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TCGA molecular classification in endometriosis-associated ovarian carcinomas: Novel data on clear cell carcinoma

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HIGHLIGHTS

- The molecular classification of endometrial carcinoma shows prognostic value in endometriosis-associated ovarian cancers.
- The difference in patient outcome between the molecular groups was more distinct in ovarian clear cell carcinoma.
- *POLE* mutated and MMR deficient ovarian clear cell carcinomas were uncommon, but carried an excellent prognosis.
- The p53 abnormal group had the poorest prognosis in both histotypes, which was particularly emphasized in clear cell tumors.
- No specific molecular profile (NSMP) was the largest group in both histotypes.

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ABSTRACT

Background. Clear cell and endometrioid ovarian carcinomas (OCC and OEC, respectively) have a presumed origin in endometriosis and share molecular alterations with each other and with their endometrial counterparts. The Cancer Genome Atlas (TCGA)-based molecular classification stratifies endometrial carcinomas into four categories: *POLE* mutated (*POLE*mut), mismatch repair deficient (MMRd), p53 abnormal (p53abn) and no specific molecular profile (NSMP) with divergent prognoses. The subsequent studies are indicative that this TCGA classification has some value in OEC, but the knowledge related to OCC is limited.

Methods. Endometrial carcinoma molecular classification was evaluated and compared in a large series of OCCs ($n = 115$) and OECs ($n = 158$). *POLE* mutation analysis and tissue microarray-based immunohistochemistry for mismatch repair and p53 proteins were performed.

Results. The distribution to the molecular groups was as follows: *POLE*mut 0.9%/3.2%, MMRd 3.5%/6.3%, p53abn 20%/30%, and NSMP 76%/60% in OCCs/OECs, respectively. The proportion of NSMP tumors was the largest in both histological types and significantly higher in OCC than OEC ($p = 0.009$). The molecular classification correlated significantly with DSS in both OCCs and OECs ($p < 0.001$ and $p = 0.009$, respectively), and with DFS in OCCs ($p = 0.001$). *POLE*mut and MMRd OCCs carried excellent prognosis, whereas MMRd OECs presented with poorer outcome. The p53abn group was associated with the poorest prognosis in both tumor types, particularly emphasized in OCCs.

Conclusions. TCGA molecular classification associated with patient outcome in both histotypes, and the difference in prognosis between the molecular groups was even more marked in OCC. The large amount of NSMP tumors highlights the need for further studies.

1. Introduction

Ovarian clear cell and endometrioid carcinomas (OCC and OEC, respectively) are the most common epithelial ovarian malignancies after high-grade serous carcinoma [1,2]. Although with distinct morphologies,

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these two subtypes share many features with one another. Occasional cases overlap morphologically, in which immunohistochemistry may be useful in diagnosis [3]. Both have a presumed origin in endometriosis and link to Lynch syndrome but not *BRCA*1/2 mutations [2,4]. These tumors share alterations in *ARID1A*, *PIK3CA* and *PTEN*, whereas activation of *HNF1B* is typical for OCC and mutations of *CTNNB1* (β-catenin gene) for OEC [2,4]. OCC is considered and treated as high-grade disease, even though the clinical course is known to vary considerably. Currently, the management of well or moderately differentiated stage IA OECs does not include adjuvant chemotherapy, whereas grade 3 stage IA OECs and all other stages receive adjuvant treatment [5]. Based on this background molecular profiling would have potential to give understanding in the divergent behavior of individual cases and help in developing more targeted treatment options.

In endometrial carcinoma, the molecular classification introduced by The Cancer Genome Atlas (TCGA) research network provided a promising new tool to stratify tumors into prognostic subgroups irrespective of traditional morphological histotype and grade [6]. The four TCGA molecular categories are DNA polymerase epsilon (*POLE*)/ultramutated, microsatellite unstable (MSI)/hypermutated, copy-number low, and copy-number high [6].

To discover the ultramutated subclass, sequencing for *POLE* exonuclease domain mutations remains the golden standard [7]. Immunohistochemistry (IHC) for mismatch repair (MMR) proteins has been demonstrated to reveal MMR deficiency as evidence of microsatellite instability and to surrogate the MSI/hypermutated category in endometrial carcinoma [8]. *TP53* mutations and aberrant immunohistochemical staining for p53 serves as currently best appreciated surrogate for the copy-number high subgroup. The copy-number low genomic type is defined by the absence of all previously mentioned alterations (no specific molecular profile, NSMP) [9].

According to the original TCGA study and following surrogate studies, *POLE* mutated (*POLE*mut) tumors present with the best outcome and copy-number high/p53 abnormal (p53abn) tumors with the worst, whereas the prognosis of MSI/MMR deficient (MMRd) and copy-number low/NSMP groups is intermediate [6,10,11]. Clear cell carcinoma of the endometrium was not studied in the original TCGA cohort, but since then it has been demonstrated to contain all four molecular categories with parallel prognostic significance in one relatively small study consisting entirely of pure clear cell tumors [12].

