was calculated, reflecting the proportion of mutations in the sample attributed to that signature.

Germline analysis

Germline annotation for likely pathogenic or pathogenic variants was performed in 76 cancer predisposition genes in the MSK-IMPACT panel, using a clinically validated pipeline applied to match normal DNA obtained from blood (12, 13). Variant annotation and assessment of pathogenicity were performed after irreversible anonymization, as not all patients were consented for identified germline analysis. Histologic type and allele-specific absolute copy number were retained prior to irreversible anonymization, permitting subsequent determination of loss of

heterozygosity. No other clinical data were retained following anonymization.

Statistical analysis

We assessed the enrichment of genomic alterations across histology and grade using Fischer's exact or χ^2 tests (where appropriate); nominal *P* values are specified. Comparisons of gene mutation prevalence across cohorts (this study compared with TCGA), and histologic subtypes, were performed on MSS samples only. Cox proportional hazards analysis and Kaplan-Meier estimation of progression-free survival (PFS) were done using the R survival package. To compare outcome between cohorts, we utilized the PFS₁. To evaluate response to therapy, we retrospectively and centrally assessed patients for clinical benefit (binary outcome: yes/no). We defined clinical benefit as at least two consecutive imaging studies showing stable disease or better and documented symptom improvement. Scans were required to be at least 30 days apart.

Annotation of somatic alterations

To determine the clinical actionability of individual genomic variants identified, we utilized the OncoKB knowledge base (OncoKB.org). OncoKB provides disease-specific levels of evidence for the actionability of individual mutant alleles, DNA copy-number alterations, and translocations (14). A level 1 alteration is an FDA-recognized biomarker in the patient's tumor type; level 2 is a biomarker routinely used to guide prescribing of an FDA-approved drug, based on tumor type (2A) or other indication (2B); level 3 demonstrates compelling clinical evidence supporting its use as a biomarker predictive. Annotations were applied on December 22, 2016.

Results

A cohort of patients with advanced endometrial cancer

We prospectively analyzed 197 samples from 189 patients, the majority (95%) with advanced disease [defined as patients with International Federation of Gynecology and Obstetrics (FIGO) stage III–IV disease at diagnosis or recurrence regardless of initial stage]. In contrast, 75% of patients profiled in TCGA study of endometrial cancer remained disease-free at last postoperative follow-up (Table 1; Supplementary Tables S1 and S2; ref. 2). The patients in our cohort (median PFS₁ 15.3 months) had worse PFS than those in TCGA (median PFS₁ not yet reached at median follow-up of 23 months; Fig. 1A). However, comparison between patients in TCGA cohort who recurred (19% of total cases) and those in our cohort revealed similar survival profiles (Fig. 1A; median PFS₁ 13.7 vs. 15.3 months, respectively; *P* = 0.1), suggesting that the subset of recurrent patients from TCGA is clinically similar to ours.

Our cohort included a higher proportion of patients with high-grade tumors (75% vs. 48%), including grade 3 endometrioid, serous, mixed, and clear cell carcinomas, and carcinosarcomas (Fig. 1B; Supplementary Table S2). As expected, patients with high-grade (FIGO grade 3) endometrioid carcinomas had worse median PFS₁ than those with low-grade (FIGO grades 1 and 2) disease (12.7 vs. 21.6 months; *P* = 0.005). The PFS₁ of patients with high-grade endometrioid carcinoma was statistically comparable with that of patients with other high-grade tumors (Supplementary Fig. S1). Other notable differences between our study and TCGA patient population were our inclusion of metastatic

Table 1. Clinical attributes of patients in MSKCC versus TCGA cohort

| | MSK cohort (<i>n</i> = 189) | TCGA cohort (<i>n</i> = 373) |
|---------------------------------------|---------------------------------|----------------------------------|
| Age at diagnosis | | |
| Median | 62 | 63 |
| Range | 38–83 | 31–90 |
| BMI at diagnosis | | |
| Median | 27.6 | 32.9 |
| Range | 17.5–48.6 | 17.4–81.6 |
| Histology and grade, <i>n</i> (%) | | |
| Endometrioid, grade 1 | 19 (10.1%) | 89 (23.9%) |
| Endometrioid, grade 2 | 26 (13.8%) | 106 (28.4%) |
| Endometrioid, grade 3 | 30 (15.9%) | 112 (30.0%) |
| Serous | 46 (24.3%) | 53 (14.2%) |
| Carcinosarcoma | 35 (18.5%) | — |
| Mixed | 15 (7.9%) | 13 (3.5%) |
| Clear cell | 13 (6.9%) | — |
| Other ^a | 5 (2.6%) | — |
| FIGO stage at diagnosis, <i>n</i> (%) | | |
| I | 56 (29.6%) | 254 (68.1%) |
| II | 14 (7.4%) | 25 (6.7%) |
| III | 46 (24.3%) | 74 (19.8%) |
| IV | 73 (38.6%) | 17 (4.6%) |
| Tumor site profiled, <i>n</i> (%) | | |
| Primary | 92 (48.7%) | 373 (100%) |
| Metastasis | 91 (48.1%) | — |
| Both | 6 (3.2%) | — |
| Disease status, <i>n</i> (%) | | |
| NED | 12 (6.3%) | 288 (77.2%) |
| AWD | 113 (59.8%) | 26 (7.0%) |
| DOD | 64 (33.9%) | 25 (6.7%) |
| DOC | 0 (0%) | 11 (2.9%) |
| PFS (months), <i>n</i> (%) | | |
| Median | 15.3 | N/A |
| Range | 1.7–186.4 | 0.4–194.5 |
| OS (months), <i>n</i> (%) | | |
| Median | 61.4 ^b | N/A |
| Range | 6.1–244.6 | 0–194.5 |

