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Comprehensive genomic profiling of recurrent endometrial cancer: Implications for selection of systemic therapy☆

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HIGHLIGHTS

- We demonstrate utility of comprehensive genomic profiling (CPG) in routine care of recurrent endometrial cancer (EC).
- CPG allows segregation of recurrent EC into the four molecular TGCA subtypes.
- CPG allows tailoring of treatments according to the molecular subtypes and clinically actionable genomic alterations.
- Matched therapies led to response in 25% and clinical benefit in 62% of cases with median treatment duration of 15 months.
- CPG may allow the identification of drug resistance markers such as JAK1/2 mutations for immunotherapy in MSI-H recurrent EC.

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ABSTRACT

Objectives. To assess whether comprehensive genomic profiling (CGP) in the setting of routine clinical care allows molecular classification of recurrent endometrial cancer (EC) into the four Cancer Genome Atlas (TCGA) categories: POLE ultramutated, microsatellite instable, copy-number low, and copy-number high and whether this approach can identify genomic alterations (GAs) which inform treatment decisions.

Methods. Archival tissues from 74 patients diagnosed with recurrent EC were prospectively analyzed using hybrid-capture-based genomic profiling. Tumor mutational burden and microsatellite instability were measured. Clinically relevant GAs (CRGAs) were defined as GAs associated with targeted therapies available on-label or in mechanism-driven clinical trials.

Results. Using POLE mutational analysis, mismatch repair status, and p53 mutational analysis as surrogate for 'copy-number' status CGP segregated all cases into four TCGA molecular subgroups. While recurrent serous ECs were predominantly copy-number high, we found no clear prevalence of a specific molecular subtype in endometrioid, clear cell or undifferentiated tumors. Every tumor sample had at least one GA and 91% (67/74) had at least one CRGA. In this series 32% (24/74) of patients received a matched therapy based on the results of CGP. Objective responses to the matched therapy were seen in 25% (6/24) of patients with an additional 37.5% (9/24) achieving stable disease leading to a clinical benefit rate of 62.5% with a median treatment duration of 14.6 months (range 4.3–69 months).

Conclusions. CGP allows molecular classification of EC into four TCGA categories and allows identification of potential biomarkers for matched therapy in the setting of routine clinical care.

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1. Introduction

Endometrial cancer is the sixth most common neoplasm in women worldwide, with the highest rates in North America and Europe [1]. Although early stage endometrial cancer is very treatable, with surgery and adjuvant therapy, variability in mortality exists based on traditional prognostic factors such as histologic subtype, tumor grade or disease stage [2]. Most importantly, however, for patients with recurrent disease, salvage rates are low and the prognosis remains poor. Annually in the United States alone, approximately 60,000 women develop endometrial cancer and 10,000 women succumb to the disease [3]. Optimal therapy for these patients remains to be established.

Large-scale genomic datasets, such as The Cancer Genome Atlas (TCGA), have increased our understanding of the molecular mechanisms driving endometrial cancer tumorigenesis and have given us novel insights into the molecular heterogeneity of the disease. The TCGA identified a genomic-based molecular classification scheme for endometrial cancers, with four molecular categories: Polymerase epsilon (*POLE*) ultramutated, microsatellite instability hypermutated (MSI-H), copy-number low (CN-L), and copy-number high (CN-H) [4,5]. Despite the recent progress in our understanding of molecular subtypes in endometrial cancer, the current standard of care for the treatment of endometrial cancer is primarily based on morphological/histological subtype, tumor stage, and tumor grade, with very few effective standard options available for patients with recurrent disease [6]. It is obvious that cancer genomic data may help us develop targeted treatment options against molecularly matched alterations that can be more effective and less toxic than traditional chemotherapeutic regimens.

In 2018 the U.S. Food and Drug Administration (FDA) approved the first comprehensive genomic profiling (CGP) assay FoundationOne CDx™, a laboratory test designed to detect genetic mutations in 324 genes and two genomic signatures in any solid tumor. Clinicians now have a CGP assay available that may help to guide personalized treatment in recurrent endometrial cancer. However, the feasibility of CGP for patients diagnosed with recurrent endometrial cancer is not well studied and its clinical utility still unclear.

