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MCM3 is a novel proliferation marker associated with longer survival for patients with tubo-ovarian high-grade serous carcinoma

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Abstract

Tubo-ovarian high-grade serous carcinomas (HGSC) are highly proliferative neoplasms that generally respond well to platinum/taxane chemotherapy. We recently identified minichromosome maintenance complex component 3 (MCM3), which is involved in the initiation of DNA replication and proliferation, as a favorable prognostic marker in HGSC. Our objective was to further validate whether MCM3 mRNA expression and possibly MCM3 protein levels are associated with survival in patients with HGSC. MCM3 mRNA expression was measured using NanoString expression profiling on formalin-fixed and paraffin-embedded tissue (N=2355 HGSC) and MCM3 protein expression was assessed by immunohistochemistry (N=522 HGSC) and compared with Ki-67. Kaplan-Meier curves and the Cox proportional hazards model were used to estimate associations with survival. Among chemotherapy-naïve HGSC, higher MCM3 mRNA expression (one standard deviation increase in the score) was associated with longer overall survival (HR=0.87, 95% CI 0.81–0.92, p<0.0001, N=1840) in multivariable analysis. MCM3 mRNA expression was highest in the HGSC C5.PRO molecular subtype, although no interaction was observed between MCM3, survival and molecular subtypes. MCM3 and Ki-67 protein levels were significantly lower after exposure to neoadjuvant chemotherapy compared to chemotherapy-naïve tumors: 37.0% versus 46.4% and 22.9% versus 34.2%, respectively. Among chemotherapy-naïve HGSC, high MCM3 protein levels were also associated with significantly longer disease-specific survival (HR=0.52, 95% CI 0.36–0.74, p=0.0003, N=392) compared to cases with low MCM3 protein levels in multivariable analysis. MCM3 immunohistochemistry is a promising surrogate marker of proliferation in HGSC.

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Not applicable.

Code availability
Not applicable.

Ethics approval
All study sites received ethics board approval. Please refer to supplementary table S1.

Consent to participate
Not applicable.

Consent for publication
Not applicable.

Keywords

High-grade serous carcinoma; proliferation; MCM3

Introduction

Tubo-ovarian high-grade serous carcinomas (HGSC) are aggressive neoplasms with unfavorable prognosis despite a generally good initial response to platinum/taxane chemotherapy [1]. Recent molecular characterization using NanoString mRNA expression profiling has defined four previously described consensus molecular subtypes of HGSC with prognostic associations: C1.MES, C2.IMM, C4.DIF, and C5.PRO [2]. Other validated prognostic factors in HGSC include *BRCA1/2* mutations, diffuse progesterone receptor expression, degree of CD8+ tumor-infiltrating lymphocytes, and CCNE1 high-level amplifications [3–5]. In a large multi-institutional study of the Ovarian Tumor Tissue Analysis (OTTA) consortium, we recently showed that a 101 gene expression signature could stratify women diagnosed with HGSC according to survival with median survival differences of more than 7 years, supporting significant heterogeneity with respect to intrinsic tumor biology and response to therapy [6].

Among the top 25 prognostic genes from the study by Millstein et al. [6], we focused on one gene: minichromosome maintenance complex component 3 (*MCM3*) for the current study. Although *MCM3* showed a hazard ratio (HR) of 0.89 (95% CI 0.85–0.93) compared to 0.84 for the top candidate *TAP1* (95% CI 0.80–0.87) [6], we selected *MCM3* for 2 reasons: first, this was one of the few top 25 gene where a high-quality antibody with nuclear staining signal was available, and second, this protein is involved in proliferation, an under-recognized aspect of cell biology in tubo-ovarian high-grade serous carcinoma. *MCM3* is a component of the pre-replication complex and involved in the initiation of DNA replication and cell cycle progression [7]. Gene microarray analyses have confirmed the association of *MCM3* with cell proliferation [8], and functional studies have shown that downregulation of *MCM3* suppresses G1/S cell cycle progression [9]. MCM3 has been utilized as a novel proliferation marker in other cancer types with better prognostication than Ki-67 [8, 10]. Although increased *MCM3* expression has previously been demonstrated to be a poor prognostic marker in ovarian carcinomas, the analyses were performed on a combination of tumor histotypes [11, 12], and no studies to date have evaluated its use specifically in HGSC.

HGSC are characterized by high proliferation rates. Mitotic count is the secondary morphologic diagnostic criterion after nuclear pleomorphism in distinguishing HGSC from ovarian low-grade serous carcinomas [13]. However, within HGSC, associations of the proliferation marker Ki-67 with survival have not been consistent. A significant association of Ki-67 with survival in ovarian carcinomas of all histotypes has been shown but this association was lost when restricted to HGSC without residual disease [14]. More recently, two studies of HGSC reported that high Ki-67 levels were associated with longer survival [15, 16]. Consistent with these findings, a high proportion of long-term survivors diagnosed

with HGSC also showed higher Ki-67 expression [17]. Therefore, assessing proliferation might improve prognostication and/or prediction of chemotherapy response in HGSC.

The aim of this study was to further validate whether *MCM3* mRNA expression correlates with survival in an independent series of samples from patients with HGSC. A secondary aim was to evaluate whether MCM3 protein levels assessed by immunohistochemistry (IHC) are also associated with survival.