Abbreviations: AWD, alive with disease; DOC, died of other causes; DOD, dead of disease; NED, no evidence of disease; OS, overall survival.

^aFour undifferentiated/dedifferentiated and one neuroendocrine.

^bTwo patients with unknown vital status, three deceased but unknown if died of disease or other causes, and 18 living with unknown disease status.

tumor samples (51%), and tumor samples collected after chemotherapy (39%) or hormonal therapy (11%; Table 1).

Comparison of TCGA and MSK cohort mutational profiles

We sought to explore whether the significant clinical differences between our cohort and TCGA population could be utilized to identify genomic alterations predictive of poor outcome. Despite substantial differences in clinical features and outcomes between these cohorts, the most frequent somatic alterations were similar, with a few notable differences (Supplementary Fig. S2). *TP53* mutations were more common in our cohort compared with TCGA (63% vs. 36%; *P* = 4×10^{-5}); *PTEN* alterations were more common in TCGA (56% vs. 22%; *P* = 8×10^{-9}). These differences were largely attributable to variability in the histologic subtypes comprising these cohorts (Fig. 1B).

Alterations in the PI3K/AKT/mTOR and RTK/RAS/β-catenin pathways were identified in 70% and 65% of patients, respectively (Fig. 1C), consistent with prior studies (2). Despite the poorer outcomes of patients with high- versus low-grade endometrioid cancers (Supplementary Fig. S1), the mutational profiles of these clinically distinct tumors were largely similar. One exception was the significantly higher frequency of *TP53* mutations in

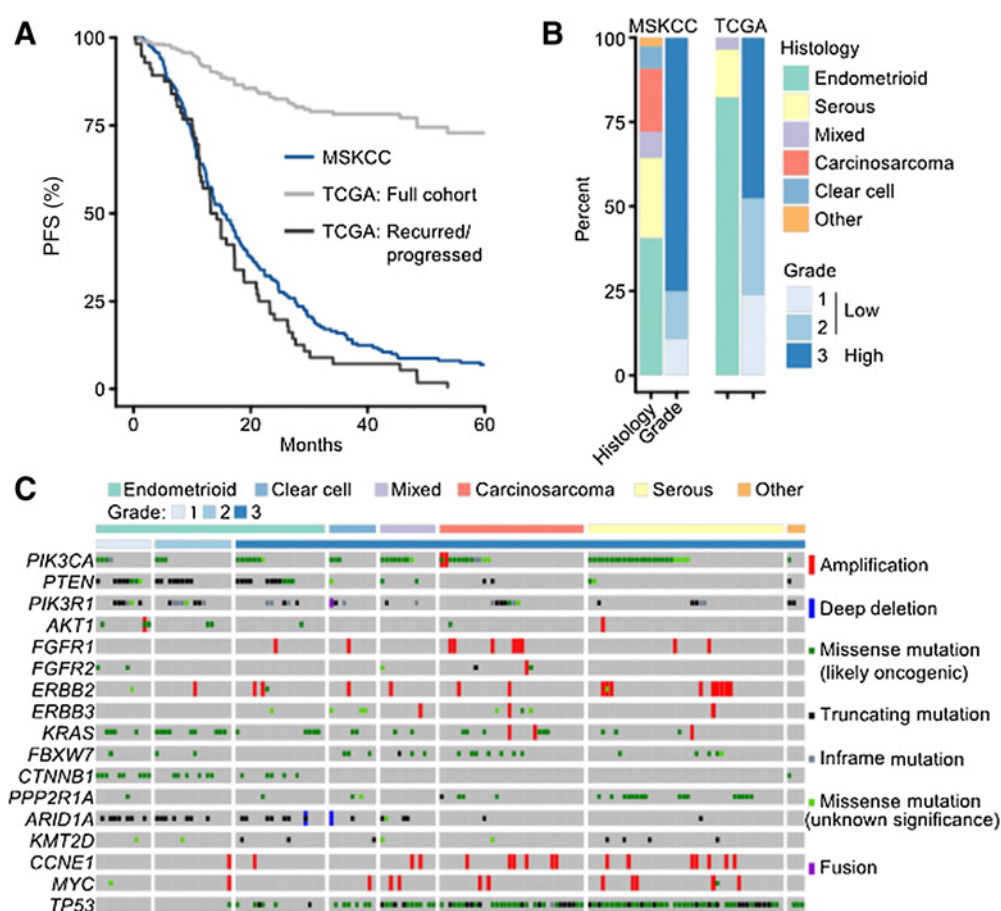


Figure 1.