As clear cell and endometrioid subtypes of ovarian carcinoma and respective histotypes of endometrial carcinoma resemble each other by morphology and molecular background, there is relevance to test the endometrial carcinoma-based TCGA classification model in both endometriosis-related histotypes of ovarian carcinoma. Parra-Herran et al. reported a series of 90 OCC cases, of which 47 were targeted to *POLE* mutation analysis. No well-established *POLE* exonuclease domain mutations were found, and probably due to the small number of cases, no conclusions could be drawn from the prognostic impact of the TCGA classification system [13]. However, four published works on OEC (number of cases ranging from 36 to 511) have reported that the TCGA classification system may have prognostic significance comparable to endometrial carcinoma [14–17].

Our aim was to evaluate and compare the value of the TCGA molecular classification of endometrial carcinoma in a large series of consecutive OCC ($n = 115$) and OEC ($n = 158$) patients treated at a single institution, including all stages of the disease and long median follow-up times.

2. Materials and methods

2.1. Patients

Initially these retrospective series consisted of 140 OCC and 249 OEC patients treated between 1989 and 2014 at the Department of Obstetrics and Gynecology, Helsinki University Hospital, Finland [18]. The above-mentioned consecutive patients, who all underwent primary surgery, were identified from the Pathology database. The associated clinical data were collected from the hospital medical records. Incomplete survival information was accomplished from the Population Register Center. Approvals from the Ethics Committee of the Helsinki University Hospital and the National Supervisory Authority of Welfare and Health were obtained. The characteristics of these cases have been described in detail in the previous publication [18].

The histopathological classification of these ovarian carcinomas was reviewed by a gynecological pathologist before including the cases in the original study series. The tumors were staged according to the year 2009 FIGO staging system. Grading (WHO) was applied only to endometrioid carcinomas. Since then the cases have been re-evaluated based on criteria set by WHO Classification of Tumors of Female Reproductive Organs (4th Edition 2014) [19]. Consequently 8 OCC and 34 OEC cases were excluded, comprising mostly WT1+/p53abn ovarian carcinomas probably representing high-grade serous carcinomas with clear cell change and endometrioid-like high-grade serous carcinomas, respectively [3,20–23]. Eventually 132 clear cell and 215 endometrioid carcinomas remained in the cohort.

2.2. Tissue microarray construction and IHC

Multicore tissue microarrays (TMAs) were constructed as described before [18,20]. Four separate cores from different areas of each tumor were included in TMAs [18]. IHC was performed on TMA slides with the following monoclonal antibodies: MLH1 (ES05; Dako), PMS2 (EPR3947; Epitomics), MSH2 (G219–1129; BD Biosciences), MSH6 (EPR3945; Abcam) and p53 (DO-7; Dako). MMR deficiency was defined as complete loss of nuclear expression of any of the MMR proteins (MLH1, PMS2, MSH2, MSH6) in carcinoma cells. p53 abnormality was defined as strong and diffuse (>80%) nuclear, completely negative ("null") or unequivocal cytoplasmic staining in carcinoma cells [24,25]. Heterogeneous nuclear staining with variable intensity was classified as wild type expression.

Stainings were scored by two pathologists blinded to clinical data. Equivocal cases were examined by a third investigator, and a whole slide section was re-stained, if necessary (e.g. samples with scarce carcinoma cells, discrepant cores or with completely negative staining without presence of positive internal control). Cases with ambiguous stainings were discarded.

2.3. POLE mutational analysis

Representative areas of tumors were selected on histologic slides by a pathologist, tumor tissue was macrodissected from the corresponding areas of formalin-fixed paraffin-embedded blocks, and DNA was extracted by proteinase K/phenol-chloroform method. Screening for the hot spots in exons 9 (c.857C > G, p.(Pro286Arg); c.890C > T, p.(Ser297Phe)), 13 (c.1231G > T/C, p.(Val411Leu)), and 14 (c.1366G > C, p.(Ala456Pro)) was performed by direct sequencing to reveal well-established *POLE* exonuclease domain mutations, as described previously [26], including the two most prevalent mutations related to ultramutated endometrial carcinomas [6,7]. Only cases with a good-quality sequence for all the examined *POLE* hot spots were accepted to the analysis. Electropherograms were analyzed both manually and with Mutation Surveyor (Softgenetics, State College, PA).

2.4. Stratification to molecular categories

On the basis of the TCGA molecular classification and its proposed surrogate markers implemented on endometrial carcinoma, the ovarian tumors were categorized into four groups according to the previously introduced molecular features in the following order: 1. *POLE*mut, 2. MMRd, 3. p53abn and 4. NSMP [7,27].

2.5. Statistical analysis

Data were analyzed using IBM SPSS version 25 software. Statistical significance was set at $p < 0.05$ (two-sided). Correlation between categorical variables was evaluated with Pearson $\chi 2$ test and Fisher's exact test. Disease-specific survival (DSS) was calculated from the date of the diagnosis (primary surgery) to the death caused by the ovarian cancer, and disease-free survival (DFS) was estimated from the date of the primary surgery to the date of the relapse of the disease. DFS was calculated only for the patients who had a complete response following the primary treatment [18]. Curves for DFS and DSS were calculated using Kaplan-Meier method, and log rank test was used to compare differences between the groups. Cox proportional hazards model was used for multivariate survival analysis.

