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Massively parallel sequencing analysis of mucinous ovarian carcinomas: genomic profiling and differential diagnoses

Jennifer J Mueller[a,1], **Brooke A Schlappe**[a,1], **Rahul Kumar**[b], **Narciso Olvera**[a,2], **Fanny Dao**[a,2], **Nadeem Abu-Rustum**[a], **Carol Aghajanian**[c], **Deborah DeLair**[b], **Yaser R Hussein**[b], **Robert A Soslow**[b], **Douglas A Levine**[a,2], and **Britta Weigelt**[b]

[a]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[b]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[c]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Abstract

Objective—Mucinous ovarian cancer (MOC) is a rare type of epithelial ovarian cancer resistant to standard chemotherapy regimens. We sought to characterize the repertoire of somatic mutations in MOCs and to define the contribution of massively parallel sequencing to the classification of tumors diagnosed as primary MOCs.

Methods—Following gynecologic pathology and chart review, DNA samples obtained from primary MOCs and matched normal tissues/blood were subjected to whole-exome (n=9) or massively parallel sequencing targeting 341 cancer genes (n=15). Immunohistochemical analysis of estrogen receptor, progesterone receptor, PTEN, ARID1A/BAF250a, and the DNA mismatch (MMR) proteins MSH6 and PMS2 was performed for all cases. Mutational frequencies of MOCs were compared to those of high-grade serous ovarian cancers (HGSOCs) and mucinous tumors from other sites.

Correspondence to: Britta Weigelt, PhD, Department of Pathology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. weigeltb@mskcc.org; Tel: +1-212-639-2332, Fax: +1-212-639-2502.

[1]These authors contributed equally.

[2]Present address: Gynecologic Oncology, Laura and Isaac Perlmutter Cancer Center, NYU Langone Health, New York, NY, USA

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

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Results—MOCs were heterogeneous at the genetic level, frequently harboring *TP53* (75%), *KRAS* (71%) mutations and/or *CDKN2A/B* homozygous deletions/mutations (33%). Although established criteria for diagnosis were employed, four cases harbored mutational and immunohistochemical profiles similar to those of endometrioid carcinomas, and one case for colorectal or endometrioid carcinoma. Significant differences in the frequencies of *KRAS, TP53, CDKN2A, FBXW7, PIK3CA* and/or *APC* mutations between the confirmed primary MOCs (n=19) and HGSOCs, mucinous gastric and/or mucinous colorectal carcinomas were found, whereas no differences in the 341 genes studied between MOCs and mucinous pancreatic carcinomas were identified.

Conclusions—Our findings suggest that the assessment of mutations affecting *TP53*, *KRAS*, *PIK3CA*, *ARID1A* and *POLE*, and DNA MMR protein expression may be used to further aid the diagnosis and treatment decision-making of primary MOC.

Keywords

mucinous ovarian cancer; massively parallel sequencing; immunohistochemistry; classification; diagnosis

1. INTRODUCTION

Mucinous adenocarcinoma of the ovary is a rare type of epithelial ovarian cancer, representing approximately 3-4% of all epithelial ovarian malignancies [1]. These tumors are distinct at the biological, clinical and genetic levels from the common high-grade serous ovarian cancers (HGSOCs). Contrary to HGSOCs, mucinous ovarian cancers (MOCs) frequently present with early stage ovarian-confined disease associated with an overall favorable prognosis (stage I, 5 year survival rate 91%) [2,3]. In advanced stage disease, however, MOC is associated with poor outcome and overall survival rates lower than those reported for advanced stage HGSOC (5-year survival 11% MOC vs 26% HGSOC) [2,4–6]. MOCs are less sensitive to standard platinum/taxane chemotherapy regimens than HGSOCs, which may contribute to the observed poor outcome when diagnosed in advanced stages [4,7]. Previous studies employing targeted and whole-exome massively parallel sequencing have revealed that primary MOCs are heterogeneous at the genomic level, with *TP53* (52-57%), *KRAS* (45-65%), *BRAF* (23%), *ERBB2* (23%) and *CDKN2A* (60%) being the genes most commonly altered [8,9], and that these tumors may be distinct from other ovarian cancer subtypes at the genomic level.

The diagnosis of primary MOC and the differentiation from metastatic mucinous tumors originating in extraovarian primary sites, most commonly from the colorectum, especially appendiceal, is challenging [10,11]. Pathology review of 44 presumed MOCs as part of a prospective Gynecological Oncology Group phase 3 trial led to the reclassification of 61% of cases as ovarian metastases from tumors originating in other primary sites [6]. Therefore, the integration of clinical history and pathologic features has been shown to be essential for the diagnosis of primary MOC and to discriminate these tumors from their metastatic mimics [6,10]. Laterality and size provide important information as primary MOCs typically present as unilateral tumors measuring >10cm as compared to metastatic lesions [10]. In addition, immunohistochemistry has been used as an ancillary diagnostic test for the

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differentiation between MOCs and adenocarcinomas from other anatomical sites, in particular those demonstrating lower intestinal differentiation [11–16]. In advanced stage mucinous ovarian disease, upper and lower endoscopy, CT or PET imaging and/or serum tumor markers are warranted to rule out the presence of an extra-ovarian primary cancer (NCCN guidelines, https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf).

In this study, we sought to characterize the repertoire of somatic genetic alterations focusing on key cancer genes in MOCs and to define the contribution of massively parallel sequencing to the classification of tumors diagnosed as MOCs based on current clinicopathologic criteria. To achieve these aims, we subjected a series of 24 MOCs to whole-exome sequencing (n=9) or massively parallel sequencing targeting all exons of 341 key cancer genes (MSK-IMPACT; n=15).