Summary of cohort and comparison with TCGA. **A**, PFS of MSKCC and TCGA cohorts. MSKCC cohort is split by low- and high-grade tumors. TCGA cohort displays the full cohort and those cases that progressed or recurred as an additional curve. **B**, Histology and grade comparison between MSKCC and TCGA cohorts. **C**, Oncoprint of genomic alterations of MSS samples split by histology and grade.

high-grade tumors (29% vs. 3%; $P = 0.013$; Fig. 1C). *PIK3CA* mutations were most prevalent in mixed histology tumors and enriched overall in high-grade tumors regardless of histologic subtype ($P = 0.035$). Amplifications of *FGFR1* and *ERBB2* were more prevalent in carcinosarcomas and serous tumors, respectively ($P = 0.007$ and $P = 0.018$; Fig. 1C). We identified three patients with truncating germline *BRCA2* mutations (one serous cancer and two carcinosarcomas). Two of the patients had the *BRCA2* c.5946delT (6174delT) founder mutation (15, 16); the third was a known recurrent frameshift deletion (c.5799_5802delCCAA). One of the carcinosarcomas had evidence of loss of heterozygosity, the serous cancer retained the wild-type *BRCA2* allele, and the other carcinosarcoma was nonevaluable due to low tumor purity. Identification of pathogenic germline mutations not historically phenotypically related to the observed cancer type has similarly been seen in other unbiased germline screening studies of patients with advanced cancer (5). We also identified a patient with grade 2 endometrioid tumor harboring a likely pathogenic, Lynch syndrome-associated, germline *MLH1* splice site mutation (c.1731+1G>T). This germline *MLH1* mutation was biallelic in the tumor due to somatic copy-neutral loss of heterozygosity; the sample was scored as MSI-H (MSIsensor 19.19). The irreversible

anonymization process required to conduct the germline analysis prevented further clinical description of germline-positive cases.

We identified no significant differences between mutational profiles of primary and metastatic tumors, with the exception of *PTEN* mutations in endometrioid carcinomas, which were significantly more common in primary tumors [67% ($n = 16/24$) vs. 34% ($n = 10/29$) of metastases; $P = 0.03$]. In 4 patients with matched primary and metastatic tumors, some genomic heterogeneity existed (Supplementary Fig. S3). This involved potentially actionable hotspot mutations, including *MTOR* L2427Q, and *PIK3R1* E468*, in two patients.

Exploring the impact of prior therapy on tumor genomic profiles, we identified five patients with endometrioid tumor with *ESR1* ligand-binding domain hotspot mutations, alterations that were previously reported in acquired resistance to endocrine therapy (17). Only two (40%) of these *ESR1* mutations were detected from samples obtained after exposure to endocrine therapy. In the remaining three patients, *ESR1* hotspot mutations arose in apparent absence of the selective pressure of endocrine therapy, including any endocrine therapy exposure that may have been delivered for a comorbid illness such as an early-stage breast cancer. The body mass index (BMI) for the patients with apparent

de novo *ESR1* mutations was similar to patients with acquired *ESR1* mutations (median: 22.9 vs. 24.7, respectively). The median BMI of these patients was nearly identical to those with *ESR1* mutations that appear to have been acquired after antiestrogen therapy. One of three with *de novo* *ESR1* mutation was treated with letrozole and had immediate progression of disease, but later achieved ongoing 18-month response to bevacizumab and fulvestrant (an estrogen receptor degrader that maintains activity in the presence of *ESR1* ligand-binding domain mutations). The other two patients have not received endocrine therapy. The presence of *de novo* *ESR1* mutations in patients with breast cancer with no prior exposure to hormonal therapy has not been reported (17, 18).