Methods

Study cohorts

Formalin-fixed and paraffin-embedded (FFPE) tissue samples of HGSC were contributed through the OTTA consortium and were independent samples from the previous OTTA study by Millstein et al. [6]. Each participating study had local ethics approval (Supplementary Table S1). A flow chart of study cases is shown in Supplementary Figure S1. *MCM3* mRNA expression was successfully measured for a total of 2355 samples. Of these, 1836 samples were obtained at the time of primary debulking surgery (referred to as “chemotherapy-naïve” cases), while 519 specimens were obtained at the time of interval debulking surgery following exposure to neoadjuvant chemotherapy (referred to as “post neoadjuvant chemotherapy” cases). 79.8% of chemotherapy-naïve and 70.3% post neoadjuvant chemotherapy samples were obtained from adnexal tumor sites. Representative hematoxylin & eosin slides were reviewed by expert pathologists to confirm HGSC histotype, and tumor areas for mRNA extraction were circled on a glass slide. Tumor cellularity was estimated in 20% intervals (0–20, 21–40, 41–60, 61–80, 81–100) and samples with 0–20% were excluded.

For IHC, one of the OTTA study sites with partial overlap with the mRNA expression analysis cohort was used (224 HGSC underwent both mRNA expression and IHC assays), which consisted of 950 ovarian carcinomas diagnosed in the two Canadian provinces British Columbia and Alberta (OVAL BC), Canada between 2001 and 2012 [18]. Contemporary histotype classification was previously established by integrating morphological review with an 8-marker IHC prediction model [18]. Rare mixed carcinomas of the ovary were excluded [19]. Tumor samples represented on tissue microarrays (TMAs) in 0.6 mm triplicate or duplicate cores were utilized for MCM3 and Ki-67 immunohistochemistry. The TMAs contained a variety of normal tissue as controls. Immunohistochemical data for MCM3 were obtained from 848 cases with ovarian carcinoma including 522 HGSC and 326 non-HGSC (154 endometrioid carcinomas (EC), 105 clear cell carcinomas (CCC), 24 low-grade serous carcinomas (LGSC), 40 mucinous carcinomas (MC), and 3 mesonephric-like adenocarcinomas). From the 522 HGSC, 393 specimens were chemotherapy-naïve and 108 post neoadjuvant chemotherapy, while 6 patients did not receive chemotherapy and the chemotherapy status was unknown for 15 patients. Ethics approval was received from the Health Research Ethics Board of Alberta (HREBA.CC-16–0161, HREBA.CC-16–0159).

mRNA analysis

MCM3 mRNA expression in FFPE tumor samples and cell lines was assessed using the NanoString nCounter technology with previously described RNA extraction methods, assay run parameters, data processing, and control/reference samples [20]. The *MCM3* target sequence was GGCTTCTGAACAATGCCTTTGAGGAGCTGGTTGCCTTCCAGCGGGCCTTAAAGGATTTTGTGGCCTCCATTGATGCTACCTATGCCAAGCAGTATGAGGA and *MCM3* mRNA data were normalized against housekeeping genes [20]. Quality assurance of the assay was previously assessed with high duplicate sample correlation [2, 6]. In addition to *MCM3* expression, we applied the PrOTYPE NanoString based assay and assigned adnexal samples to gene expression based molecular subtypes of HGSC: C1.MES, C2.IMM, C4.DIF, and C5.PRO [2].

MCM3 and Ki-67 immunohistochemistry

IHC was performed on tissue microarray sections of 4 μm thickness using a DAKO Omnis platform (Agilent Technologies, Santa Clara, CA). Heat-induced epitope retrieval was performed on board, followed by antibody incubation in Dako EnVision FLEX (Agilent Technologies, Santa Clara, CA). For MCM3, the DAKO Omnis protocol H30-X-30 was utilized with recombinant rabbit monoclonal anti-MCM3 antibody (dilution 1/1000, clone EPR7080, catalogue # ab128923; Abcam, Cambridge, UK). For Ki-67, the DAKO Omnis protocol L20-X-20 was used with mouse monoclonal anti-Ki-67 antibody (ready-to-use, clone Mib-1, catalogue # GA626; Agilent Technologies, Santa Clara, CA). Nuclear expression in tumor cells was scored in 5% increments. Based on the distribution, no naturally occurring cut-off was apparent. Therefore, scores were categorized into 3 relatively equally sized groups after several iterations to optimize for prognostic stratification (For MCM3: <40%, 40% to 75%, >75%; for Ki-67 <20%, 20% to 30%, >30%). TMA cores with less than 10% tumor content were excluded from study. A subset of cases was scored again by a second observer blinded to the initial scores. Previously generated immunohistochemical and chromogenic *in situ* hybridization (CISH) data for p53, p16, RB1, and CCNE1 were used for correlative analysis [5, 18, 21].

Statistical analyses

For the mRNA expression analysis (OTTA cohort), survival analysis was carried out for overall survival (OS) with right censoring at 10 years and left truncation of prevalent cases. Kaplan-Meier plots displayed survival for tertiles or quintiles of patients categorized according to gene expression. The p-value corresponded to a log-rank test of differences between groups. Cox proportional hazards regression models were stratified by study site, included a b-spline on age with a knot at median age to account for non-linear effects, and included stage as an adjustment covariate. Further Cox proportional hazards regression models were stratified by molecular subtype. Genes were scaled to have a standard deviation of one, so hazard ratios (HRs) correspond to a change of one standard deviation. For these analyses, R software v4.0.3 was used.