2. MATERIAL AND METHODS

Case selection

All primary MOCs diagnosed between July 2001–July 2013 with available tissue slides and blocks were retrieved from the files of the Department of Pathology at Memorial Sloan Kettering Cancer Center (MSKCC). This study was approved by the Institutional Review Board (IRB) of MSKCC, and patient consent was obtained where appropriate. Representative sections of each case diagnosed as primary MOC were re-reviewed by gynecologic pathologists (YRH, RAS, DD), and clinical information, including age, stage, laterality, therapy, endoscopy and follow-up, was obtained from the medical records. Invasive MOC was defined as the presence of confluent tumor cells with intracytoplasmic mucin, measuring >10mm^2 and at least 5mm in one linear dimension [3]. Unilateral tumor size ≥10cm and expression of CK7 with or without CK20 expression were used to confirm the diagnosis of primary MOC along with normal upper and lower endoscopy and prolonged clinical follow-up without evidence of gastrointestinal involvement in patients with advanced stage disease, and the absence of the development of a second primary tumor during follow-up; in addition, cases with a unilateral tumor size <10cm or bilateral disease with a tumor size ≥10cm were acceptable if PAX8 was expressed [10,12,13,15,17]. The ovarian origin of the stage I carcinomas as opposed to a gastrointestinal carcinoma metastatic to the ovary was further confirmed by the prolonged survival, as 71% (12/17) of the stage I tumors had no evidence of disease at a median follow-up of 82 months (range 41-185 months), 12% (2/17) were alive with disease (40 and 326 months follow-up), and 18% (3/17) died of unknown cause (62, 98 and 102 months follow-up; Table 1). Following this review, 24 primary MOCs (15 formalin-fixed paraffin-embedded (FFPE), 9 fresh frozen) were included in this study.

Immunohistochemistry

Immunohistochemistry (IHC) for CK7, CK20 and PAX8, was performed in the diagnostic work-up of the tumors, following previously validated protocols [15,18]. For CK7 and CK20 only cytoplasmic staining was considered positive, and for PAX8 only nuclear staining was considered positive [15,18]. After sequencing, additional immunohistochemical analysis was performed for estrogen receptor (ER) and progesterone receptor (PR), PTEN, ARID1A/

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BAF250a and the DNA mismatch repair proteins MSH6 and PMS2 using previously described protocol [19–22]. ER and PR expression was defined as positive when >1% of the tumor nuclei showed immunoreactivity, following the ASCO/CAP guidelines for breast cancer [23]. Loss of MSH6, PMS2 and ARID1A expression was defined as complete absence of protein expression in unequivocal tumor cell nuclei [24]. Normal epithelium and stroma were used as internal controls for PTEN, MSH6, PMS2 and ARID1A expression; PTEN expression was defined as lost if tumor cells displayed no immunoreactivity or less than the internal control.

DNA extraction

Tumor sections were reviewed by two gynecologic pathologists (YRH, RAS) to ensure >20% neoplastic cells. Matched normal DNA was extracted from peripheral blood lymphocytes or normal tissue sections (benign lymph node), confirmed to be devoid of any neoplastic cells. Genomic DNA from tumor- and matched normal samples was extracted using the DNeasy Blood & Tissue kit (Qiagen).

Whole-exome and targeted massively parallel sequencing

Tumor and matched normal DNA samples were subjected to whole-exome sequencing (n=9) or massively parallel sequencing (n=15) using the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay targeting all exons, selected intronic and regulatory regions of 341 key cancer genes, as previously described [25,26]. Sequencing data were analyzed as previously described (Supplementary Methods) [24,26]. Cancer cell fractions of each mutation were inferred using ABSOLUTE (v1.0.6) [27], as previously described [24,26]. Mutational signatures were defined for cases with at least 20 somatic mutations using deconstructSigs [28].

Comparison with high-grade serous ovarian, colorectal, gastric and pancreatic carcinomas

The mutational frequencies of the 341 genes in our targeted sequencing panel of MOCs were compared to those of HGSOCs from The Cancer Genome Atlas (TCGA; n=316) [29], mucinous colorectal carcinomas (TCGA; n=32) [30], mucinous gastric adenocarcinomas (TCGA; n=18)[31], pancreatic adenocarcinomas from the International Cancer Genome Consortium (ICGC; mucinous cystadenocarcinoma/intraductal papillary mucinous neoplasm with invasion, n=11; pancreatic ductal adenocarcinoma, n=177) [32] and to MOCs described by Ryland et al (n=12) [9]. The whole-exome sequencing-derived mutational data of the mucinous colorectal carcinomas and mucinous gastric adenocarcinomas were retrieved from GDAC Firehose (https://gdac.broadinstitute.org/; Mutation Annotation File) and of the HGSOCs and pancreatic adenocarcinomas from cBioPortal (http://www.cbioportal.org/) [33]. We restricted the comparison to the 341 genes targeted by our sequencing panel. Comparisons were performed using Fisher’s exact tests, and p-values <0.01 were deemed statistically significant.

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3. RESULTS

The repertoire somatic mutations and copy number alterations of mucinous ovarian cancers

After secondary specialist gynecologic pathology and chart review, a final diagnosis of primary MOC was rendered in 24 cases (Table 1, Fig. 1). Whole-exome sequencing was performed at a median depth of coverage of 127x (range 92x-141x) for tumor and 119x (81x-156x) for normal samples, respectively, and targeted massively parallel sequencing of 266x (range 27x-620x) and 180x (range 27x-485x) for tumor and normal samples, respectively (Supplementary Table S1). When focusing on the 341 cancer-related genes, the 24 MOCs studied here harbored a median of 3.5 (range 1-189) non-synonymous somatic mutations; the mutational burden affecting these 341 genes that was significantly higher than that of HGSOCs (2, range 0-9 non-synonymous mutations; $p<0.001$, Mann-Whitney U test) but significantly lower than that of mucinous colorectal carcinomas (12, range 4-206 non-synonymous mutations; $p<0.001$, Mann-Whitney U test) from the TCGA datasets [29,30]. One of the MOCs analyzed here (MOC62) harbored a pathogenic *POLE* A456P exonuclease domain mutation, and a high mutational burden with 189 non-synonymous somatic mutations in the 341 genes tested (Supplementary Table S2).