Complementary measures of high mutational burden endometrial cancers

In our cohort, the median number of mutations per sample was 5.7 mutations/Mb (range, 0–419.8 mutations/Mb; Fig. 2A). A total of 15.9% (30/189 cases) had ≥ 10 single-nucleotide variants, permitting mutational signature decomposition. Notably, the two patients with *POLE* exonuclease domain hotspot mutations

had tumors with an ultramutator phenotype (V411L, 419.8 mutations/Mb and P286R, 152.8 mutations/Mb). Seven additional patients with *POLE* mutations of uncertain significance, occurring outside of the exonuclease domain, lacked a similar ultramutator phenotype. Twenty-four tumors (13% vs. 39% in TCGA; $P = 5 \times 10^{-11}$), including one hotspot *POLE*-mutant tumor, were MMR-D based on IHC for MLH1, PMS2, MSH2, and MSH6 (Fig. 2A). Excluding the *POLE* hotspot-mutant tumor, the remaining 23 had a median burden of 31.6 mutations/Mb (range, 19.8–107.6 mutations/Mb) compared with 4.7 mutations/Mb (range, 0–32.7 mutations/Mb) in the MMR-proficient (MMR-P) samples ($P = 1.9 \times 10^{-14}$). The majority (19/24) of these MMR-D tumors were endometrioid carcinomas; the remainder included clear cell ($n = 2$), mixed ($n = 2$), and undifferentiated ($n = 1$) carcinomas. In two MMR-D tumors scored as MSI-indeterminate by MSIsensor, both the sequenced tumor specimens were of low purity, potentially accounting for this finding. In addition to 24 MMR-D tumors identified by conventional IHC testing, six in the MMR-P and not tested categories had high somatic mutational burden (TMB ≥ 20), including the other *POLE*-mutant tumor

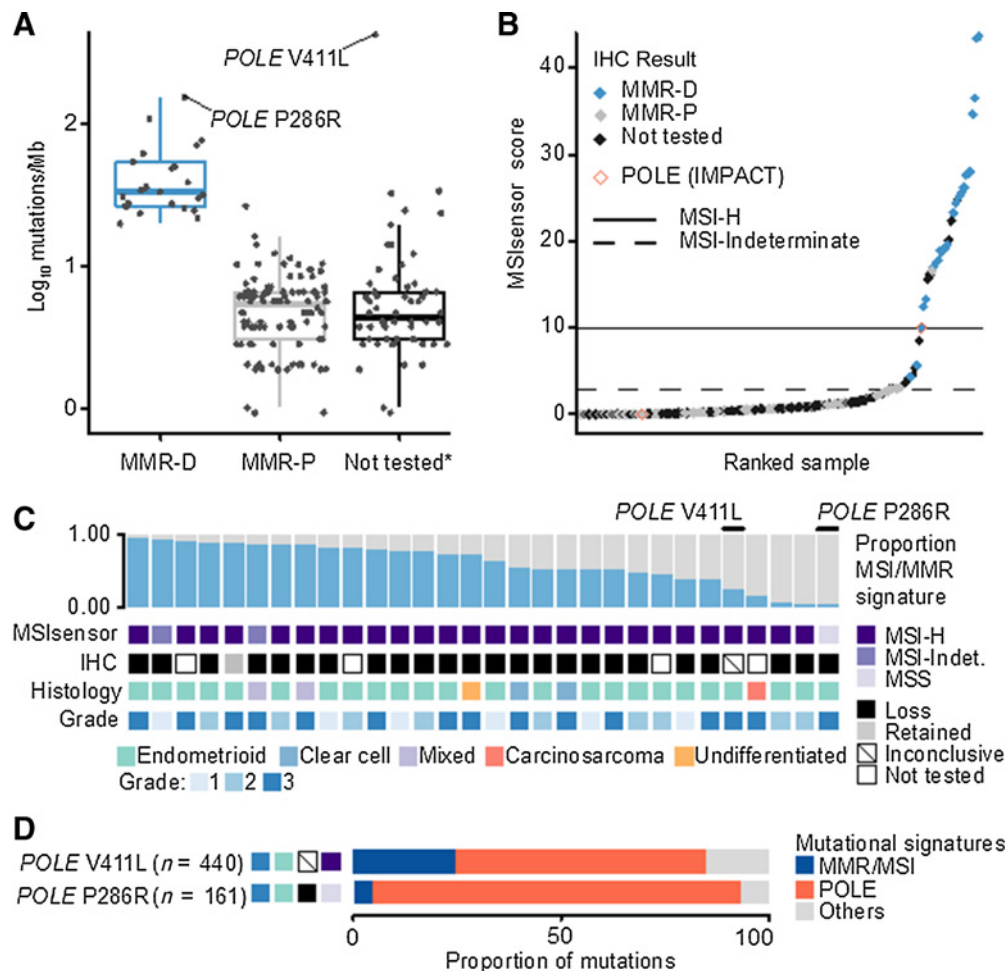


Figure 2.

Mutational signatures. **A**, MSK-IMPACT sequencing mutational burden, split by clinical MMR-D IHC results, *POLE* cases identified by MSK-IMPACT sequencing; *includes one inconclusive result. **B**, MSIsensor scores for the full cohort. **C**, Signature decomposition of hypermutated cases. **D**, Signature decomposition of *POLE*-mutant cases.