3. Results

3.1. Molecular classification and clinicopathological features

Within OCCs, *POLE* hotspot mutation analysis was successful in 115/132 (87%) cases and IHC against molecular classification surrogate markers succeeded in all of them. Similarly, 159/215 (74%) OECs were successfully analyzed for *POLE* mutations. In one of these cases, IHC against MMR and p53 proteins failed leading to its exclusion from this study. Consequently, 115 OCCs and 158 OECs were included in the study. Median follow-up time (without patients who died of the disease) was 118 (range 20–304) months for OCCs and 83 (range 5–274) months for OECs.

POLE mutation was found in one (0.9%) clear cell and five (3.2%) endometrioid tumors (Table 1A-1B). All mutations found in the endometrioid cases represented the two most common *POLE* exonuclease domain mutations (i.e. c.857C > G, p.(Pro286Arg) and c.2131G > C/T, p.(Val411Leu)) in the pivotal TCGA study. *POLE* mutation of the only clear cell tumor (c.1366G > C, p.(Ala456Pro)) was also introduced by the TCGA research group in one ultramutated endometrial carcinoma [6].

*POLE*mut clear cell tumor also showed loss of MSH2 and MSH6. According to the selected classifying model, *POLE* mutation defined the molecular category for this case. Additional four (3.5%) OCCs presented with deficient MMR status: three MSH2-MSH6-negative and one MSH6-negative. All endometrioid *POLE*mut cases were MMR proficient. Ten (6.3%) OECs harbored MMR deficiency: six MLH1-PMS2-negative, two MSH2-MSH6-negative, and isolated loss of PMS2 or MSH6 was detected in one case each. Two OEC patients had a family history of Lynch syndrome, and one OCC patient was later identified to have Lynch syndrome.

In the absence of *POLE* and MMR alterations, abnormal p53 staining was detected in 23 (20%) clear cell and 48 (30%) endometrioid cases, forming p53abn molecular group. p53 overexpression was observed in 16 (70%) and 34 (71%), "null" pattern in six (26%) and 13 (27%), and cytoplasmic staining in one (4.3%) and one (2.1%) clear cell and endometrioid p53abn tumors, respectively. None of the MMRd cases showed abnormal p53 staining, but in one *POLE*mut OEC aberrant p53 overexpression was detected (=*POLE*mut). The remaining 87 (76%) clear cell and 95 (60%) endometrioid tumors were classified to the NSMP subgroup. Stratification to the molecular categories is summarized in the flow chart (Fig. 1).

The proportion of the cases distributed to the NSMP molecular group was significantly higher in OCC than OEC ($p = 0.009$). In contrast, the relative amounts of *POLE*mut, MMRd and p53abn tumors tended to be lower in OCC compared to OEC, but the differences within these groups ($p = 0.406$, $p = 0.407$ and $p = 0.069$ respectively) did not reach statistical significance.

FIGO stage (low-stage I-II versus high-stage III-IV) differed significantly between the molecular categories (Table 1A-1B). In OCC 83%

Table 1
A. Clinicopathological characteristics within molecular subgroups of ovarian clear cell carcinoma.

	Molecular subgroup					
	*POLE*mut	MMRd	p53abn	NSMP	All OCCs	*p*-value[a]
Patients						
n (%)	1 (0,9%)	4 (3,5%)	23 (20,0%)	87 (75,6%)	115 (100%)	
Age (years)						
Median	49	49	62	58	58	0.490[a]
Range	-	45–59	35–84	27–83	27–84	
FIGO Stage						
I-II	1/1 (100%)	4/4 (100%)	4/23 (17,4%)	63/87 (72,4%)	72/115 (62,6%)	**<0.001**
III-IV	0	0	19/23 (82,6%)	24/87 (27,6%)	43/115 (37,4%)	
Synchronous endometrial carcinoma	1/1 (100%)	1/4 (25,0%)	1/23 (4,3%)	1/87 (1,1%)	4/115 (3,5%)	**<0.001**

B. Clinicopathological characteristics within molecular subgroups of ovarian endometrioid carcinoma.

	Molecular subgroups					
	*POLE*mut	MMRd	p53abn	NSMP	All OECs	*p*-value[a]
Patients						
n (%)	5 (3,2%)	10 (6,3%)	48 (30,4%)	95 (60,1%)	158 (100%)	
Age (years)						
Median	51	54	61	57	58	**0.034**[b]
Range	45–55	44–78	41–90	27–100	27–100	
FIGO Stage						
I-II	3/5 (60,0%)	8/10 (80,0%)	25/47 (53,2%)	72/94 (76,6%)	108/156 (69,2%)	**0.032**
III-IV	2/5 (40,0%)	2/10 (20,0%)	22/47 (46,8%)	22/94 (23,4%)	48/156 (30,8%)	
Grade						
1	2/5 (40,0%)	7/10 (70,0%)	8/48 (16,7%)	65/95 (67,4%)	81/158 (51,3%)	**<0.001**
2	3/5 (60,0%)	2/10 (20,0%)	20/48 (41,7%)	27/95 (28,4%)	52/158 (32,9%)	
3	0	1/10 (10,0%)	20/48 (41,7%)	4/95 (4,2%)	25/158 (15,8%)	
Synchronous endometrial carcinoma	2/5 (40,0%)	7/10 70,0%)	3/48 (6,3%)	26/95 (27,4%)	38/158 (24,1%)	**<0.001**