The genes most frequently affected by somatic mutations were *TP53* (18/24; 75%) and *KRAS* (17/24; 71%), which co-occurred in 13 MOCs (54%; Fig. 2), consistent with previously reported results [8,9,34]. Of the 18 *TP53* mutations, 14 were found to be clonal (i.e. to be present in all cancer cells within a sample), 14 affected *TP53* hotspot codons (58%), and 12 (50%) *TP53* mutations displayed loss of heterozygosity (LOH) of the wild-type allele. All *KRAS* mutations affected the hotspot codons G12 (13/17), G13 (1/17) or Q61 (3/17), and all but three *KRAS* mutation were clonal (Fig. 2). Additional recurrent somatic mutations found in these tumors were *SETD2* (4/24), *GNAS* (3/24) and *ERBB3* (3/24), amongst others.

MOCs displayed a heterogeneous pattern of copy number alterations, with 25% (6/24) cases harboring *CDKN2A/B* homozygous deletions (Fig. 2; Supplementary Fig. S1). Of note, two cases lacking *CDKN2A/B* homozygous deletions had a *CDKN2A* hotspot mutations coupled with LOH of the wild-type allele (MOC1) or a *CDKN2A* in-frame indel (MOC67). In addition, single cases harbored amplification of *ERBB2* (MOC05), *MYC* (MOC04) or *AKT2* (MOC25), or homozygous deletions of *RB1* (MOC31) or *SMAD4* (MOC67, Fig. 2; Supplementary Fig. S1).

Mutational signature analysis for the cases with at least 20 somatic mutations (n=11) revealed that all but two MOCs displayed a dominant signature 1 associated with aging (Fig. 3A) [35]. We further noted that of the nine MOCs with a signature 1, seven displayed an underlying mutational signature 3 associated with defective homologous recombination DNA repair (Fig. 3A) [35]. MOC62, the case with a pathogenic *POLE* A456P exonuclease domain mutation, had a *POLE* mutational signature (signature 10) and MOC38 a mutational signature associated with defective DNA MMR (mutational signature 6; Fig. 3B).

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Re-classification of presumed primary MOCs using ancillary markers

Whilst the majority of MOCs included in this study harbored alterations affecting the most common drivers of these lesions (i.e. *TP53*, *KRAS* and/or *CDKN2A*), we did identify six MOCs either lacking *TP53*, *KRAS* or *CDKN2A* mutations/homozygous deletions and/or harboring mutations in genes reported to be commonly mutated in endometrioid ovarian or endometrioid endometrial cancers [36,37], including *PIK3CA* or *CTNNB1* hotspot mutations and/or *ARID1A* truncating/frameshift mutations (i.e. MOC10, MOC17, MOC19, MOC38, MOC46, MOC62; Fig. 2). To define whether these MOCs diagnosed upon re-review were truly primary MOCs, we performed a detailed analysis of the mutational profiles of these six tumors, and subjected them to immunohistochemical analysis using ER, PR, DNA MMR proteins, PTEN and ARID1A.

MOC46, which harbored *KRAS* and *CTNNB1* hotspot mutations, was ER- and PR-negative and retained DNA MMR, PTEN and ARID1A expression; these features are consistent with a diagnosis of primary MOC. MOC62, however, a 12cm unilateral ER-negative, PR-negative, PAX8-negative tumor, harbored a *POLE* exonuclease domain mutation, a *POLE* mutational signature (signature 10) and also one truncating hotspot (p.R1114*) and two missense mutations affecting the tumor suppressor gene *APC* (Table 1; Figs. 2 and 3; Supplementary Table S2), suggesting that this tumor is unlikely to constitute a primary MOC. The differential diagnosis includes a primary ovarian carcinoma that lacks PAX8 expression or a metastasis from an occult extraovarian carcinoma (e.g. mucinous carcinoma of colorectal origin), however the patient did not develop an extraovarian carcinoma during surveillance following oophorectomy, and was alive without evidence of disease at 61 months follow-up (Table 1). Primary MOCs frequently lack PAX8 expression [18], as do many adenocarcinomas that arise in teratomas. Rare endometrioid carcinomas with or without mucinous differentiation may also, on occasion, be PAX8-negative.

Four MOCs displayed features consistent with a diagnosis of an endometrioid ovarian carcinoma with mucinous differentiation: 1) MOC38, a 7cm unilateral tumor, which harbored 28 non-synonymous mutations in the 341 genes tested, including a *TP53*, *PIK3CA* hotspot and *ARID1A* frameshift mutation, and the mutational signature 6 associated with defective DNA MMR and microsatellite instability (Fig. 2), was found to be ER-positive and to lack ARID1A and MLH1/PMS2 protein expression. The uterine pathology of this case revealed a complex atypical hyperplasia, however no invasive component was found. 2) MOC17, a 20cm unilateral lesion, which lacked *KRAS* or *TP53* mutations presented with a *PIK3CA* hotspot mutation, was ER- and PR-positive and showed PTEN loss of expression (Fig. 2; Table 2). 3) MOC10, a 11cm unilateral tumor, harbored a *KRAS* mutation but also an *ARID1A* truncating mutation, a *PTEN* missense mutation and a *PTEN* frameshift insertion, expressed ER and PR, and showed equivocal PTEN expression by immunohistochemistry. 4) MOC19, which harbored a *KRAS* hotspot and *ARID1A* truncating mutation coupled with loss of ARID1A protein expression and was PR-positive.

Our findings indicate that in addition to tumor size, laterality, CK7, CK20, ER and PR expression, the repertoire of mutations affecting a small panel of genes, including *TP53*, *KRAS*, *PIK3CA*, *ARID1A* and *POLE*, coupled with the assessment of DNA MMR protein

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expression may provide useful information for the differential diagnosis of mucinous tumors affecting the ovary.