For the IHC analysis (OVAL BC cohort), paired interobserver reproducibility was analyzed using Cohen’s kappa scores. Categorical data were compared using Pearson’s chi-squared

test and continuous data using analysis of variance (ANOVA) or Spearman's correlation coefficient. Disease-specific survival was used as the endpoint with right censoring at 10 years. Kaplan-Meier plots displayed survival for three categories according to protein level. Covariates in multivariable Cox proportional hazards regression and parametric survival fit models included province, age (continuous), International Federation of Gynaecology and Obstetrics (FIGO) stage (I, II, III, IV), and residual disease (absent, ≤ 1 cm, and > 1 cm). For these analyses, JMP14.0 software was used.

Results

MCM3 mRNA expression and prognosis across molecular subtypes in high-grade serous carcinoma

As mentioned in the introduction, *MCM3* mRNA expression was significantly associated with a lower risk of death in our previous NanoString study [6]. In a multivariable model, one standard deviation increase in *MCM3* expression score was associated with an HR of 0.89 (N=3561) [6]. Re-analyzing the data with updated survival information obtained since publication did not change the estimate (HR=0.89, Supplementary Table S2).

Clinical characteristics for the new 2355 HGSC from the OTTA consortium used in the current NanoString study are shown in Table 1. *MCM3* mRNA expression levels relative to housekeeping genes varied from −3.2 (down-regulated) to 4.25 (up-regulated). In chemotherapy-naïve HGSC categorized into tertiles, there was a significant difference in overall survival (log-rank p<0.001; Figure 1). In multivariable models, one standard deviation increase in *MCM3* expression score corresponded to an HR of 0.87 (95% CI 0.81–0.92, p<0.0001, N=1820; Supplementary Table S2). This estimate did not meaningfully change when restricted to chemotherapy-naïve adnexal specimens (HR=0.89, 95% CI 0.83–0.95, p=0.0010, N=1436). No significant survival association was observed for post neoadjuvant chemotherapy cases (HR=1.06, 95% CI 0.95–1.19, p=0.30, N=515; Supplementary Table S2, Supplementary Figure S2), suggesting a modifying effect of chemotherapy exposure.

Analysis stratified by the HGSC molecular subtypes restricted to chemotherapy-naïve and adnexal specimens (N=1436) revealed significant differential expression across molecular subtypes with C5.PRO showing the highest, and C1.MES the lowest, *MCM3* expression (p<0.001; Figure 2). MCM3 mRNA expression was significantly associated with tumor cellularity (Supplementary Table S3). C1.MES had the lowest tumor cellularity (63.9% of cases with ≥ 61% tumor cellularity compared to 86.8% with ≥ 61% tumor cellularity for the other 3 HGSC molecular subtypes combined, Supplementary Table S3). In univariable Kaplan-Meier survival analysis, significant associations were seen for C4.DIF (log-rank p=0.021) and C5.PRO subtypes (log-rank p=0.018, Supplementary Figure S3). In multivariable models stratified by molecular subtype, a significant association was observed within C4.DIF (HR=0.86, 95% CI 0.75–0.98, p=0.021, N=503) and suggestive for C1.MES (HR=0.90, 95% CI 0.75–1.07, p=0.23, N=291) with both showing consistent hazard ratios compared to the first NanoString analysis (Supplementary Table S4). Results for C2.IMM were inconsistent between the studies (Supplementary Table S4). No significant association between *MCM3* expression and survival was seen within C5.PRO. The p-value

for interaction between the molecular subtype and *MCM3*/survival associations was not significant.

MCM3 expression in normal tissue

Normal tissue controls on TMAs were assessed for MCM3 protein levels by IHC. MCM3 was highly expressed in highly proliferative compartments such as germinal centers of the tonsil and intestinal crypts. Focal expression was observed in normal fallopian tube epithelium and cytotrophoblasts in the placenta, while no expression was seen in normal liver or kidney (Supplementary Figure S4).

MCM3 protein expression and prognosis

We performed MCM3 IHC to compare the prognostic significance of MCM3 expression to the standard proliferation marker Ki-67. MCM3 IHC data were available for 393 chemotherapy-naïve and 108 post neoadjuvant chemotherapy HGSC cases. The correlation between IHC scores and mRNA expression computed with data collected from 224 specimens yielded a moderate correlation (Spearman's r_s=0.44; Supplementary Figure S5). Interobserver agreement for MCM3 and Ki-67 scoring was assessed in 111 and 114 cases, respectively, and achieved substantial Cohen's kappa coefficients of 0.79 and 0.72 for categorized scores and Spearman's correlation coefficients of 0.96 and 0.93 for the 5% increment scores, respectively. When considering the timing of chemotherapy, specimens from patients who received neoadjuvant chemotherapy had a significantly lower percentage of MCM3-positive tumor nuclei compared to chemotherapy-naïve tumors (mean 37.0% versus 46.4%, p=0.0057; Figure 3A). A similar difference was seen for Ki-67 (22.9% versus 34.2%, p<0.001; Figure 3B). To avoid the modifying effects of neoadjuvant chemotherapy, we restricted the subsequent analyses to chemotherapy-naïve samples.