Percentages (%) were calculated without missing values.
Abbreviations: OCC, ovarian clear cell carcinoma; OEC, ovarian endometrioid carcinoma; *POLE*mut, *POLE* mutated; MMRd, mismatch repair deficient; p53abn, p53 abnormal; NSMP, no specific molecular profile; FIGO, International Federation of Gynecology and Obstetrics.
[a] Significant *p*-values (<0.05) are bolded.
[b] Age: *p*-value for the difference between molecular groups was calculated using the approximate median age of the whole cohort (≥58 years vs. <58 years).

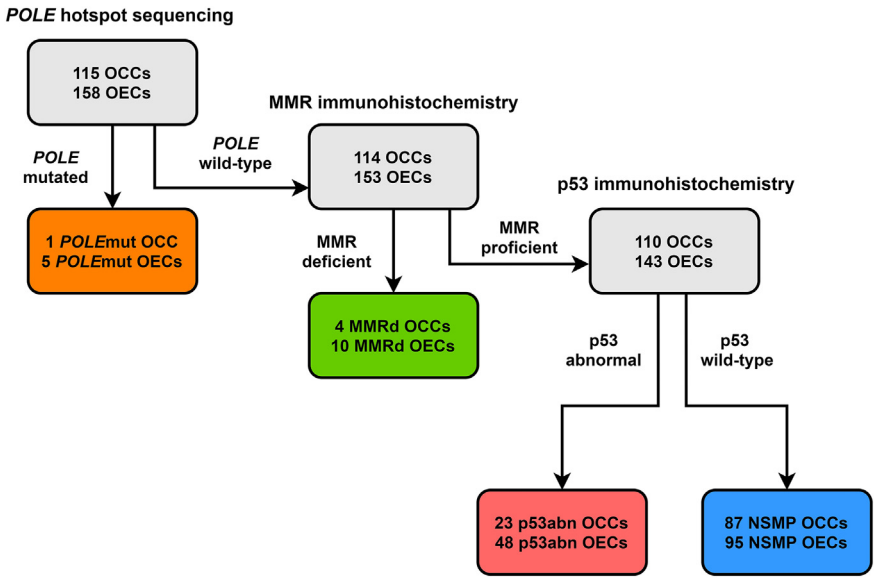

Fig. 1. Stratification of ovarian clear cell and endometrioid carcinomas to the molecular categories, i.e. *POLE*mut, MMRd, p53abn and NSMP groups, is summarized in the flow chart. Abbreviations: OCC, ovarian clear cell carcinoma; OEC, ovarian endometrioid carcinoma; *POLE*mut, *POLE* mutated; MMRd, mismatch repair deficient; p53abn, p53 abnormal; NSMP, no specific molecular profile.

and in OEC 47% of the p53abn tumors were high-stage, with significant difference between OCCs and OECs ($p = 0.005$). MMRd tumors were most commonly low-stage, as all four OCCs (100%) and eight (80%) OECs were localized within pelvis. The only *POLE*mut OCC was limited to ovary (stage I), but a synchronous stage I endometrial carcinoma was also detected. Interestingly, two (40%) *POLE*mut OECs presented at advanced stage.

In OEC, FIGO grade (G1-G3) strongly associated with molecular groups (Table 1A-1B). 42% of the p53abn cases were G3, whereas four NSMP (4%), one MMRd (10%) and none of *POLE*mut (0%) tumors were high-grade. However, still over half of the p53abn tumors were low-grade: 42% of G2 and 17% of G1.

The prevalence of synchronous endometrial and ovarian tumors varied significantly between molecular groups (Table 1A-1B). It was higher in *POLE*mut and MMRd compared to the other molecular subgroups, especially p53abn group. In addition to one *POLE*mut OCC, a synchronous endometrial carcinoma was found in one (25%) MMRd OCC, two (40%) *POLE*mut OECs and seven (70%) MMRd OECs, whereas only one (4.3%) and three (6.3%) p53abn OCCs and OECs co-occurred with endometrial carcinoma. In three of the OCC cases, the concurrent endometrial tumor was of clear cell type and in one case of endometrioid type. In all OEC cases, the synchronous endometrial carcinoma was of endometrioid type.

Median age at diagnosis in this patient series was 58 years for both OCCs and OECs. Patients in the *POLE*mut and MMRd groups tended to be younger than in the two other groups (Table 1A-1B). The difference between the molecular groups was statistically significant for OECs but not for OCCs, due to small numbers of *POLE*mut and MMRd OCCs.