Comparison of the mutational repertoire of MOCs with mucinous carcinomas from other sites

We next compared the repertoire of somatic mutations affecting the 341 genes included in the MSK-IMPACT assay in the MOCs studied here and in HGSOCs and mucinous cancers from other sites. For this analysis, only the tumors with a mutational/immunohistochemical profile suggestive of *bona fide* primary MOC were included in the comparisons (i.e. MOC10, MOC17, MOC19, MOC38 and MOC62 were excluded), whose pattern of mutations affecting the 341 cancer-related genes studied here did not significantly differ from those of the invasive MOCs reported in Ryland et al [9] (Supplementary Table S3). Compared to HGSOCs (n=316; TCGA)[29], the 19 *bona fide* MOCs significantly more frequently harbored mutations affecting *KRAS* and *CDKN2A* (*KRAS*, 79.0% vs 0.6%, p<0.001; *CDKN2A*, 10.5% vs 0%, p=0.003, Fisher's exact test; Fig. 4). In addition, MOCs significantly more frequently harbored *TP53* and *KRAS* mutations than mucinous gastric carcinomas (n=18; TCGA)[31] (*TP53*, 89.5% vs 16.7%, p<0.001; *KRAS*, 79.0% vs 11.1%, p<0.001, Fisher's exact test). Compared to mucinous colorectal cancers (n=32; TCGA)[30], MOCs more frequently had *TP53* mutations (89.5% vs 28.1%, p<0.001) but less frequently *APC*, *PIK3CA* and *FBXW7* mutations (*APC*, 0% vs 65.6%, p<0.001; *PIK3CA* and *FBXW7*, 0% vs 31.3%, p=0.008, Fisher's exact test; Fig. 4). Also pancreatic ductal carcinomas (n=177; ICGC)[32] were found to less frequently harbor *TP53* and *ERBB2* mutations than MOCs (*TP53*, 55.4% vs 89.5%, p=0.006; *ERBB2*, 0% vs 10.5%, p=0.009, Fisher's exact test), whilst no statistically significant differences in the mutational repertoire of the 341 genes examined were found between the *bona fide* primary MOCs studied here and pancreatic mucinous carcinomas (n=11; Fig. 4).

4. DISCUSSION

Here we demonstrate that massively parallel sequencing resulted in the reclassification of a subset of tumors initially classified as MOCs based on clinicopathologic parameters. Furthermore, we show that MOCs display a heterogeneous repertoire of somatic genetic alterations, and confirm that these tumors are underpinned by *TP53* mutations, *KRAS* mutations and/or *CDKN2A* alterations [8,9,34]. Similar frequencies of mutations affecting the 341 genes studied here were found between MOCs and pancreatic mucinous carcinomas, however at the mutational level, MOCs are an entity distinct from common-type epithelial ovarian cancers and mucinous colorectal or gastric cancers. This notion is further corroborated by the observation that HGSOCs and primary MOCs are characterized by the presence of recurrent *TP53* mutations, however whilst HGSOCs lack *KRAS* mutations and harbor high numbers of copy number alterations, MOCs frequently harbor *KRAS* mutations and fewer gene copy number changes (Fig. 1, Supplementary Fig. S1). In addition, it has been reported that MOCs display risk factor profiles distinct from that of other ovarian cancers [38], providing evidence to suggest that MOCs have a different etiology than other types of ovarian cancer.

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The heterogeneity of primary MOC is further reflected by the prevalence of *ERBB2* amplifications. Primary MOCs have been reported to harbor *ERBB2* amplification in up to 20% of cases [39], however only one of the MOCs studied here was *ERBB2* amplified (4.2%). It should be noted that one other case harbored a hotspot *ERBB2* p.R678Q mutation. Further studies are warranted to define whether *ERBB2* altered primary MOCs constitute a subgroup of these lesions associated with distinct clinico-pathologic characteristics, stage at presentation, response to treatment and/or outcome. In addition, Ryland and colleagues reported that deleterious somatic mutations of the tumor suppressor gene *RNF43* would play a role in a subset of primary MOCs [9], however none of the cases studied here harbored mutations or homozygous deletions affecting *RNF43*. Hence, the clinical and biological relevance of alterations affecting *RNF43* in MOCs remains to be determined.

Despite pathologic and clinical re-review, 21% of the cases diagnosed as primary MOCs here are, based on their immunohistochemical and mutational profiles, more consistent with a diagnosis of endometrioid ovarian cancers with mucinous differentiation or a mucinous carcinoma of colorectal type. The clinical outcome of these patients, however, is consistent with the diagnosis of primary ovarian cancers. Given the challenges associated with the diagnosis of primary MOC and their distinction from other types of ovarian cancer, such endometrioid carcinoma with mucinous differentiation, and metastatic mucinous tumors of the colorectum, the implementation of a small gene panel and/or immunohistochemistry set of markers, including *TP53*/p53, *KRAS*, *PIK3CA*, *ARID1A*/ARID1A, and DNA MMR proteins, may be useful to guide the diagnosis of these lesions. Such an approach would not only ensure an accurate diagnosis, but also facilitate the delivery of precision medicine-based treatments for patients with MOCs. In fact, based upon current practice, primary MOCs deemed eligible for chemotherapy due either to advanced stage or high-grade histology are treated using a standard epithelial ovarian cancer regimen using carboplatin plus paclitaxel or oxaliplatin containing GI type regimens depending on the clinician preference (NCCN guidelines, https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf). Importantly, however, by subjecting MOCs to a targeted capture sequencing approach, patients whose tumors display a hypermutator phenotype, such as the DNA MMR-deficient MOC38 or the *POLE*-mutant MOC62, would potentially be eligible for checkpoint blockage immunotherapy [40]. Also, we identified MOCs with *ERBB2* amplification or mutations, which may benefit from anti-HER2 agents, and a case with *EGFR* amplification (MOC59).

Our study has several limitations. The sequencing coverage was low for a few FFPE samples, which may have affected the mutation and/or copy number analyses performed here. The frequency of cancer genes mutated in the MOCs studied here is, however, similar to that previously reported [9]. In addition, with the exception of the tumors harboring *POLE* exonuclease domain mutations or DNA MMR alterations, the number of somatic mutations in the MOCs subjected to targeted massively parallel sequencing (MSK-IMPACT) was too low to assess the mutational signatures in these lesions.