In this study, we aimed to investigate whether available CGP testing would permit a molecular classification of endometrial cancer in clinical practice and how this might affect treatment for women diagnosed with recurrent disease in the setting of routine clinical care. We also investigated whether a molecular classification scheme is distinct from the histological classification traditionally used in endometrial cancer. Most importantly we asked whether this precision medicine approach could identify “actionable” genomic alterations and inform treatment decisions for individual patients diagnosed with recurrent endometrial cancer in the setting of standard clinical care.

2. Methods

This was an IRB approved retrospective analysis of prospectively collected CGP data in patients with histologically confirmed recurrent endometrial cancer, who underwent prospective CGP between August 2012 and September 2017 and were treated at the University of California Los Angeles and the University of Oklahoma. CGP was prospectively performed in archival tumor specimens from the primary surgery or in specimens obtained from biopsies of recurrent tumors of various endometrial histologic subtypes including endometrioid, papillary serous, clear cell and undifferentiated subtypes. Testing for these patients was performed to help identify potential targeted therapies available on-label or in mechanism-driven clinical trials. Patient and tumor characteristics, as well as treatment data, were retrospectively collected for each identified patient. All patients were known to have relapsed metastatic disease. Clinician use of treatments where targeted therapy was utilized were captured. Responses were based on standard Response Evaluation Criteria in Solid Tumors (RECIST) criteria including the following: complete response (CR), defined as the complete disappearance of all target lesions; partial response (PR), defined as a ≥ 30% decrease in tumor size from baseline; progressive disease (PD), defined as a ≥ 20% increase in tumor size; and stable disease (SD), defined as small changes that do not meet the above criteria [7].

The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin (H&E) stained slides and all samples forwarded for DNA extraction contained a minimum of 20% tumor nuclear area, compared with benign nuclear area. Profiling was performed at Foundation Medicine on DNA extracted from formalin-fixed paraffin embedded (FFPE) samples using a hybrid capture-based next generation sequencing platform (FoundationOne®) [8]. In brief, ≥ 50 ng DNAs was extracted from 40 μm of tumor samples in FFPE tissue blocks. The samples were assayed by CGP using adaptor-ligation and hybrid capture performed for all coding exons of 315 cancer related genes plus select introns from 28 genes frequently rearranged in cancer. Sequencing of captured libraries was performed using the Illumina HiSeq technology to a mean exon coverage depth of >500×, and resultant sequences were analyzed for base substitutions, insertions, deletions, copy number alterations (focal amplifications and homozygous deletions), and select gene fusions, as previously described [8,9]. Tumor mutational burden (TMB) was determined on 1.1megabases (Mb) of sequenced DNA for each case based on the number of somatic base substitution or indel alterations per Mb after filtering to remove known somatic and deleterious mutations, as previously described [10]. Patients were classified as TMB high (TMB—H) or low, using the top quartile threshold and microsatellite instable (MSI-H) or stable (MSS), using a computational algorithm developed by Foundation Medicine. TMB status is reported as TMB-High (≥20 Muts/Mb), TMB-Intermediate (6–19 Muts/Mb) or TMB-Low (≤5 Muts/Mb). The MSI-H/MSS designation is based on genome wide analysis of 95 microsatellite loci and the threshold for MSI-H/MSS was determined by analytical concordance to comparator assays (IHC and PCR) using uterine and colorectal cancer FFPE tissue. Tumors were categorized into the following molecular subtypes: POLE ultramutated, MSI-H, CN-L and CN-H using POLE mutational analysis, mismatch repair status, and p53 mutational analysis as surrogate for ‘copy-number’ status similar to an algorithm developed by Talhouk et al. [5]. TP53 sequencing (if TMB <20) was used to classify the CN high and low groups following determination of microsatellite and POLE groups.