Residual tumor after primary surgery was most often reported in patients with p53abn tumors (78% of OCCs and 43% of OECs), but also in a proportion of patients with NSMP tumors (17% of OCCs and 11% of OECs) and MMRd endometrioid tumors (11%), whereas none of the *POLE*mut or MMRd OCCs had residual tumor at surgery (Table 2A-2B). Within the p53abn molecular group, residual tumor was observed significantly more often in OCCs than OECs ($p = 0.006$).

The highest frequency of complete response after primary treatment was achieved in *POLE*mut and MMRd OCCs (100% for both), and the lowest for p53abn OCCs (52%) and OECs (74%)(Table 2A-2B). The difference between frequencies of primary response in molecular subtypes was significant for OCC, but not for OEC.

3.2. Survival analyses

Recurrences were most common in p53abn subgroup, but were detected also among NSMP category and endometrioid MMRd tumors (Table 2A-2B). None of the clear cell MMRd tumors relapsed. All *POLE*mut cases of either histotype with complete primary response remained disease-free.

As with recurrences, the deaths caused by the ovarian cancer were percentually highest among p53abn molecular group; within OCCs 83% and OECs 48% of patients deceased (Table 2A-2B). All four MMRd OCC patients stayed alive with no evidence of the disease during the follow-up period, whereas among MMRd OECs the disease-related deaths were fairly common with 44% rate. Only one *POLE*mut patient succumbed to her ovarian cancer. In this case, the tumor was of endometrioid type, disease presented at advanced stage and was found unexpectedly.

Molecular classification correlated significantly with DSS in both OCCs and OECs ($p < 0.001$ and $p = 0.009$, respectively), whereas with DFS its prognostic impact was statistically confirmed in OCCs only ($p = 0.001$) (Fig. 2A-2D). The *POLE*mut OCC patient stayed alive and disease-free, and also in OECs the *POLE*mut tumors carried the best prognosis with 5-year DSS and DFS of 75% and 100%. MMRd tumors had variable prognosis: in OCCs no recurrences or deaths were reported, while in OECs 5-year DSS and DFS were 56% and 71%. p53abn tumors had the poorest 5-year survival percentages of 10% and 25% in OCCs and 43% and 56% in OECs (DSS and DFS, respectively). NSMP subgroup held intermediate prognosis of 65% and 73% in OCCs and 64% and 79% in OECs (5-year DSS and DFS, respectively). The outcome of p53abn OCCs was significantly poorer than that of p53abn OECs (DSS log rank $p = 0.004$, DFS log rank $p = 0.030$), whereas there was no statistical difference between the survivals of the other groups.

In multivariate Cox regression analysis adjusted with stage, molecular classification retained its independent prognostic significance with DSS but not with DFS in OECs ($p = 0.035$ and $p = 0.317$). When

Table 2
A. Clinical and survival data within molecular subgroups of ovarian clear cell carcinoma.

	Molecular subgroup					
	*POLE*mut	MMRd	p53abn	NSMP	All OCCs	*p*-value[a]
Residual tumor	0/1 (0%)	0/4 (0%)	18/23 (78,3%)	15/86 (17,4%)	33/114 (28,9%)	**<0.001**
Primary response						
CR	1/1 (100%)	4/4 (100%)	12/23 (52,2%)	70/86 (81,4%)	87/114 (76,3%)	**0.017**
PR/SD/progression	0/1 (0%)	0/4 (0%)	11/23 (47,8%)	16/86 (18,6)	27/114 (23,7%)	
Recurred	0/1 (0%)	0/4 (0%)	8/12 (66,7%)	15/70 (21,4%)	23/87 (26,4%)	**<0.001**[b]
Died of disease	0/1 (0%)	0/4 (0%)	19/23 (82,6%)	27/87 (31,0%)	46/115 (40,0%)	**<0.001**[b]
5-year DSS	100%	100%	10%	65%	54%	

B. Clinical and survival data within molecular subgroups of ovarian endometrioid carcinoma.

	Molecular subgroup					
	*POLE*mut	MMRd	p53abn	NSMP	All OECs	*p*-value[a]
Residual tumor	0/4 (0%)	1/9 (11,1%)	20/47 (42,6%)	10/94 (10,6%)	31/154 (20,1%)	**<0.001**
Primary response						
CR	4/5 (80,0%)	8/10 (80,0%)	35/47 (74,5%)	83/94 (88,3%)	130/156 (83,3%)	0.217
PR/SD/progression	1/5 (20,0%)	2/10 (20,0%)	12/47 (25,5%)	11/94 (11,7%)	26/156 (16,7%)	
Recurred	0/3 (0%)	2/7 (28,6%)	12/35 (34,3%)	15/82 (18,3%)	29/127 (22,8%)	0.109[b]
Died of disease	1/4 (25,0%)	4/9 (44,4%)	22/46 (47,8%)	23/93 (24,7%)	50/152 (32,9%)	**0.009**[b]
5-year DSS	75%	56%	43%	64%	57%	

Percentages (%) were calculated without missing values.
Abbreviations: OCC, ovarian clear cell carcinoma; OEC, ovarian endometrioid carcinoma; *POLE*mut, *POLE* mutated; MMRd, mismatch repair deficient; p53abn, p53 abnormal; NSMP, no specific molecular profile; CR, complete response; PR, partial response; SD, stable disease; DSS, Disease-specific survival.
[a] Significant *p*-values (<0.05) are bolded.
[b] Log rank *p*-values from Kaplan-Meier analyses (Fig. 2A-2D).

grade was added to the multivariate analysis, only stage remained significant. In OCCs, molecular classification did not reach statistical overall significance in multivariate analysis for DSS or DFS adjusted with stage.