Despite these limitations, our data demonstrate that primary MOCs are heterogeneous at the genetic level and suggest that the assessment of mutations affecting *TP53*, *KRAS*, *PIK3CA*, *ARID1A* and *POLE*, and DNA MMR protein expression may be used in conjunction with

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current established criteria to guide the diagnosis and, potentially, the treatment of patients with primary MOCs. In addition, our genomic analysis of tumors diagnosed as primary MOCs based on current clinico-pathologic guidelines may in fact constitute endometrioid carcinomas with mucinous differentiation, given their repertoire of somatic genetic alterations and immunohistochemical profiles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- MOCs are heterogeneous at the mutational level
- Mucinous ovarian cancers (MOCs) frequently harbor *TP53* and *KRAS* mutations
- The current pathologic criteria to diagnose MOCs may result in misclassifications
- Mutation analysis of a small gene panel may help improve the accuracy of MOC diagnosis

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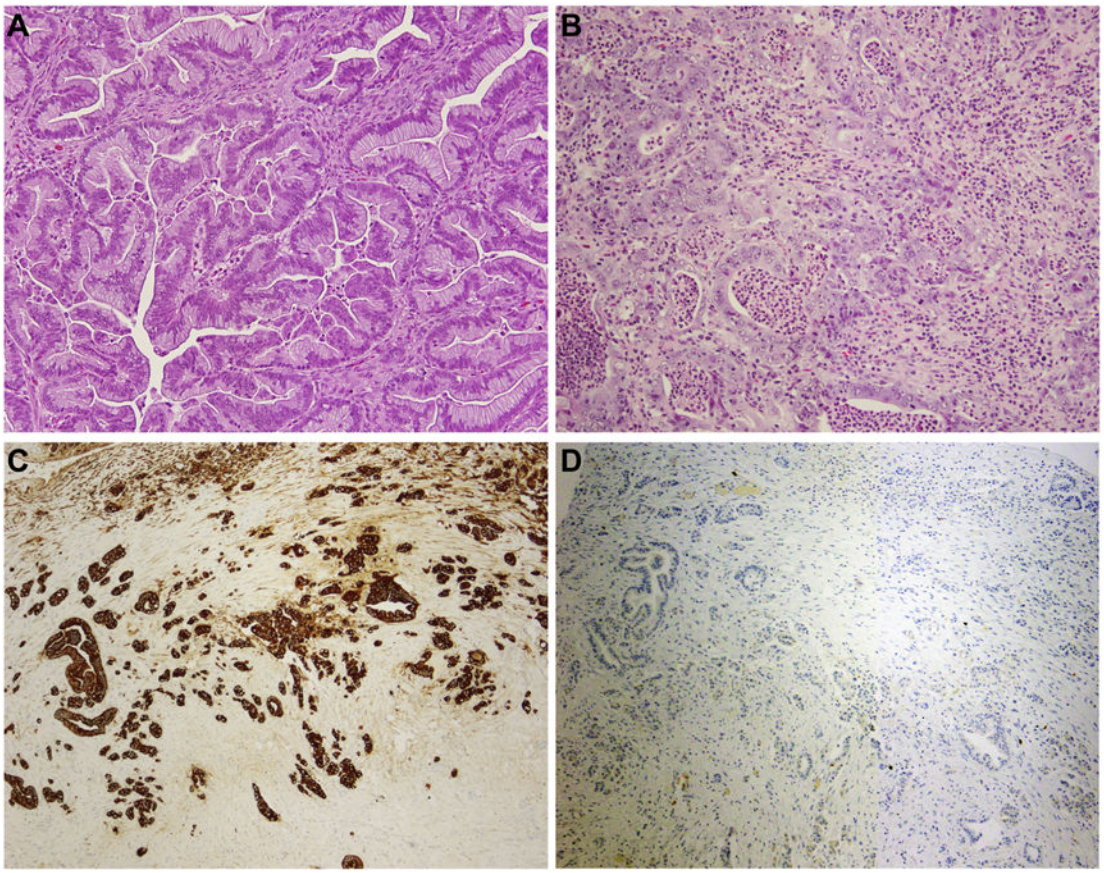

Figure 1. Histologic features of primary mucinous ovarian carcinomas

Representative hematoxylin and eosin stained sections of (**A**) a mucinous adenocarcinoma with an expansile pattern of growth and (**B**) a poorly differentiated mucinous adenocarcinoma with destructive stromal invasion. (**C**) Poorly differentiated mucinous adenocarcinoma showing strong CK7 expression. (**D**) Poorly differentiated mucinous adenocarcinoma displaying lack of CK20 expression. Magnification 400x.