(five endometrioid carcinomas, one carcinosarcoma; range, 23.5–419.8 mutations/Mb). Although four of these cases had insufficient tissue for standard MMR testing, and one had an inconclusive result, the remaining case was MMR-P by IHC, suggesting possible occult MMR deficiency. To address these discrepancies and compare MMR as defined by IHC versus orthogonal genomic approaches, we performed a combination MSI analysis and mutational signature decomposition analysis to determine the underlying mutational process in each of 30 cases with known or presumed MMR-D (Fig. 2B–D). All but one (97%, 29/30) displayed a high MSIsensor score, including the MMR-competent case (by IHC) with high somatic mutation burden. This case lacked clear function *MMR* gene mutations and was not consented for germline analysis (Fig. 2C). The one MSS case by MSIsensor harbored a *POLE* exonuclease domain hotspot mutation, accounting for the ultramutated phenotype (Fig. 2C and D). In total, these results indicate that MMR assessment using IHC is highly sensitive for detecting MMR-D/MSI tumors and that bioinformatic detection methods based on NGS can have excellent concordance when applied to tumors of adequate purity while permitting simultaneous detection of mutational processes driven by *POLE*.

Pattern of somatic copy-number alterations identifies a prognostically distinct subset of endometrial cancers

Endometrial cancers have previously been categorized into four groups, based on their pattern of somatic copy-number alterations (SCNA; ref. 2). To ascertain whether a similar pattern was evident in our cohort, we performed hierarchical clustering of ploidy and purity-corrected genome-wide DNA copy-number profiles. Three distinct clusters emerged (labeled A–C; Fig. 3A). More detailed characteristics of each cluster, as well as the most common alterations identified in each can be found in Supplementary Table S3. Cluster A was enriched for *TP53* mutant, predominantly high-grade tumors of various histologies, and underwent whole-genome doubling. Cluster B comprised mostly endometrioid carcinomas, including most FIGO grade 1/2 tumors, and was enriched for *PTEN* mutations. This cluster had attributes similar to clusters 2 and 3 from TCGA cohort, with gains of chromosomes 1q, 8, and 10, although we were unable to distinguish two separate clusters. Cluster C comprised high-grade endometrioid and nonendometrioid tumors. These lack 1q gains and have a copy-number profile characterized largely by heterozygous losses

across the genome. The combination of clusters A and C from our cohort resembled the copy-number high cluster 4 from TCGA. TCGA cluster 1, characterized by the absence of SCNAs and consisting largely (93%) of patients who did not progress/recr, was not present in our cohort, likely reflecting clinical differences between the two cohorts. Notably, the copy-number clusters that we identified reflected outcome differences among patients. Specifically, median PFS₁ in cluster C was significantly lower than that in the other two clusters (median PFS of 9.6 months, cluster C vs. 17.4 and 17 months, clusters A and B, respectively, $P = 0.006$; Fig. 3B). This suggests that a copy-number alteration pattern driven by large-scale chromosomal losses may be associated with poor outcomes even when compared with a subset of *TP53*-mutant patients with whole-genome doubling, both poor prognostic factors in other contexts. To further validate this finding, we performed a reanalysis of TCGA cluster 4 (the "SCNA high" cohort). In TCGA cluster 4, we identified two approximately equal sized subgroups. One subgroup was cluster A-like and characterized by whole-genome duplication and thus primarily by copy-number gains over diploid. The second subgroup was cluster C-like characterized primarily by heterozygous losses (Supplementary Fig. S4A). Heavy censoring in the TCGA cluster 4 cohort (only 21 progression events in 81 patients) prevented robust comparison of outcomes (Supplementary Fig. S4B). An additional cohort of patients with advanced endometrial cancer will be required to more definitively determine the prognostic impact of heterozygous copy-number losses in this tumor type.

Therapeutic actionability and clinical benefit

Excluding *RAS* mutations that have not been successfully targeted by novel therapies to date, 67% of patients (127/189) had at least one likely therapeutically actionable alteration, for which therapy was FDA approved or under clinical investigation. The most common were *PIK3CA* mutation ($n = 66/189$, 35%), *PTEN* mutation ($n = 54/189$, 29%), MSI high (any evidence; $n = 30/189$ cases, 16%), and *ERBB2* amplifications ($n = 16/189$ cases, 16%; Fig. 4A). In total, 27% (34/127) of patients with potentially actionable alterations were enrolled in matched clinical trials. Reasons for lack of matching included the lack of trials targeting a specific alteration, ongoing response to standard-of-care treatment, alternative unmatched clinical trial options (i.e., those including immunotherapy agents), clinical deterioration, or