4. Discussion

In the present study we have established the existence of all four endometrial carcinoma-based TCGA molecular categories in OCC as well as in OEC. We found that NSMP was the most prevalent subgroup in both OCCs and OECs (76%, 60%), followed by p53abn class (20%, 30%, respectively). The number of tumors stratified to MMRd category was lower, comprising 3.5% of OCCs and 6.3% of OECs. *POLE*mut tumors were relatively uncommon, as only one (0.9%) case constituted the subgroup in OCCs, and 3.2% belonged to the corresponding subgroup in OECs. For the first time, the prognostic value of this molecular categorization was established in OCC. In OEC, the molecular categorization also presented with prognostic value, which supports and strengthens the findings of the previous studies [14–17]. The difference in the patient outcome between the molecular subgroups was even more marked in OCC than in OEC.

While *POLE* variants of unknown significance have been described to occur in OCC [13], to our knowledge, this is the first report of a well-established *POLE* exonuclease domain mutation in OCC. The *POLE* mutation (c.1366G > C, p.(Ala456Pro)) we found in one OCC has been introduced by the TCGA research group in one ultramutated endometrial carcinoma, and although it is not among the most common *POLE* mutations, its significance is widely acknowledged [4,6,7].

The prevalence of the *POLE*mut molecular subgroup in OEC (3.2%) was very similar to the largest study so far by Krämer et al., reporting 3.5% rate of *POLE*mut tumors [17]. In the other studies on OEC, *POLE* mutation rate has varied from 3% to 10% [14–16]. The differencies in the observed frequencies may be partly due to methodological issues, such as sequencing methods, covered exons and hotspots of *POLE*, and the practice evaluating the variants [7].

We performed direct sequencing to detect mutations in the hotspots of *POLE* exons 9, 13 and 14. Our method revealed the two most common *POLE* mutations related to endometrial carcinoma molecular classification (p.(Pro286Arg) and p.(Val411Leu)), along with two other well established but less common *POLE* mutations (p.(Ser297Phe) and p.(Ala456Pro)), together covering four of the five most significant mutations found in endometrial carcinoma [4,7]. It is possible that there is a minor underestimation of the true number of *POLE*mut/ultramutated cases, but it is not likely to be substantial considering the rarity of the potentially missed variants [7].

The proportion of MMRd tumors was relatively low, 3.5% of OCCs and 6.3% of OECs. Corresponding rates, 2% of OCCs and 8% of OECs, have been reported earlier [13,14]. However, the other studies on OECs found somewhat higher number of MMRd/MSI cases ranging between 14%–19% [15–17]. One study with the higher prevalence consisted of low-stage tumors only [16]. Another study reported inclusion of subclonal loss of MMR proteins, and they also used full slides in part of their series [17].

In our series, 20% of OCCs and 30% of OECs were stratified into p53abn group, which is slightly more than reported earlier, 7% and 10–24%, respectively [13–17]. The few studies evaluating only p53 status in OCCs have presented with discordant findings: p53 abnormality rate varying from 5% up to 82% [3,28,29]. The lowest percentage, 5%, was found in a study consisting of early-stage tumors only, which is in line with our findings (6% of low-stage OCCs in our cohort) [28]. Some of the variation may be explained by the methodology, e.g. different laboratory protocols or use of TMAs, and the interpretation of the immunohistochemical staining [18,24,30]. Among OECs, the different composition of the series may also partly explain the differences, as some groups used WT1-positivity [14] or WT1+/p53abn immunohistochemical combination [17] as a definitive exclusion criteria. However, despite this fact, our proportion of p53abn tumors in OECs was not substantially higher compared to that of Parra-Herrans group (24%) [14]. This does not either explain our higher proportion (20%) of p53abn OCCs compared to Parra-Herrans group (7%) [13], as in our series only three p53abn OCC cases showed focal WT1 staining. In clear cell carcinoma of the endometrium, the prevalence of p53abn group is even higher (43%) [12]. Similar to its endometrial counterpart, >80% of the p53abn OCCs in our cohort presented with advanced stage and poor outcome. However, in a study consisting of early-stage OCCs only, p53 abnormality was associated with poor prognosis as well [28].

NSMP was by far the most common group in OCCs (76%), but, although less frequent, it also established the largest category in OECs (60%). In line with this, the proportions of the other subgroups, i.e.