MCM3 and Ki-67 protein expression was significantly higher in HGSC compared to other ovarian tumor histotypes (Figure 4) and this was more pronounced for MCM3. In HGSC, the mean MCM3 labelling index of 46.4% (median 45%) was higher compared to Ki-67 with 34.5% (median 27.5%) and showed a wider distribution (MCM3 SD=31.0 versus Ki-67 SD=21.2). There was moderate correlation between MCM3 and Ki-67 levels (r_s=0.50). With categorization of MCM3 protein levels, we observed a significant difference in disease-specific survival (log-rank p=0.012; Figure 5A). Cases with high MCM3 levels (>75%) showed a lower risk of death compared to low MCM3 levels (<40%, HR=0.52, 95% CI 0.37–0.74, p=0.0003) as well as compared to intermediate MCM3 levels (40–75%, HR=0.61, 95% CI 0.42–0.87, p=0.0074) in a multivariable analysis adjusted for age, stage, and residual disease. There was no significant difference between intermediate and low MCM3 levels (HR=0.86, 95% CI 0.64–1.15, p=0.30). When combining low and intermediate MCM3 expressing HGSC as a reference, high expressing cases showed an HR of 0.56 (95% CI 0.40–0.77, p=0.0005). For Ki-67, we did not observe a significant association of high Ki-67 expression with survival in univariable analysis (p=0.72; Figure 5B) or multivariable analysis using low and intermediate expressing cases as a reference (HR=0.83, 95% CI 0.65–1.07, p=0.16). We also performed a parametric survival fit model using the more continuous 5% increments of MCM3 and Ki-67 scores including age, stage, residual disease, and study site. Significant parameters in this model were residual disease

(p<0.0001), MCM3 (p=0.0003), and stage (p=0.0052) but not Ki-67 (p=0.78), age (p=0.52), or study site (p=0.90).

Patients with HGSC and low MCM3 protein levels tended to be diagnosed at higher stage (p=0.048; Table 2). MCM3 protein levels were not significantly associated with residual disease status after surgery, nor with *TP53* mutation classification as previously assessed by surrogate IHC [18, 21]. However, as expected, MCM3 protein levels were significantly and directly associated with diffuse strong p16 block expression (p=0.005) and loss of RB1 (p<0.0001; Table 2). Surprisingly, MCM3 protein levels were not associated with the binary status of previously assessed CCNE1 high-level amplification or protein overexpression. Therefore, we compared in more detail the correlation between MCM3 and CCNE1 protein expression on the continuous scale. However, there was only a weak correlation between MCM3 and CCNE1 (r_s=0.132, p=0.0089). Likewise, the Ki-67 labelling index also weakly correlated with CCNE1 (r_s=0.199, p<0.001).

Discussion

The results of this study of 1820 cases independently validate the previous finding based on 3561 cases [6] that high *MCM3* mRNA expression is associated with longer survival for women diagnosed with HGSC. We observed differences in *MCM3* expression across HGSC molecular subtypes: the highest expression was seen in C5.PRO, which has been named after its association with high expression of proliferation-related genes. Since MCM3 is highly expressed in tumor epithelium and lymphocytes but lowly expressed in the associated stroma, the lower MCM3 expression in C1.MES could be explained by their higher stromal content without dense lymphocytic infiltrate. Bulk tumor analysis can be influenced by the epithelial purity of the specimen [22], however, we mitigated against this by macrodissecting areas with high tumor cellularity. Our data show no interaction between HGSC molecular subtype and the association of *MCM3* expression with survival.

The association of MCM3 with survival was also found when measuring MCM3 protein levels by IHC despite only a modest correlation of *MCM3* mRNA expression with MCM3 protein levels by IHC. There could be a number of reasons for the limited correlation. MCM3 protein levels could be controlled more at the translational or post-translational level than at the transcriptional level. The half-life of the translated protein could be independent of mRNA levels depending on the rate of degradation [23]. A further contribution might be error or noise in the experiments. For the NanoString assay, we standardized specimen handling, pre-processing, and normalization [20]. An intrinsic limitation of the conventional IHC we used is that most cells expressing the protein reached a saturated signal due to highly sensitive polymer-based detection systems and estimating the percentage of stained tumor cells did not allow for accurate quantification of protein expression. Immunofluorescence or quantitative IHC with a linear dynamic range would allow for better quantification of the protein and direct comparison preferentially on the same samples [24]. Ultimately, the action of the protein is what matters and perhaps in future studies, a combination of mRNA expression and spatial protein levels may predict clinical outcomes more accurately than either alone [25].

Chemotherapy is known to have a modifying effect on the proliferation status, illustrated here by the significantly lower MCM3 and Ki-67 expression in post neoadjuvant chemotherapy specimens. This finding parallels the previously reported association of chemotherapy with lower mitotic rates and Ki-67 labelling indices [26, 27] and suggests that proliferation indices are best assessed on chemotherapy-naïve tumor samples. This idea is further supported by the lack of prognostic association of *MCM3* mRNA expression in post neoadjuvant chemotherapy specimens. In contrast, changes in expression following chemotherapy have not been observed with other biomarkers such as WT1 and p53, which is reassuring given their importance as diagnostic markers of HGSC [28].