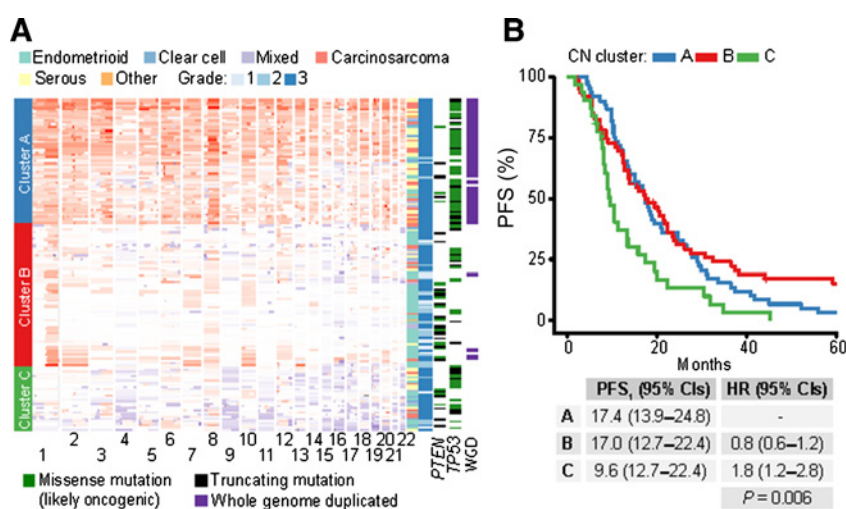


Figure 3. Somatic copy-number alterations in endometrial carcinomas. **A**, Clustering of SCNAs. **B**, Kaplan-Meier curves of PFS for each CN cluster.

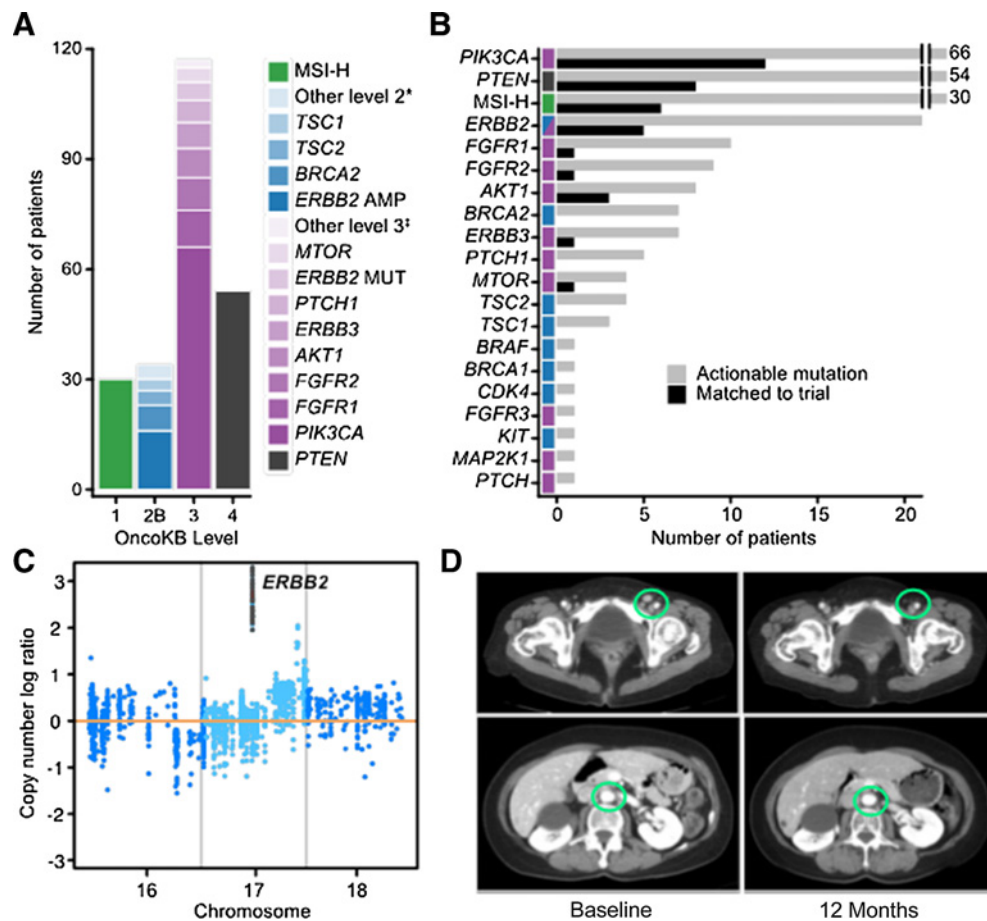


Figure 4.