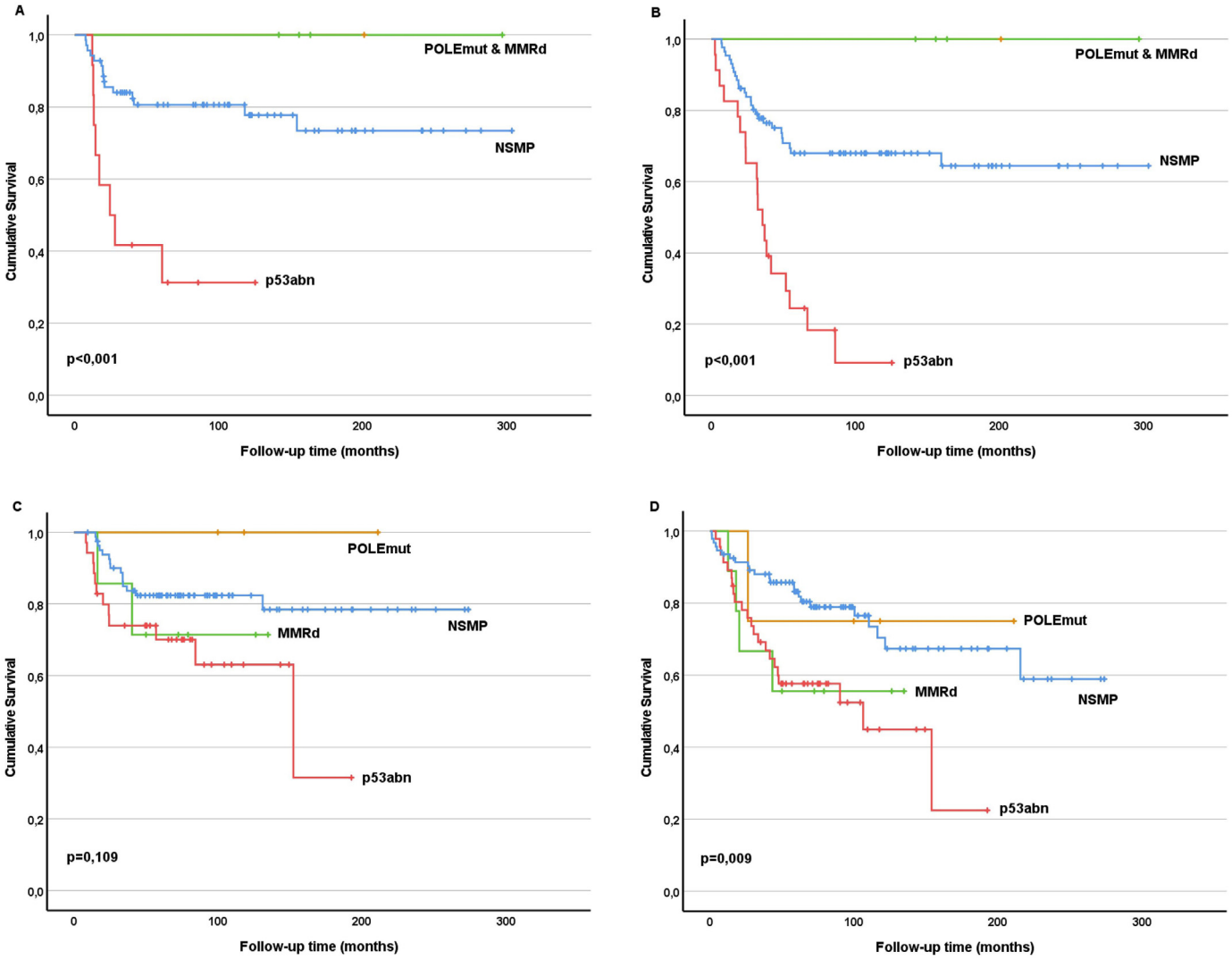

Fig. 2. Disease-free (A) and disease-specific (B) survival according to the molecular subgroups in clear cell ovarian carcinoma patients, and disease-free (C) and disease-specific (D) survival according to the molecular subgroups in endometrioid ovarian carcinoma patients.
Abbreviations: *POLE*mut, *POLE* mutated; MMRd, mismatch repair deficient; p53abn, p53 abnormal; NSMP, no specific molecular profile.

*POLE*mut (0.9% vs. 3.2%), MMRd (3.5% vs. 6.3%) and p53abn (20% vs. 30%), tended to be lower in clear cell than endometrioid histotype. The NSMP category has also been the largest group in the previous TCGA reports on OCC and OEC [13–17]. As the possible molecular drivers in NSMP tumors are not established, there is need for further studies.

*POLE*mut and MMRd tumors appear to be less common in the ovary than in the uterus (endometrial clear cell carcinoma 3.8% and 9.8% [31], endometrial endometrioid carcinoma 7.6% and 29% [32]). In the endometrium, *POLE*mut and MMRd cases constitute a significant proportion of grade 3 endometrioid tumors, 13% and 36%, respectively [33]. No high-grade *POLE*mut tumors were found in our cohort, and only one of ten MMRd OEC cases was grade 3, which is similar to the findings of the other ovarian cancer studies [14–17,32]. MMRd ovarian tumors were more likely to present with synchronous endometrial carcinoma (in 70% of OEC and 25% of OCC MMRd subgroup), which is consistent with literature reporting synchronous endometrial and ovarian tumors especially in relation to microsatellite instability or related hereditary syndromes (75%) [34]. Interestingly, we found synchronous carcinomas to be relatively common also in *POLE*mut category, as 40% of *POLE*mut OECs and the only *POLE*mut OCC (100%) co-occurred with endometrial carcinoma.