Despite the diagnostic utility of assessing proliferation using Ki-67 in serous tubal intraepithelial carcinoma, the precursor lesion of HGSC, there is currently no biomarker in use to assess this important aspect of the tumor biology of HGSC. The use of Ki-67 has been implemented for prognostication of gastrointestinal neuroendocrine tumors and carcinomas of the breast, but this study shows that in HGSC, MCM3 allows for prognostication while Ki-67 does not. This result contrasts a previous study of 318 chemotherapy-naïve HGSC patients, which found that high Ki-67 levels were correlated with longer survival [16]. While we observed a similar trend, the association was not statistically significant. Both studies were of similar size and analyzed chemotherapy-naïve samples but differed with respect to the median Ki-67 labelling index, which was higher in the other study: 40% [16] compared to 27.5% in our study. One potential explanation is the use of full sections in the other study [16], which allows for an assessment of hot spots, versus use of TMAs in our study, which randomly samples 2 or 3 small tumor areas. Another explanation could be the challenges around interlaboratory standardization of Ki-67 IHC assays that were encountered with assessment of breast cancers [29].

MCM3 has also been shown to be a good surrogate marker of proliferation in tumors from other sites [8–10, 30–34]. We observed the expected differences of MCM3 and Ki67 levels across high and low proliferating histotypes. Interestingly, even though both MCM3 and Ki-67 have been used as proliferation markers, expression levels only showed moderate correlation in this study (r_s=0.50), which is similar to a previous study in squamous cell carcinomas (r_s=0.52) but lower compared to a study of cutaneous T-cell lymphoma (r=0.91) [35, 36]. To date, the function of Ki-67 remains unclear, although some studies suggest a role in ribosome biosynthesis during cell proliferation rather than a direct association with the cell cycle [37]. Ki-67 expression also appears to be more profoundly influenced by proinflammatory factors compared to MCM3 [10, 38]. Conversely, MCM proteins are expressed throughout the cell cycle and maintained for longer than Ki-67, continuing until the transition between G_0 and G_1, which could explain why we observed higher protein expression of MCM3 compared to Ki-67 [39, 40]. Additionally, loss of MCM3 expression is seen in quiescent or differentiated cells [39]. Therefore, it is possible that MCM3 more accurately reflects the proliferative status of tumor cells without being influenced by other factors and thus is a more reliable proliferation marker for HGSC than Ki-67. In one study evaluating mitotic counts and PHH3 and Ki-67 expression by IHC in invasive breast cancer, mitotic count strongly correlated with PHH3 (r=0.94), but Ki-67 showed weaker associations with PHH3 (r=0.79) and mitotic count (r=0.83) [41]. It would be interesting to evaluate whether MCM3 expression more strongly correlates with mitotic counts and PHH3

expression in future studies. Additionally, future studies could test a potential diagnostic use of MCM3 for serous tubal intraepithelial carcinoma and explore the prognostic utility in other histotypes of ovarian carcinoma.

Previously reported studies evaluating MCM3 in cohorts with multiple combined ovarian carcinoma histotypes showed that higher expression of MCM3 is associated with shorter survival [11, 12]. In contrast, we show that in HGSC, high MCM3 expression is associated with longer survival. This difference can be explained by the fact that in studies including all histotypes, due to its higher expression in HGSC, MCM3 mainly serves as a surrogate marker for HGSC (the most aggressive histotype). As we have previously shown, histotype is an effect modifier for biomarker studies and analyses should be stratified by histotype [14]. When we restricted the analysis to HGSC, the direction of the association reversed. Given that almost all patients with HGSC are uniformly treated with a standard chemotherapy regimen and that rapidly proliferating tumors have been shown previously to respond better to chemotherapy than slowly growing tumors [42], we can indirectly infer that longer overall survival seen in cases of HGSC with high MCM3 expression is likely a reflection of good response to platinum/taxane chemotherapy. MCM3 may thus be a useful predictive marker of chemotherapy response in HGSC. Future studies may evaluate the predictive value of MCM3 in a “window of opportunity” approach by studying MCM3 expression in paired pre- and post-neoadjuvant chemotherapy samples of HGSC and correlating changes in expression levels with the chemotherapy response score to determine whether MCM3 would be an early predictive marker for platinum/taxane chemotherapy resistance in the context of *BRCA1/2*, homologous repair deficiency, and molecular subtype status. A prerequisite would be to study the intertumoral heterogeneity of MCM3 expression to assure comparability between adnexal and omental specimens.

In this study, we also explored the associations of other cell cycle related proteins such as p16, RB1, and CCNE1 with MCM3 protein levels. As expected, RB1 loss and diffuse strong block positivity for p16 were strongly associated with MCM3 expression. This corresponds with the normal role of RB1 as a negative regulator of proliferation and the futility of p16 block staining as a proliferation suppressor in this context [43]. Surprisingly, there was only a weak association between proliferation and CCNE1 expression, suggesting that low levels of CCNE1 are sufficient to enter the G1/S phase but the relationship is not dose-dependent, and high levels of CCNE1 protein expression are not strongly associated with proliferation. Given the opposite prognostic association between MCM3 expression/tumor proliferation and CCNE1 high-level amplification, a mechanism for chemoresistance of CCNE1 amplification that is unrelated to proliferation seems likely [5][9]. For example, upregulation of CCNE1 has also been shown to induce centrosome amplification, resulting in polyploid cells with multiple centers of partially completed mitosis [44].