Clinical actionability and genomic study matching. **A**, Number of actionable alterations by OncoKB level; **BRAF*, *BRCA1*, *CDK4*, *KIT* each x1; †*FGFR3*, *MAP2K1* each x1. **B**, Rates of matching to genomically targeted therapy by each actionable gene. **C**, Copy-number tumor/normal log ratio highlighting an *ERBB2* amplification. **D**, CT scans showing a complete response in a patient with *ERBB2*-amplified serous carcinoma enrolled to a study of trastuzumab emtansine.

patient choice. The most common matched alteration was *PIK3CA* mutation ($n = 12$; Fig. 4B). Patients were enrolled in clinical trials of PI3K ($n = 12$), dual PI3K/mTOR ($n = 6$), *ERBB2* ($n = 6$), *AKT* ($n = 4$), mTOR ($n = 2$), and/or FGFR inhibitors ($n = 2$). None of the patients with germline *BRCA1/2* mutations were enrolled to a PARP inhibitor study. Of 34 matched patients, 31 (91.2%) were enrolled studies involving targeted therapy alone; the remaining three patients (8.8%) were enrolled to studies that also incorporated chemotherapy. Responses were observed in patients with multiple actionable alterations treated with targeted agents, including one heavily pretreated patient whose serous tumor harbored an *ERBB2* amplification (Fig. 4C), and who achieved a durable (14 months, ongoing) complete response to trastuzumab emtansine (TDM1; Fig. 4D). Overall, the rate of clinical benefit to patients in clinical trials was 47% ($n = 16/34$), exceeding historic expectations. In patients matched on the basis of the two most common actionable alterations, *PIK3CA* and *PTEN*, clinical benefit rates were numerically similar at 42% (8/19). In total, 8.5% (16/189) of patients achieved clinical benefit to matched targeted therapy when accounting for all patients sequenced.

Tumor mutational burden is currently being evaluated as a predictive biomarker for immune checkpoint inhibitors in several cancer types. In our cohort, samples with high mutational burden were attributed to either MSI ($n = 28$), *POLE*-mediated ($n = 1$), or MSI and *POLE*-mediated ($n = 1$) defects, suggesting that no new hypermutation mechanisms were adopted despite advanced-stage disease and prior treatment history. We identified 30 (16%) patients as MSI-H by conventional (IHC) and/or genomic means. No other MSS patients with high mutational burden were identified, suggesting that MSI accounts for the vast majority of high tumor mutational burden in patients with endometrial cancer. Although this analysis was performed prior to FDA approval of pembrolizumab for MSI-H tumors (19), 17% (5/30) of MSI-H patients were treated with immune checkpoint inhibitors; two experienced clinical benefits (40%). Two additional patients were treated with other immunotherapeutic agents, but none responded. Neither of the *POLE* hotspot-mutant patients harboring the ultramutator phenotype was treated with an immune checkpoint inhibitor. One patient whose *POLE* mutation was detected in their primary tumor has not recurred in 2.5 years of follow-up; the other patient whose *POLE* mutation

was detected in an isolated metastatic lesion had this tumor site resected, and remains without evidence of disease after 2 years. Both the low absolute rate of *POLE* exonuclease–domain hotspot mutant cases observed in our cohort of advanced cancers, as well as the observation that neither of the two patients identified here required systemic therapy for recurrent disease, provides additional support that the *POLE*-mediated ultramutation is associated with a favorable prognosis.

Discussion

We demonstrate that prospective molecular characterization yields potentially clinically relevant information beyond the standard variables of tumor grade and histology and can facilitate prognostication and treatment decision making for patients with advanced endometrial cancer. Since the time of this analysis, the immune checkpoint inhibitor pembrolizumab has been approved for MSI-H cancers. This alteration was present in 16% of our cohort, expanding the therapeutically actionable patient population that can be identified (as we have done here) and establishing the lower boundary for percentage of patients with advanced endometrial cancer whose treatment could ultimately be guided by this testing strategy. Moreover, prospective sequencing identified MSI in a tumor with retained staining of MMR proteins by IHC, indicating that a genomic approach can improve upon current standard screening methodology (20). Furthermore, the clinical sequencing performed in our study simultaneously detected germline cancer predispositions (such as Lynch syndrome) that underlie MSI, as well as many additional somatic mutations, *ERBB2* amplifications, *FGFR2/3* fusions, and alterations in the PI3K pathway that may be of therapeutic relevance.

Overall, 67% of patients harbored at least one potentially actionable genomic alteration. Only 27% of patients with potentially qualifying genomic alterations were subsequently matched to investigational therapy based on these results. As real-world experience with genomically allocated study enrollment grows, substantial patient attrition from identification to match has emerged as a consistent feature (5, 21–23). This finding again demonstrates the need for improvements in ensuring patient access to the relevant targeted therapy in the context of clinical studies that contribute to generalizable scientific understanding. As with prior efforts, we identified lack of matched study availability, alternative routine and unmatched investigational options, and clinical deterioration as important barriers to achieving a higher match rate.