3. Results

The study included 74 patients diagnosed with recurrent metastatic endometrial cancer who underwent genomic profiling as part of standard clinical care. Patient and disease characteristics are shown in Table 1. Tumor histologies were endometrioid in 51% (38/74) of the cases, in serous 28% (21/74), clear cell in 5% (4/74), and undifferentiated in 15% (11/74). The median patient age was 61 years (range 27–86). The majority of patients were initially diagnosed at advanced stages (38% FIGO stage III and 32% FIGO stage IV). High grade tumors were found in 60% (44/74) of the cases. Patients had received extensive prior chemotherapy treatment with a median of 2 (range 0–6) prior lines of chemotherapy. CGP was performed on the archival tissue specimen from the initial surgery in 20% (15/74) and in biopsy specimens obtained from the recurrent tumor in 80% (59/74) of the cases (Table 1)

In this series of patients diagnosed with recurrent metastatic endometrial cancer every tumor sample examined had at least one genomic alteration (GA). Clinically relevant genomic alterations (CRGA) were defined as GA associated with on-label targeted therapies and targeted therapies available in mechanism-driven clinical trials at the time of testing. Among these patients 91% (67/74) had at least one CRGA. The median number of GAs per patient was 6 (range 1–29) and the median number of CRGA was 3 (range 0–7). In this series of patients 32% (24/74) received a matched therapy based on the results of CGP (Table 1).

Using *POLE* mutational analysis, mismatch repair status, and p53 mutational analysis as a surrogate for ‘copy-number’ status, CGP

Table 1
Patient and disease characteristics ($n = 74$).

	N = 74	%
Median age at diagnosis (range)	61 (27–85)	
Histology		
Endometrioid	38	51.4
Serous	21	28.4
Clear cell	4	5.4
Undifferentiated	11	14.9
FIGO stage		
I	12	16.2
II	7	9.5
III	28	37.8
IV	24	32.4
Grade[a]		
1	12	16.2
2	15	20.3
3	44	59.5
Unknown[a]	3	4.1
Median number of prior lines of chemotherapy (range)	2 (0–6)	
Molecular subtype		
POLE ultramutated	1	1.4
MSI-H	13	17.6
Copy number high (CN-H)	32	43.2
Copy number low (CN-L)	28	37.8
Genomic alterations (GAs)		
Patients with a GA	74	100
Patients with a clinically relevant GA (CRGA)[a]	67	90.5
Median GAs per patient (range)	6 (1–29)	
Median CRGAs per patient (range)	3 (0–7)	
Matched therapy		
Patients with matched therapy	24	32.4

[a] CRGA were defined as GA associated with on-label targeted therapies and targeted therapies in mechanism-driven clinical trials.

Table 2
Genomic alterations per molecular subtype.

	POLE (N = 1)	MSI-H (N = 13)	CN-L (N = 28)	CN-H (N = 32)
Median GAs per patient (Range)	29	12 (5–27)	5 (1−11)	4 (1–9)
Median CRGAs per patient (range)	6	5 (3–7)	3 (0–6)	1 (0–5)
Median TMB	425	24.3 (11.2–48)	3.15 (0–22.8)	3.26 (0.9–8.1)
TMB				
Low (<5 Muts/Mb)	0	0	18 (64.3%)	23 (71.9%)
Intermediate (5– < 20 Muts/Mb)	0	5 (38.5%)	9 (32.1%)	9 (28.1%)
High (>20 Muts/Mb)	1	8 (61.5%)	1 (3.6%)	0
Histologic subtype*				
Endometrioid (N = 38)	1 (2.6%)	10 (26.3%)	19 (50.0%)	8 (21.1%)
Serous (N = 21))	0	0	4 (19.1%)	17 (80.9%)
Clear cell (N = 4)	0	1 (20.0%)	2 (50.0%)	2 (50.0%)
Undifferentiated (N = 11)	0	3 (27.3%)	3 (27.3%)	5 (45.5%)
Grade**				
1 (N = 12)	0	4 (33.3%)	6 (50.0%)	2 (16.7%)
2 (N = 15)	1 (6.7%)	3 (20.0%)	9 (60.0%)	2 (13.3%)
3 (N = 44)	0	4 (9.1%)	12 (27.3%)	28 (63.6%)