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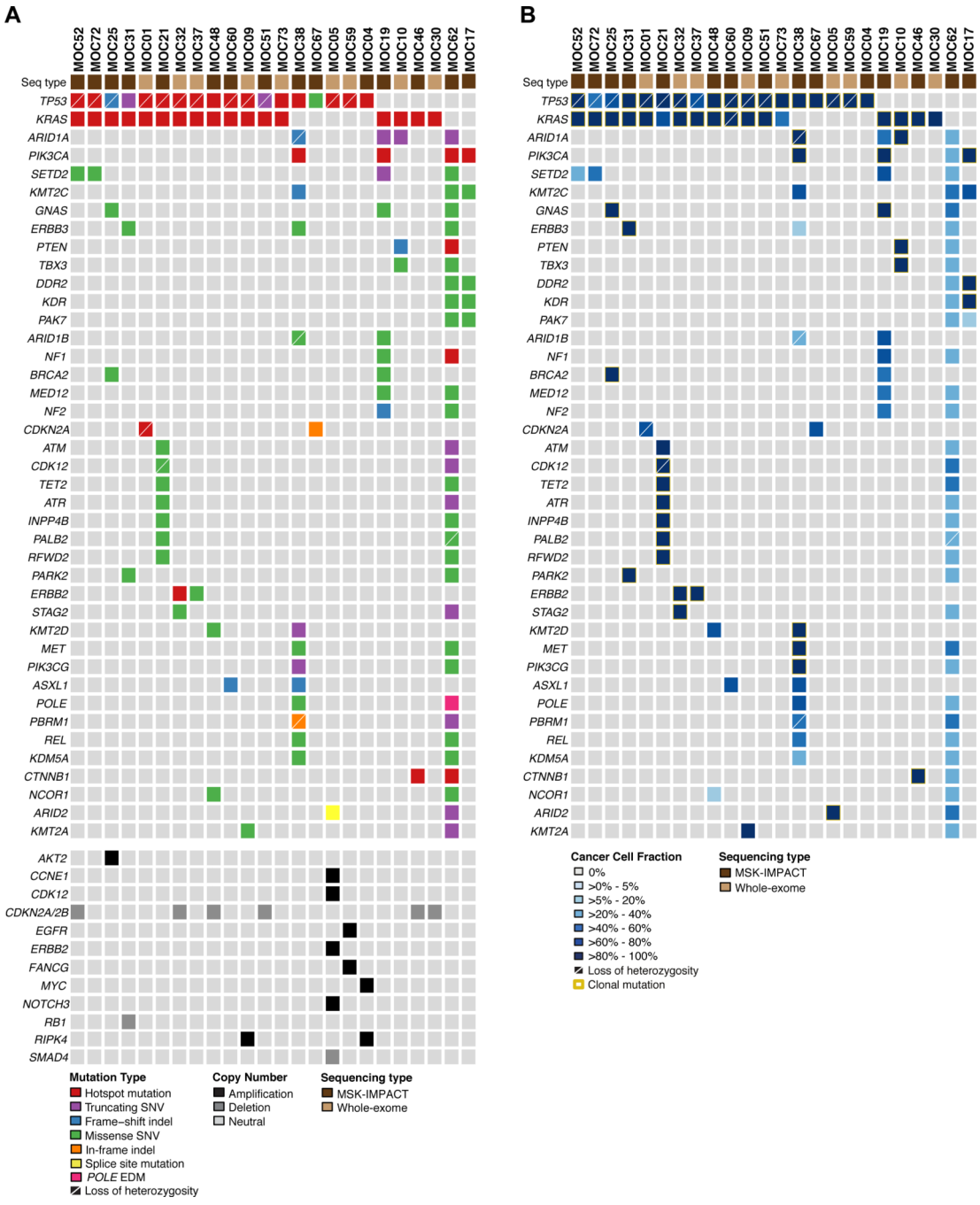

Figure 2. Recurrent non-synonymous somatic genetic alterations detected by massively parallel sequencing in primary mucinous ovarian cancers

(**A**) Recurrent (n≥2) non-synonymous somatic mutations (top) and amplifications and homozygous deletions of interest (bottom) identified in 24 tumors initially diagnosed as primary mucinous ovarian cancer in 341 cancer-related genes. Mutation types, gene copy number alterations and sequencing type are color-coded according to the legend. Loss of heterozygosity of the wild-type allele in association with a somatic mutation is depicted by a diagonal bar. EDM, exonuclease domain; Indel, small insertion/deletion; Seq, sequencing;

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SNV, single nucleotide variant. (**B**) Cancer cell fractions of non-synonymous somatic mutations as defined by ABSOLUTE [27] in tumors initially diagnosed as primary mucinous ovarian cancer. Cancer cell fractions and sequencing type are color-coded according to the legend, and clonal mutations are depicted by an orange box. Loss of heterozygosity of the wild-type allele in association with a somatic mutation is depicted by a diagonal bar. Seq, sequencing.

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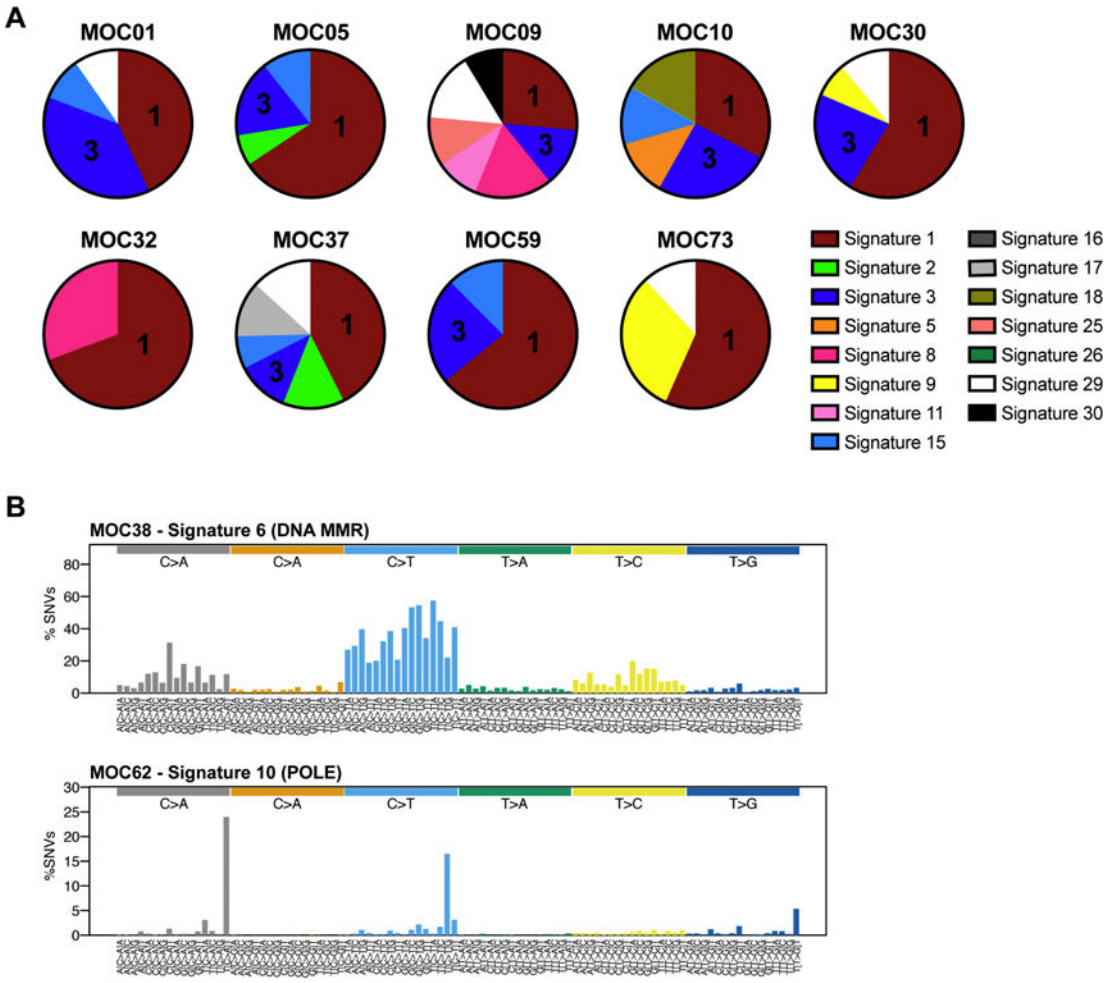