Our results demonstrated distinct DFS and DSS for the TCGA molecular subgroups in both OCC and OEC. The survival differences between the subgroups were clearer in OCC than in OEC. In OCC, the *POLE*mut and MMRd tumors carried excellent prognosis with no recurrences or deaths. The p53abn cases showed extremely poor outcome with 10% 5-year DSS rate, whereas the prognosis of NSMP cases was in the middle with 65% 5-year DSS rate. The only previous TCGA molecular classification study on OCC was limited by the small number of cases and comparison of only p53abn and p53 wild type cases, where the difference was significant for DFS, but not for DSS [13]. Thus, our findings provide new evidence for the prognostic use of molecular classification system in OCC.

In OEC, our findings are in line with the previous studies showing the most favorable survival in *POLE*mut group, whereas the prognosis of p53abn cases was poor and NSMP cases intermediate [14–17]. However, MMRd subgroup carried relatively poor prognosis in our OEC cohort. In the other ovarian studies the prognosis of MMRd cases varied between favorable and poorer than NSMP subgroup [14–17]. The poor prognosis of the MMRd cases is apparently in contrast to the reported good outcome of ovarian cancer patients with Lynch syndrome (10-year survival >85%) [35], but it is known that Lynch syndrome patients represent only a small proportion of MMRd cases. In our series, only two

of the ten OEC MMRd patients were known carriers of the Lynch syndrome.

In endometrial endometrioid carcinoma, the proportion of MLH1 deficiency is far more common (>80%) than the deficiency of the other three MMR proteins (<20%), and defective MLH1 is mostly (>90%) due to the promoter hypermethylation instead of a somatic/germline mutation in the gene itself [36]. In a recent study MLH1-methylated MMR deficient tumors had significantly worse outcome compared to other MMR deficient and MMR proficient endometrial endometrioid carcinomas, and the patients were also significantly older at the time of diagnosis [36]. We do not have methylation testing data for our ovarian carcinoma series, but three of four disease-related deaths among endometrioid MMRd subgroup occurred in cases with MLH1 deficiency, a tendency to older age and concurrent endometrial and ovarian tumors, offering a potential explanation for the poor performance of the OEC MMRd subgroup.

None of four OCC MMRd tumors, compared to 60% in OEC MMRd group, showed loss of both MLH1 and PMS2, which is usually caused by MLH1 promoter hypermethylation also in ovarian tumors. Other aberrant profiles (loss of MSH2/MSH6 in combination, MSH6 or PMS2 alone) are mostly caused by somatic or germline mutation in the respective gene [37]. According to hospital records one OCC patient was later identified to have Lynch syndrome. As germline analysis of MMR genes was not systematically performed, the true proportion of Lynch syndrome associated tumors cannot be assessed. However, the prognosis of all MMRd OCCs was favorable (5-year DSS 100%).

Currently, anti-PD1-based immune therapy with pembrolizumab is recommended as an option for second-line therapy of MSI/MMRd uterine carcinomas [38], and anti-PD1-antibody dostarlimab has shown efficacy for recurrent or advanced MMRd endometrial cancer that has progressed on platinum-based therapy [39]. In the ovary, immune checkpoint inhibitors are also a promising group of therapy for MMRd cases with aggressive behavior, as they have been accepted by FDA to treat microsatellite unstable tumors at advanced stage irrespective of the anatomic origin of the tumor [38]. Even though the overall prognosis of *POLE*mut tumors is excellent, in our series one patient with *POLE*mut OEC died of her ovarian cancer. Similarly, a small proportion of *POLE*mut OECs with adverse outcome has been reported by Hollis et al. [40]. Thus, immune therapy may also be a future option for rare advanced *POLE*mut tumors due to the high mutation burden and neoantigen load [41].

In conclusion, this study demonstrates distinct survival characteristics for the molecular subgroups in both endometriosis-associated histotypes of ovarian cancer, i.e. ovarian clear cell and endometrioid carcinoma. For the first time, these findings show prognostic value in OCC, and add evidence for the feasibility of the molecular classification for estimating patient outcome in OEC. The distribution and the prognosis of the molecular subgroups in ovarian clear cell and endometrioid carcinomas resemble each other to some extent, but also show certain differences. *POLE*mut, MMRd and p53abn tumors appear to be less frequent in the clear cell than endometrioid histotype. The prognosis of MMRd OCCs was excellent, whereas the relatively poor prognosis of MMRd OECs associated with certain clinical characteristics, raising a question of the significance of MLH1 promoter hypermethylation in the pathogenesis. In addition, the poor prognosis of p53abn tumors was particularly emphasized in OCCs. Still, by far the most common subgroup was NSMP, especially in OCCs, and more data is needed to better characterize this group.

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Author contributions

All authors contributed to the article and approved the submitted version.

Declaration of Competing Interest

All authors declare no conflict of interest related to this work.

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