* Chi Square Test: $p = 0.001$.
** Missing n = 3, Chi Square Test: $p = 0.003$.

segregated all cases into four TCGA molecular subgroups. One case was found to have very high TMB (425 Muts/Mb) with micro-satellite stable status and confirmed to have a mutation in *POLE*. This was a 63-year-old patient diagnosed with recurrent endometrial cancer in April 2011 following an initial diagnosis of a moderately differentiated FIGO Stage IA endometrioid endometrial cancer 3 years earlier. Prior to CGP in July 2013 she had by then failed 2 lines of carboplatin paclitaxel based chemotherapy, as well as pelvic radiation. CPG demonstrated 29 GAs and 7 CRGAs (*PIK3CA*, *PTEN*, *NF1*, *NF2*, *PTCH1*, *BRCA2* and *KIT*). In August 2013, the patient subsequently started treatment with pazopanib, a multi-targeted kinase inhibitors which inhibits signaling in a variety of receptors such as VEGFR-1,-2 and −3, platelet-derived growth factor receptors (PDGFR) and c-KIT. Of note, she experienced a complete remission lasting for >5 years until a small para aortic lymph node recurrence was diagnosed again in December 2018.

MSI-H status was reported in 18% (13/74) of patient's tumors which demonstrated a median TMB of 24.3 (range 11.2–48) Muts/Mb. The median number of GAs and CRGAs were 12 (range 5–27) and 5 (range 3–7), respectively (Table 2). High TMB (>20 Muts/Mb) was only seen in the MSI-H and POLE subgroups (Table 2). Notably, of the 13 patients categorized as MSI-H, only 5 (38%) had mutations in MMR genes confirming that other mechanism, such as epigenetic silencing of MMR genes likewise contribute to MSI [11].

CGP identified 81% (60/74) as MSS and separated 39% (28/74) of these into the CN-L subgroup and 43% (32/74) into the CN-H subgroup with each subgroup demonstrating a low median TMB of 3 (range 0–-23) Muts/Mb and 4 (range 1–8) Muts/Mb, respectively (Table 2). However, although the median TMB appeared low in both CN-L and CN-H cases, intermediate TMB (5– < 20 Muts/Mb) was found in 32% (9/28) and 28% (9/32) of the CN-L and CN-H cases, respectively, underscoring the potential importance of evaluating immunotherapy in molecular subtypes other than MSI-H cases specifically if they are found to have intermediate or high TMB. In the CN-L group the median number of GAs and CRGAs were 5 (range 1–11) and 3 (range 0–6), respectively. In the CN-H group the median number of GAs and CRGAs were 4 (range 1–9) and 1 (range 0–5), respectively (Table 2).

While 81% (17/21) of recurrent serous endometrial cancers were predominantly found to be CN-H, we found no clear prevalence of a specific molecular subtype in endometrioid, clear cell or undifferentiated tumors (Table 2). In fact, of the 38 endometrioid endometrial cancers 50% (19/38) were CN-L, 26% (10/38) MSI-H, and 21% (8/38) CN-H. Similarly, although high grade tumors were more likely to be associated with the CN-H subgroup, and low or moderate grade tumors were more likely to be found in the MSI-H and CN-L group the observed overlap was not accurate (Table 2). More importantly neither grade nor histology provide specific information on molecular alterations that may have therapeutic implications for patients with recurrent endometrial cancer.

Distinct patters of mutations were seen between the four molecular subgroups. While the frequency of mutations in phosphatidylinositol 3-kinase catalytic subunit alpha (*PIK3CA*) was comparable across molecular subgroups (MSI-H: 46%, CN-L: 50%, CN-H: 41%) clear differences were seen for most other genes. The frequency of mutations in the phosphatase and tensin homolog (*PTEN*) gene varied between subgroups (MSI-H: 92%, CN-L: 61%, CN-H: 19%). Likewise, the frequency of mutations in AT-rich interactive domain 1A (*ARID1A*) varied similarly (MSI-H: 77%, CN-L: 46%, CN-H: 6%). *PIK3R1*, *KRAS*, *JAK1* and *PTCH1* mutations were more common in MSI-H tumors, whereas *PPP2R1A* mutations or *HER2* and *CCNE1* amplifications were more commonly seen in CN-H tumors. Mutations in Catenin (Cadherin-Associated Protein) Beta 1 (*CTNNB1*) were most common in the CN-L group (Table 3).