A limitation of our study is that, we evaluated a lower number of cases with IHC, precluding a definitive conclusion as to whether mRNA or protein is the better prognosticator. Of note, we also used different analytical approaches for mRNA expression (HR reflects one change in standard deviation) and protein (HR compares 3 categories), which should be kept in mind when comparing the HRs. Despite different endpoints for mRNA (overall survival) versus protein expression (disease-specific survival), we arrived at the same conclusion and

believe that both results serve as cross validation of the biomarker itself. Since most patients with HGSC die of the disease, and we mitigated against death from other causes by right censoring at 10 years. Disease-specific survival data are more important for other cancer types with better outcome. We acknowledge possible selection bias in the IHC analysis when excluding TMA cores with less than 10% tumor. However, restricting the analysis to chemotherapy-naïve cases excludes the possibility that low tumor content is an effect of neoadjuvant chemotherapy.

In conclusion, we demonstrated that high MCM3 tumor expression, assessed either on mRNA or protein level, is associated with longer survival in patients with HGSC, suggesting that high MCM3 expressing HGSC respond better to standard chemotherapy. MCM3 IHC is a promising surrogate assay to evaluate proliferation in HGSC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Reference

1. Peres LC, Cushing-Haugen KL, Kobel M, Harris HR, Berchuck A, Rossing MA, Schildkraut JM, Doherty JA (2019) Invasive Epithelial Ovarian Cancer Survival by Histotype and Disease Stage J. Natl. Cancer Inst 111:60–68. doi: 10.1093/jnci/djy071 [PubMed: 29718305]
2. Talhouk A, George J, Wang C, Budden T, Tan TZ, Chiu DS, Kommoss S, Leong HS, Chen S, Intermaggio MP, Gilks B, Nazeran TM, Volchek M, Elatre W, Bentley RC, Senz J, Lum A, Chow V, Sudderuddin H, Mackenzie R, Leong SCY, Liu G, Johnson D, Chen B, Group A, Alsop J, Banerjee SN, Behrens S, Bodelon C, Brand AH, Brinton L, Carney ME, Chiew YE, Cushing-Haugen KL, Cybulski C, Ennis D, Fereday S, Fortner RT, García-Donas J, Gentry-Maharaj A, Glasspool R, Goranova T, Greene CS, Haluska P, Harris HR, Hendley J, Hernandez BY, Herpel E, Jimenez-Linan M, Karpinskyj C, Kaufmann SH, Keeney GL, Kennedy CJ, Köbel M, Koziak JM, Larson MC, Lester J, Lewsley LA, Lissowska J, Lubiński J, Luk H, Macintyre G, Mahner S, McNeish IA, Menkiszak J, Nevins N, Osorio A, Oszurek O, Palacios J, Hinsley S, Pearce CL, Pike MC, Piskorz AM, Ray-Coquard I, Rhenius V, Rodriguez-Antona C, Sharma R, Sherman ME, De Silva D, Singh N, Sinn P, Slamon D, Song H, Steed H, Stronach EA, Thompson PJ, Tołoczko A, Trabert B, Traficante N, Tseng CC, Widschwendter M, Wilkens LR, Winham SJ, Winterhoff B, Beeghly-Fadiel A, Benitez J, Berchuck A, Brenton JD, Brown R, Chang-Claude J, Chenevix-Trench G, deFazio A, Fasching PA, García MJ, Gayther SA, Goodman MT, Gronwald J, Henderson MJ, Karlan BY, Kelemen LE, Menon U, Orsulic S, Pharoah PDP, Wentzensen N, Wu AH, Schildkraut JM, Rossing MA, Konecny GE, Huntsman DG, Huang RY, Goode EL, Ramus SJ, Doherty JA, Bowtell DD, Anglesio MS (2020) Development and Validation of the Gene Expression Predictor of High-grade Serous Ovarian Carcinoma Molecular SubTYPE (PrOTYPE) Clin. Cancer Res 26:5411–5423. doi: 10.1158/1078-0432.Ccr-20-0103 [PubMed: 32554541]

3. Bolton KL, Chenevix-Trench G, Goh C, Sadetzki S, Ramus SJ, Karlan BY, Lambrechts D, Despierre E, Barrowdale D, McGuffog L, Healey S, Easton DF, Sinilnikova O, Benítez J, García MJ, Neuhausen S, Gail MH, Hartge P, Peock S, Frost D, Evans DG, Eeles R, Godwin AK, Daly MB, Kwong A, Ma ES, Lázaro C, Blanco I, Montagna M, D'Andrea E, Nicoletto MO, Johnatty SE, Kjær SK, Jensen A, Høgdall E, Goode EL, Fridley BL, Loud JT, Greene MH, Mai PL, Chetrit A, Lubin F, Hirsh-Yechezkel G, Glendon G, Andrulis IL, Toland AE, Senter L, Gore ME, Gourley C, Michie CO, Song H, Tyrer J, Whittemore AS, McGuire V, Sieh W, Kristoffersson U, Olsson H, Borg Å, Levine DA, Steele L, Beattie MS, Chan S, Nussbaum RL, Moysich KB, Gross J, Cass I, Walsh C, Li AJ, Leuchter R, Gordon O, Garcia-Closas M, Gayther SA, Chanock SJ, Antoniou AC, Pharoah PD (2012) Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer JAMA 307:382–390. doi: 10.1001/jama.2012.20 [PubMed: 22274685]