Figure 3. Mutational signatures in mucinous ovarian cancers

(**A**) Mutational signatures of all somatic single nucleotide variants (SNVs) identified in the nine mucinous ovarian cancers subjected to whole-exome sequencing using deconstructSigs [28]. Signature 1 associated with aging is the dominant signature in the MOCs studied. Signatures are color-coded according to the legend, including signature 1 (aging), signature 2 (APOBEC), signature 3 (defective homologous recombination DNA repair), and signatures 15 and 26 (defective DNA mismatch repair). (**B**) Mutational signatures of all SNVs in MOC38 and MOC62 subjected to MSK-IMPACT sequencing, displayed according to the 96 substitution classification defined by the substitution classes (C>A, C>G, C>T, T>A, T>C, and T>G bins), the 5’ and 3’ sequence context, normalized to the trinucleotide frequency of the human genome. MMR, mismatch repair.

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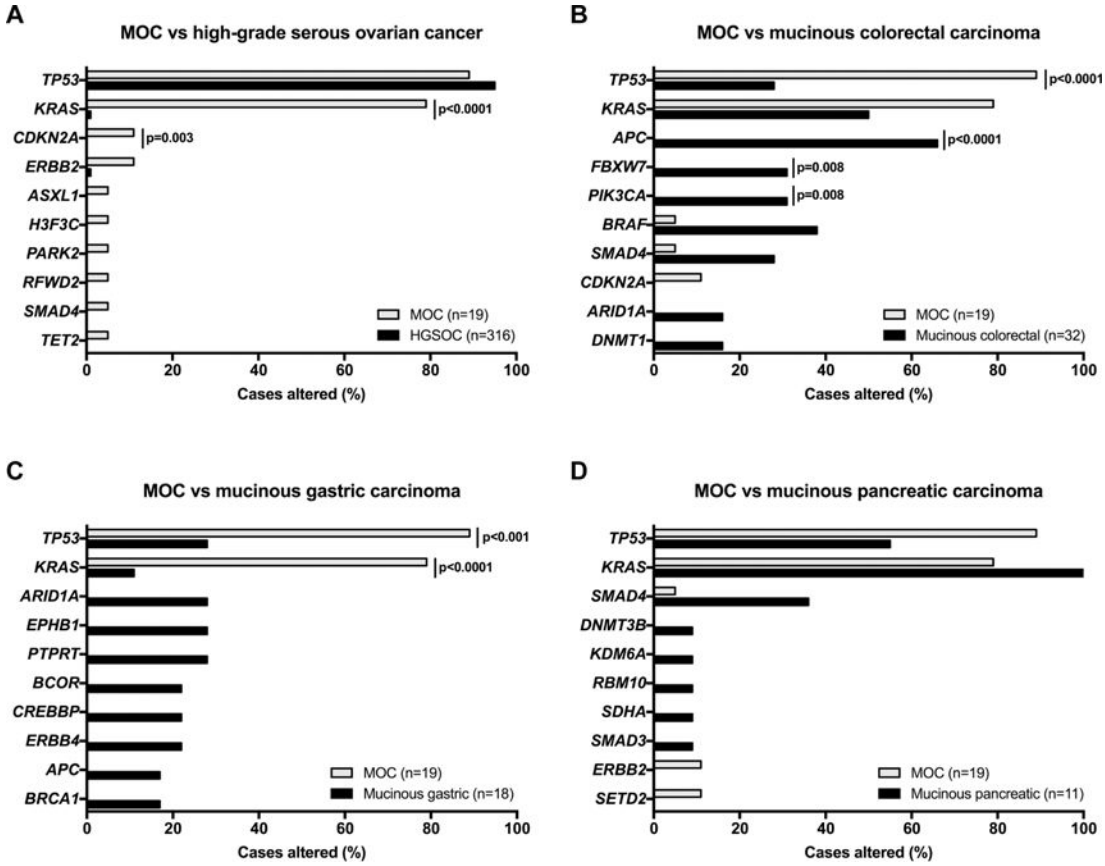

Figure 4. Comparison of the mutational profiles of mucinous ovarian cancers with high-grade serous ovarian cancers and mucinous carcinomas from other sites

The repertoire of somatic mutations affecting the 341 genes included in the MSK-IMPACT assay in the *bone fide* mucinous ovarian cancers (MOCs) studied here (n=19) and in (**A**) high-grade serous ovarian cancers (HGSOCs) from The Cancer Genome Atlas (TCGA; n=316)[29], (**B**) mucinous colorectal carcinomas (TCGA; n=32)[30], (**C**) mucinous gastric carcinomas (TCGA; n=18)[31] and (**D**) mucinous pancreatic carcinomas from the International Cancer Genome Consortium (ICGC; n=11)[32]. The top most frequently mutated genes of a given cancer type and *TP53* and *KRAS* are shown. Comparisons were performed using Fisher's exact test and statistically significant p-values are shown.