Although we utilized a large gene panel covering the entire coding regions of up to 410 genes, currently only a minority of this genomic content can be considered even potentially actionable. On the basis of current clinical- and variant-specific knowledge bases, we believe 30 to 50 genes, provided they are capable of detecting all classes of genomic alterations, are likely to cover the vast majority of the currently "actionable genome" (14). Although panels of this size are generally not well-suited for establishing MSI status or tumor mutational burden, orthogonal approaches are available for determination of MSI/MMR status. Similarly, although the inclusion of matched normal DNA in our assay provided the opportunity for simultaneous germline diagnosis, commercial germline testing panels are also available to fill this role, although not always readily reimbursed. Thus, we acknowledge that incorporation of NGS testing may not currently be

appropriate for every practice setting and that, for many others, a more selective and focused testing strategy may ultimately be a better fit.

This analysis has several other important limitations. First, due to the overall sample size and retrospective nature of the analysis, our findings will need to be validated in additional unrelated cohorts. In addition, this study was conducted at a large dedicated cancer center with access to a diverse and active clinical trial portfolio. The rate at which patients are successfully matched to clinical trials is dependent not only on assay characteristics but also the study portfolio and therefore is expected to vary significantly by practice setting. Finally, it is important to acknowledge that despite the combination of broad testing and access to studies, only 18% of patients were enrolled to matched targeted therapy, and an even smaller proportion, 8.5% of all patients tested, benefit.

In summary, we show that clinical sequencing of endometrial cancers provides an efficient means of simultaneously detecting the presence of MSI, germline cancer predisposition syndromes, and potentially actionable somatic variants. In total, 18% of patients went on to receive matched therapy; 47% of these patients achieved clinical benefit. Despite these observational results, further study will be needed to understand the true extent of benefit of NGS in this patient population. Despite this, with the recent approval of pembrolizumab for MSI-H cancers, we anticipate that the cost and operational efficacy of NGS will drive increasing adoption of this technology in the treatment of endometrial cancer.

Disclosure of Potential Conflicts of Interest

R. Grisham is a consultant/advisory board member for Clovis. R.E. O'Ceirhaill is a consultant/advisory board member for Clovis and Tesaro. R.A. Soslow reports receiving speakers bureau honoraria from Ebix/Oakstone and reports receiving other remuneration from Springer Publishing and Cambridge University Press. C. Aghajanian is a consultant/advisory board member for ImmunoGen, Clovis, Tesaro, Mateon Therapeutics, Cerulean Pharma, Bayer, and VentiRx. D.B. Solit is a consultant/advisory board member for Loxo Oncology and Pfizer. D.M. Hyman is a consultant/advisory board member for Atara Biotherapeutics, Chugai Pharma, CytomX Therapeutics, Boehringer Ingelheim, AstraZeneca, Pfizer, Bayer, and Genentech. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: T.E. Soumerai, M.T.A. Donoghue, C. Aghajanian, D.B. Solit, B.S. Taylor, D.M. Hyman

Development of methodology: M.T.A. Donoghue, V. Makker, M. Ladanyi, B.T. Li, A. Zehir, C. Aghajanian, D.B. Solit

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T.E. Soumerai, C. Bandlamudi, D. Zamarin, K.A. Cadoo, R.N. Grisham, W.P. Tew, J.A. Konner, M.L. Hensley, V. Makker, C. Friedman, M. Gorsky, S.J. Schweber, R. Murali, S. Chiang, R.A. Soslow, M. Ladanyi, B.T. Li, B. Weigelt, A. Zehir, N.R. Abu-Rustum, C. Aghajanian, D.F. DeLair, D.B. Solit, D.M. Hyman

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T.E. Soumerai, M.T.A. Donoghue, C. Bandlamudi, P. Srinivasan, M.T. Chang, R.E. O'Ceirhaill, W.P. Tew, V. Makker, S. Middha, R. Murali, S. Chiang, R.A. Soslow, M. Ladanyi, B.T. Li, B. Weigelt, A. Zehir, M.F. Berger, C. Aghajanian, D.B. Solit, B.S. Taylor, D.M. Hyman

Writing, review, and/or revision of the manuscript: T.E. Soumerai, M.T.A. Donoghue, C. Bandlamudi, D. Zamarin, K.A. Cadoo, R.N. Grisham, R.E. O'Ceirhaill, W.P. Tew, J.A. Konner, M.L. Hensley, V. Makker, P. Sabbatini, D.R. Spriggs, T.A. Troso-Sandoval, A. Snyder Charen, C. Friedman, S. Middha, R. Murali, S. Chiang, K.J. Park, R.A. Soslow, B.T. Li, J. Mueller, B. Weigelt, A. Zehir, N.R. Abu-Rustum, C. Aghajanian, D.F. DeLair, D.B. Solit, B.S. Taylor, D.M. Hyman

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.T.A. Donoghue, B.T. Li, D.B. Solit, D.M. Hyman

Study supervision: R.E. O'Ceirbhail, C. Aghajanian, D.B. Solit, B.S. Taylor, D.M. Hyman

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