4. Sieh W, Köbel M, Longacre TA, Bowtell DD, deFazio A, Goodman MT, Høgdall E, Deen S, Wentzensen N, Moysich KB, Brenton JD, Clarke BA, Menon U, Gilks CB, Kim A, Madore J, Fereday S, George J, Galletta L, Lurie G, Wilkens LR, Carney ME, Thompson PJ, Matsuno RK, Kjær SK, Jensen A, Høgdall C, Kalli KR, Fridley BL, Keeney GL, Vierkant RA, Cunningham JM, Brinton LA, Yang HP, Sherman ME, García-Closas M, Lissowska J, Odunsi K, Morrison C, Lele S, Bshara W, Sucheston L, Jimenez-Linan M, Driver K, Alsop J, Mack M, McGuire V, Rothstein JH, Rosen BP, Bernardini MQ, Mackay H, Oza A, Wozniak EL, Benjamin E, Gentry-Maharaj A, Gayther SA, Tinker AV, Prentice LM, Chow C, Anglesio MS, Johnatty SE, Chenevix-Trench G, Whittemore AS, Pharoah PDP, Goode EL, Huntsman DG, Ramus SJ (2013) Hormone-receptor expression and ovarian cancer survival: an Ovarian Tumor Tissue Analysis consortium study The Lancet Oncology 14:853–862. doi: 10.1016/s1470-2045(13)70253-5 [PubMed: 23845225]

5. Chan AM, Enwere E, McIntyre JB, Wilson H, Nwaroh C, Wiebe N, Ou Y, Liu S, Wiedemeyer K, Rambau PF, Grevers X, Morris DG, Neri P, Gilks CB, Visser F, Le N, Luo L, Cook LS, Kobel M (2020) Combined CCNE1 high-level amplification and overexpression is associated with unfavourable outcome in tubo-ovarian high-grade serous carcinoma The journal of pathology. Clinical research 6:252–262. doi: 10.1002/cjp2.168 [PubMed: 32391646]

6. Millstein J, Budden T, Goode EL, Anglesio MS, Talhouk A, Intermaggio MP, Leong HS, Chen S, Elatre W, Gilks B, Nazeran T, Volchek M, Bentley RC, Wang C, Chiu DS, Kommoss S, Leung SCY, Senz J, Lum A, Chow V, Sudderuddin H, Mackenzie R, George J, Group A, Fereday S, Hendley J, Traficante N, Steed H, Koziak JM, Kobel M, McNeish IA, Goranova T, Ennis D, Macintyre G, Silva De Silva D, Ramon YCT, Garcia-Donas J, Hernando Polo S, Rodriguez GC, Cushing-Haugen KL, Harris HR, Greene CS, Zelaya RA, Behrens S, Fortner RT, Sinn P, Herpel E, Lester J, Lubinski J, Oszurek O, Toloczko A, Cybulski C, Menkiszak J, Pearce CL, Pike MC, Tseng C, Alsop J, Rhenius V, Song H, Jimenez-Linan M, Piskorz AM, Gentry-Maharaj A, Karpinskyj C, Widschwendter M, Singh N, Kennedy CJ, Sharma R, Harnett PR, Gao B, Johnatty SE, Sayer R, Boros J, Winham SJ, Keeney GL, Kaufmann SH, Larson MC, Luk H, Hernandez BY, Thompson PJ, Wilkens LR, Carney ME, Trabert B, Lissowska J, Brinton L, Sherman ME, Bodelon C, Hinsley S, Lewsley LA, Glasspool R, Banerjee SN, Stronach EA, Haluska P, Ray-Coquard I, Mahner S, Winterhoff B, Slamon D, Levine DA, Kelemen LE, Benitez J, Chang-Claude J, Gronwald J, Wu AH, Menon U, Goodman MT, Schildkraut JM, Wentzensen N, Brown R, Berchuck A, Chenevix-Trench G, deFazio A, Gayther SA, Garcia MJ, Henderson MJ, Rossing MA, Beeghly-Fadiel A, Fasching PA, Orsulic S, Karlan BY, Konecny GE, Huntsman DG, Bowtell DD, Brenton JD, Doherty JA, Pharoah PDP, Ramus SJ (2020) Prognostic gene expression signature for high-grade serous ovarian cancer Ann. Oncol 31:1240–1250. doi: 10.1016/j.annonc.2020.05.019 [PubMed: 32473302]

7. Forsburg SL (2004) Eukaryotic MCM proteins: beyond replication initiation Microbiol. Mol. Biol. Rev 68:109–131. doi: 10.1128/mmbr.68.1.109-131.2004 [PubMed: 15007098]

8. Zhao Y, Wang Y, Zhu F, Zhang J, Ma X, Zhang D (2020) Gene expression profiling revealed MCM3 to be a better marker than Ki67 in prognosis of invasive ductal breast carcinoma patients Clin. Exp. Med 20:249–259. doi: 10.1007/s10238-019-00604-4 [PubMed: 31980982]

9. Zhou H, Xiong Y, Zhang G, Liu Z, Li L, Hou S, Zhou T (2020) Elevated expression of minichromosome maintenance 3 indicates poor outcomes and promotes G1/S cell cycle progression, proliferation, migration and invasion in colorectal cancer Biosci. Rep 40. doi: 10.1042/bsr20201503

10. Valverde LF, de Freitas RD, Pereira TA, de Resende MF, Agra IMG, Dos Santos JN, Dos Reis MG, Sales CBS, Gurgel Rocha CA (2018) MCM3: A Novel Proliferation Marker in Oral

Squamous Cell Carcinoma Appl. Immunohistochem. Mol. Morphol 26:120–125. doi: 10.1097/pai.0000000000000397 [PubMed: 27258565]