CGP allowed us to select a targeted treatment option in 32% (24/74) of patients (see Fig. 1). These patients had received prior treatments for their recurrent disease with a median number of 2 (range 1–4) prior chemotherapy regimens. The most commonly utilized therapies were agents targeting the PI3K/PTEN/mTOR pathway and immune therapy with pembrolizumab. Less commonly used inhibitors targeted *HER2* and *PTCH1*. A summary of matched treatments with the respective target and the best responses and treatment duration is summarized in Table 4. Objective responses to the matched targeted therapy were seen in 25% (6/24) of patients with 37.5% (9/24) achieving stable disease (SD) leading to a clinical benefit rate of 62.5% with a median treatment duration in these patients of 14.6 months (range 4.3–69 months). Moreover, of the 6 patients treated with immunotherapy 2 experienced a partial response and 3 demonstrated disease stabilization with a median treatment duration in these patients of 17 months (range 5.7–-28 months) (Table 5).

Table 3
Copy number variations and short variants per molecular subgroup.

	All (n = 74)		MSI-H (N = 13)		CN-L (N = 28)		CN-H (N = 32)	
Copy number variations								
CCNE1	6	8.11%	–	0%	1	3.57%	5	15.63%
HER2	6	8.11%	–	0%	–	0%	6	18.75%
MYC	5	6.76%	–	0%	2	7.14%	3	9.38%
MCL1	3	4.05%	2	15.38%	1	3.57%	–	0%
CDKN2A	2	2.70%	–	0%	2	7.14%	–	0%
HER3	3	4.05%	–	0%	–	0%	3	9.38%
AKT2	2	2.70%	–	0%	–	0%	2	6.25%
Short variants								
PTEN	36	48.65%	12	92.31%	17	60.71%	6	18.75%
TP53	35	47.30%	–	0%	–	0%	31	96.88%
PIK3CA	34	45.95%	6	46.15%	14	50.00%	13	40.63%
ARID1A	26	35.14%	10	76.92	13	46.43%	2	6.25%
CTNNB1	15	20.27%	3	23.08%	11	39.29%	1	3.13%
PIK3R1	13	17.57%	5	38.46%	4	14.29%	4	12.50%
KRAS	11	14.86%	4	30.77%	4	14.29%	3	9.38%
PPP2R1A	9	12.16%	–	0%	2	7.14%	7	21.88%
PPP2R1A	9	12.16%	–	0%	2	7.14%	7	21.88%
FBXW7	8	10.81%	1	7.69%	2	7.14%	5	15.63%
KMT2C	8	10.81%	5	38.46%	2	7.14%	1	3.13%
KMT2D	7	9.46%	3	23.08%	2	7.14%	2	6.25%
BCOR	7	9.46%	2	15.38%	3	10.71%	2	6.25%
BRCA2	7	9.46%	2	15.38%	2	7.14%	2	6.25%
KMT2D	7	9.46%	3	23.08%	2	7.14%	2	6.25%
CREBBP	6	8.11%	2	15.38%	3	10.71%	–	0%
FGFR2	6	8.11%	3	23.08%	3	10.71%	–	0%
JAK1	6	8.11%	6	46.15%	–	0%	–	0%
CTCF	5	6.76%	4	30.77%	1	3.57%	–	0%
RNF43	5	6.76%	4	30.77%	1	3.57%	–	0%
NOTCH3	4	5.41%	2	15.38%	1	3.57%	1	3.13%
PTCH1	4	5.41%	3	23.08%	–	0%	–	3.13%
ESR	3	4.05%	1	7.69%	2	7.14%	–	0%
BRCA1	1	1.35%	1	7.69%	–	0%	–	0%

Potential predictors of drug resistance were also identified. For example, loss-of-function mutations in Janus Kinase (*JAK1/2*) may lead to acquired resistance to anti-programmed death protein 1 (PD-1)