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Table 1

Clinicopathologic information of the 24 primary mucinous ovarian cancers included in this study.

ID	Age at diagnosis (years)	FIGO Stage	Tumor size (cm)	Laterality	CK7	CK20	PAX8	Adjuvant chemo-therapy	Follow-up (months, status)	Sequencing type
MOC01	38	IA	13	Unilateral (right)	Strong positive	Positive	Positive	No	150, NED	WES
MOC04	68	IIIA	20	Unilateral (right)	Strong positive	Positive	Focal positive	Yes	4.2, DOD	IMPACT
MOC05	76	IA	15	Unilateral (left)	Strong positive	Positive	Negative	Yes	101.5, DUC	WES
MOC09	64	IA	12	Unilateral (right)	Strong positive	Positive	Positive	No	185.4, NED	WES
MOC10	59	IIC	11	Unilateral (left)	Strong positive	Negative	Positive	Yes	91.3, NED	WES
MOC17	77	IA	20	Unilateral (left)	Strong positive	Negative	Focal positive	No	98.1, DUC	IMPACT
MOC19*	62	IIC	9	Unilateral (right)	Strong positive	Positive	Positive	Yes	244.4, DUC	IMPACT
MOC21*	38	IC	11	Unilateral (NA)	Strong positive	Negative	Positive	Yes	326.1, AWD	IMPACT
MOC25	20	IA	21	Unilateral (left)	Strong positive	Positive	Focal positive	No	83.7, NED	IMPACT
MOC30	59	IC	30	Unilateral (right)	Strong positive	Positive	Focal positive	No	133.6, NED	IMPACT
MOC31	37	IA	20	Unilateral (right)	Strong positive	Positive	Focal positive	No	53.3, NED	IMPACT
MOC32	60	IIIC	13, 3	Bilateral	Strong positive	Negative	Positive	Yes	96.6, DOD	WES
MOC37	46	IC	11	Unilateral (left)	Strong positive	Negative	Focal positive	Yes	117.6, NED	WES
MOC38	86	IC	7	Unilateral (right)	Strong positive	Positive	Positive	No	61.6, DUC	IMPACT
MOC46	46	IC	25	Unilateral (right)	Strong positive	Positive	Positive	Yes	90.8, NED	IMPACT
MOC48	78	IA	8	Unilateral (left)	Strong positive	Focal positive	Positive	No	76.7, NED	IMPACT
MOC51	70	IA	16	Unilateral (left)	Strong positive	Focal positive	Positive	No	41.3, NED	IMPACT
MOC52	45	IA	29	Unilateral (right)	Strong positive	Negative	Focal positive	No	79.5, NED	IMPACT
MOC59	54	IIIC	30	Unilateral (right)	Negative	Strong positive	Negative	Yes	35.0, AWD	WES
MOC60	37	IA	15	Unilateral (right)	Strong positive	Negative	Positive	No	54.3, NED	IMPACT
MOC62	53	IA	12	Unilateral (left)	Strong positive	Focal positive	Negative	Yes	61.0, NED	IMPACT
MOC67*	59	IIB	12	Unilateral (left)	Weak positive	Positive	Positive	Yes	31.6, DOD	IMPACT
MOC72	50	IA	32	Unilateral (right)	Strong positive	Focal positive	Positive	No	39.8, AWD	IMPACT
MOC73	41	IIIB	11	Unilateral (right)	Strong positive	Negative	Focal positive	No	47.9, DOD	WES

AWD, alive with disease; CK, cytokeratin; DOD, dead of disease; DUC, dead of unknown cause; NA, not available; NED, no evidence of disease; WES, whole-exome sequencing;

*recurrences.

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Table 2

Results of immunohistochemical analysis and mutation status of key genes to guide the diagnosis of primary mucinous ovarian cancer.

ID	Immunohistochemical markers					Mutation status				
	ER	PR	DNA MMR	PTEN	ARID1A	*TP53*	*KRAS*	*PIK3CA*	*ARID1A*	*POLE* EDM
MOC01	Negative	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT
MOC04	Negative	Negative	Retained	Retained	Retained	MUT	WT	WT	WT	WT
MOC05	Negative	Negative	Retained	Retained	Retained	MUT	WT	WT	WT	WT
MOC09	Negative	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT
MOC10	Positive	Positive	Retained	Equivocal	Retained	WT	MUT	WT	MUT	WT
MOC17	Positive	Positive	Retained	Loss	Retained	WT	WT	MUT	WT	WT
MOC19	Negative	Positive	Retained	Retained	Loss	WT	MUT	MUT	MUT	WT
MOC21	Negative	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT
MOC25	Negative	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT
MOC30	Negative	Negative	Retained	Retained	Retained	WT	MUT	WT	WT	WT
MOC31	Positive	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT
MOC32	Negative	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT
MOC37	Positive	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT
MOC38	Positive	Negative	PMS2/MLH1 loss	Retained	Loss	MUT	WT	MUT	MUT	WT
MOC46	Negative	Negative	Retained	Retained	Retained	WT	MUT	WT	WT	WT
MOC48	Negative	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT
MOC51	Negative	Negative	Retained	Retained	Retained	WT	MUT	WT	WT	WT
MOC52	Negative	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT
MOC59	Negative	Negative	Retained	Retained	Retained	MUT	WT	WT	WT	WT
MOC60	Negative	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT
MOC62	Negative	Negative	Retained	Retained	Loss	WT	WT	MUT	MUT	MUT
MOC67	Negative	Negative	Retained	Retained	Retained	MUT	WT	WT	WT	WT
MOC72	Positive	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT
MOC73	Negative	Negative	Retained	Retained	Retained	MUT	MUT	WT	WT	WT

EDM, exonuclease domain; ER, estrogen receptor; MMR, mismatch repair; MUT, mutant; PR, progesterone receptor; WT, wild-type.