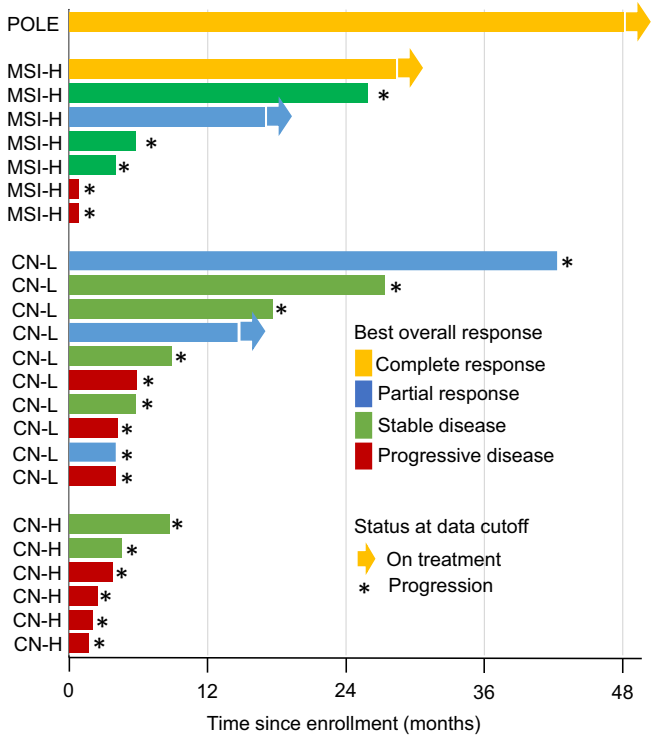

Fig. 1. Treatment duration and best response for all patients achieving a clinical benefit grouped by molecular subtype.

Table 4
Matched therapies.

Molecular subtype	TMB	Matched therapy	CRGA	Best response	Treatment duration in months
POLE	425	Pazopanib	KIT	CR	69
MSI-H	48	Pembrolizumab	MSI-H	PR	16.9
	45	Temsirolimus	PI3K/PTEN	PD	0.8
	30.3	Everolimus + aromatase inhibitor (AI)	PI3K/PTEN	SD	4.03
	24.3	Pembrolizumab	MSI-H	CR	28.3
	15.7	Pembrolizumab	MSI-H	PD	0.77
	14	Pembrolizumab	MSI-H	SD	25.9
	11.2	Everolimus + AI	PI3K/PTEN	SD	5.7
	6	BGJ398	FGFR2	PD	4
CN-L	22.8	Pembrolizumab	TMB	SD	11.5
	5.4	Temsirolimus	PI3K	SD	8.9
	4.5	Temsirolimus	PI3K	SD	17.6
	4.4	Palbociclib + AI	KRAS	PR	14.6
	3.6	GDC-0032 (PIK3 Inhibitor)	PI3K/PTEN	PD	5.8
	2.7	MLN0128 (mTOR Inhibitor)	PTEN	PR	4.0
	2.7	Prexasertib + LY3023414 (mTOR Inhibitor)	PI3K	PD	4.2
	1.3	Copanlisib	PI3K/PTEN	SD	27.4
	0	BYL719 AMG479	PI3K/PTEN	PR	42.3
CN-H	8.1	Everolimus	PI3K/PTEN	PD	2.0
	7.2	Pembrolizumab	TMB	SD	8.7
	4.5	TDM1	HER2	SD	4.6
	3.6	Trastuzumab	HER2	PD	1.7
	3.6	Vismodegib	SMO	PD	3.7
	0.9	Everolimus + AI	PI3K/PTEN	PD	2.4

therapy in malignant melanoma [12]. It has been proposed that *JAK1/2* loss-of-function mutations are a genetic mechanism of lack of reactive PD-L1 expression and response to interferon gamma, leading to primary resistance to PD-1 blockade therapy (12). Of note, 46% (6/13) of MSI-H tumors harbored a mutation in *JAK1/2* whereas none were found in either the CN-H or CN-L groups (Table 3). It is worth further study, that of the 4 patients in the MSI-H group that received pembrolizumab the 2 that demonstrated an ongoing objective response to pembrolizumab did not have a mutation in *JAK1/2*, whereas the 2 that did not respond to immunotherapy had a mutation in *JAK1*. Evaluation of Estrogen receptor (*ESR1*) mutations have been associated with resistance to aromatase inhibitors and decreased sensitivity to fulvestrant in breast cancer [13,14]. *ESR-1* mutations were found in 23% (3/13) of MSI-H and 11% (3/28) of CN-L cases, respectively.

4. Discussion

The value of genomic data is apparent in multiple cancers where molecular alterations define distinct clinical groups. For example, in non–small cell lung cancer (NSCLC), alterations in 8 genes are

Table 5
Matched therapy response rates.

Best response to targeted therapy	(N = 24)	%
Complete response	2	8.3
Partial response	4	16.7
Stable disease	9	37.5
Progressive disease	9	37.5
Median duration of targeted therapy in months	14.6 (4.3–69)	
Best response to immunotherapy	**(N = 6)**	
Complete response	1	16.7
Partial response	1	16.7
Stable disease	3	50
Progressive disease	1	16.7
Median duration of therapy immunotherapy in months	17 (5.7–28.3)	

associated with sensitivity to targeted inhibitors and genomic analyses of these targets is now recommended in treatment guidelines [15]. Despite of the now well understood molecular heterogeneity of endometrial cancer and its promise for treatment stratification, this knowledge has not yet been introduced into clinical practice nor has it been broadly integrated into clinical trial designs. The purpose of this study was to demonstrate the clinical utility of prospective next generation sequencing in patients with advanced endometrial cancer and to define the value of this in matching patients to molecularly-driven therapeutics. With the implementation of a next generation sequencing platform in the clinic, we prospectively interrogated 74 patients diagnosed with recurrent endometrial cancer treated at UCLA and the University of Oklahoma. In this study, we demonstrate the utility of CGP for molecular classification of relapsed endometrial cancer into the four Cancer Genome Atlas categories: POLE ultramutated, MSI-H, CN-L, and CN-H. Moreover, we confirm genomic findings derived from retrospectively analyzed cohorts of surgically-resected, early-stage, endometrial cancers and extend these observations to an advanced patient population more representative of those treated as part of routine clinical practice.

Using POLE mutational analysis, mismatch repair status, and p53 mutational analysis as a surrogate for 'copy-number' status CGP segregated all cases into four TCGA molecular subgroups. The fact that this classification, however, did not demonstrate prognostic relevance may have been due to the advanced nature of our patient cohort. Importantly, however, our data are consistent with prior reports regarding the frequency and overall distribution of oncogenic alterations in endometrial cancer. Consistent with earlier reports, mutations in *PTEN* and *ARID1A* were the highest in the MSI-H group, mutations in *CTNNB1* were the highest in the CN-L group and mutations in p53 and *PPP2R1A* were the highest in the CN-H group [4]. We did not observe the high frequency of MSI-H patients in this prospective series. This may have also been due to the advanced nature of our patient cohort, as MSI-H has been shown to be a favorable prognostic indicator [11]. While recurrent serous endometrial cancers were predominantly found to be CN-H, we found no clear prevalence of a specific molecular subtype in endometrioid, clear cell or undifferentiated tumors. This underscores the potential advantages of a molecular classification when compared to the traditional histologic separation specifically for endometrioid, clear cell and undifferentiated endometrial cancers because it provides a more accurate means for treatment stratification due to enrichment of subtype specific CRGAs and due to the broad genomic feature of microsatellite instability as biomarker of sensitivity for immune checkpoint inhibitors. In addition, deficiency in homologous recombination (HR) which is more likely to be found in CN-H tumors may also become a potential biomarker for sensitivity toward PARP inhibitors [16,17].

As our sampling and analysis strategy occurred in parallel with clinical care, we were able to explore potential predictive genetic markers for targeted therapeutics, such PI3K/mTOR and immune checkpoint inhibitors. In this series 32% (24/74) of patients received a matched therapy based on the results of CGP. The most commonly utilized therapies were agents targeting the PI3K/PTEN/mTOR pathway and immune therapy with pembrolizumab. Objective responses to the matched targeted therapy were seen in 25% (6/24) of patients with 37.5% (9/24) of patients achieving stable disease leading to a clinical benefit rate of 62.5%. These patients received a targeted therapy for a median time of 14.6 months (range 4.3–69 months) which compares very favorably with previously reported activity for chemotherapy and other anticancer agents in patients with previously treated recurrent endometrial cancer [18–20]. Immunotherapy with PD1 – blockade has shown efficacy in endometrial cancer with MSI-H status [21]. Although the number is small in the current series we were able to confirm a notable anti-tumor activity in heavily pretreated endometrial cancer patients who received pembrolizumab based on microsatellite instability or increased TMB. Notably, of those patients categorized as MSI-H, only 38% of these had mutations in MMR genes confirming that other mechanism such as epigenetic silencing of MMR genes frequently contribute to MSI [11]. Despite of the notable clinical activity of PD-1 blockade in MSI-H endometrial cancer drug resistance still poses a clinical challenge. In our series 46% (6/13) of MSI-H tumors harbored a mutation in *JAK1/2* whereas none were found in either the CN-H or CN-L groups. Because it has been proposed that *JAK1/2* loss-of-function mutations are a genetic mechanism of lack of reactive PD-L1 expression and response to interferon gamma, leading to primary resistance to PD-1 blockade therapy further investigation of *JAK1/2* loss-of-function mutations in MSI-H endometrial cancer is clearly warranted.

Other actionable mutations were identified only in a small subset of patients but CGP permitted real-time interpretation and utilization of these results in clinical practice. As illustrated in the presented patient with a POLE mutation, identification of a POLE mutation with high TMB and increased number of CRGAs in endometrioid endometrial cancer provides ample opportunity for targeted therapy that would not otherwise be recognized. CGP also detected mutations in BRCA1/2 in 11% (8/74) of the cases. Despite these insights, PARP inhibitors have not yet been broadly assessed in endometrial cancer patients.

Our study has several notable limitations. First, the targeted capture approach used in this study could not by definition detect alterations in genes not included in the assay design. Secondly, epigenetic mechanisms of gene suppression can also not be detected by CGP. Thirdly, tumor heterogeneity is well documented in advanced cancer and sampling a single site of disease can never fully assess clonal complexity in patients with multisite metastatic disease [22]. Fourth, the molecular subtypes of endometrial cancer have been developed in primary endometrial cancer and it is not clear whether and how the subtypes change in recurrent disease. The current study will not allow us to address this issue. Nevertheless, despite these limitations, our data represent the first attempt to link real time NGS to clinical practice in patients diagnosed with and treated for advanced endometrial cancer and suggest that CGP done in routine clinical care may benefit patient diagnosed with recurrent endometrial cancer. Although the direct clinical impact of prospective CGP for endometrial cancer currently has less utility than in other solid tumors such as lung cancer and melanoma our data suggest that most patients diagnosed with recurrent endometrial cancer harbor a CRGA or broad genomic features which are potentially actionable alterations that could be targets for currently available FDA approved drugs, or agents in active clinical development. Linking CGP to routine clinical care has the potential to identify those endometrial cancer patients most likely to benefit from select standard therapies and should be used in an investigational context to match patients to genome-directed targeted therapies.

Author contributions

Emily N. Prendergast, Laura L. Holman, Kathleen Moore, Julia A. Elvin, Joshua Cohen and Gottfried E Konecny conceived project, collected data, analyzed data, prepared manuscript and figures. Annie Y. Liu, Tiffany S. Lai, Maira P. Campos, Jacquline N. Fahey, Nabilah Abdelaal and Jian Yu Rao collected data. Xiaoyan Wang analyzed data and prepared figures and reviewed the manuscript. All authors approved the final manuscript.

Declaration of Competing Interest

All of the authors have completed the disclosure form. Julia Elvin is an employee of Foundation Medicine and all authors have no conflict of interest with the content of the manuscript.

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