11. Ehlén Å, Nodin B, Rexhepaj E, Brändstedt J, Uhlén M, Alvarado-Kristensson M, Pontén F, Brennan DJ, Jirström K (2011) RBM3-regulated genes promote DNA integrity and affect clinical outcome in epithelial ovarian cancer Transl. Oncol. 4:212–221. doi: 10.1593/tlo.11106
12. Kobierzycki C, Pula B, Skiba M, Jablonska K, Latkowski K, Zabel M, Nowak-Markwitz E, Spaczynski M, Kedzia W, Podhorska-Okolow M, Dziegiel P (2013) Comparison of minichromosome maintenance proteins (MCM-3, MCM-7) and metallothioneins (MT-I/II, MT-III) expression in relation to clinicopathological data in ovarian cancer Anticancer Res. 33:5375–5383 [PubMed: 24324072]
13. Malpica A, Deavers MT, Tornos C, Kurman RJ, Soslow R, Seidman JD, Munsell MF, Gaertner E, Frishberg D, Silva EG (2007) Interobserver and intraobserver variability of a two-tier system for grading ovarian serous carcinoma Am. J. Surg. Pathol 31:1168–1174. doi: 10.1097/PAS.0b013e31803199b0 [PubMed: 17667538]
14. Kobel M, Kalloger SE, Boyd N, McKinney S, Mehl E, Palmer C, Leung S, Bowen NJ, Ionescu DN, Rajput A, Prentice LM, Miller D, Santos J, Swenerton K, Gilks CB, Huntsman D (2008) Ovarian carcinoma subtypes are different diseases: implications for biomarker studies PLoS Med. 5:e232. doi: 10.1371/journal.pmed.0050232 [PubMed: 19053170]
15. Feng Z, Wen H, Bi R, Ju X, Chen X, Yang W, Wu X (2016) A clinically applicable molecular classification for high-grade serous ovarian cancer based on hormone receptor expression Sci. Rep 6:25408. doi: 10.1038/srep25408 [PubMed: 27139372]
16. Chen M, Yao S, Cao Q, Xia M, Liu J, He M (2017) The prognostic value of Ki67 in ovarian high-grade serous carcinoma: an 11-year cohort study of Chinese patients Oncotarget 8:107877–107885. doi: 10.18632/oncotarget.14112
17. Garsed DW, Alsop K, Fereday S, Emmanuel C, Kennedy CJ, Etemadmoghadam D, Gao B, Gebski V, Gares V, Christie EL, Wouters MCA, Milne K, George J, Patch AM, Li J, Arnau GM, Semple T, Gadipally SR, Chiew YE, Hendley J, Mikeska T, Zapparoli GV, Amarasinghe K, Grimmond SM, Pearson JV, Waddell N, Hung J, Stewart CJR, Sharma R, Allan PE, Rambau PF, McNally O, Mileshkin L, Hamilton A, Ananda S, Grossi M, Cohen PA, Leung YC, Rome RM, Beale P, Blomfield P, Friedlander M, Brand A, Dobrovic A, Kobel M, Harnett P, Nelson BH, Bowtell DDL, deFazio A, Nadia Traficante ftAOCSG (2018) Homologous Recombination DNA Repair Pathway Disruption and Retinoblastoma Protein Loss Are Associated with Exceptional Survival in High-Grade Serous Ovarian Cancer Clin. Cancer Res 24:569–580. doi: 10.1158/1078-0432.CCR-17-1621 [PubMed: 29061645]
18. Kobel M, Luo L, Grevers X, Lee S, Brooks-Wilson A, Gilks CB, Le ND, Cook LS (2019) Ovarian Carcinoma Histotype: Strengths and Limitations of Integrating Morphology With Immunohistochemical Predictions Int. J. Gynecol. Pathol 38:353–362. doi: 10.1097/pgp.0000000000000530 [PubMed: 29901523]
19. Mackenzie R, Talhouk A, Eshragh S, Lau S, Cheung D, Chow C, Le N, Cook LS, Wilkinson N, McDermott J, Singh N, Kommoss F, Pfisterer J, Huntsman DG, Kobel M, Kommoss S, Gilks CB, Anglesio MS (2015) Morphologic and Molecular Characteristics of Mixed Epithelial Ovarian Cancers Am. J. Surg. Pathol 39:1548–1557. doi: 10.1097/pas.0000000000000476 [PubMed: 26099008]
20. Talhouk A, Kommoss S, Mackenzie R, Cheung M, Leung S, Chiu DS, Kalloger SE, Huntsman DG, Chen S, Intermaggio M, Gronwald J, Chan FC, Ramus SJ, Steidl C, Scott DW, Anglesio MS (2016) Single-Patient Molecular Testing with NanoString nCounter Data Using a Reference-Based Strategy for Batch Effect Correction PLoS One 11:e0153844. doi: 10.1371/journal.pone.0153844 [PubMed: 27096160]
21. Kobel M, Piskorz AM, Lee S, Lui S, LePage C, Marass F, Rosenfeld N, Mes Masson AM, Brenton JD (2016) Optimized p53 immunohistochemistry is an accurate predictor of TP53 mutation in ovarian carcinoma The journal of pathology. Clinical research 2:247–258. doi: 10.1002/cjp2.53 [PubMed: 27840695]
22. Hunt AL, Bateman NW, Barakat W, Makohon-Moore S, Hood BL, Conrads KA, Zhou M, Calvert V, Pierobon M, Loffredo J, Litzi TJ, Oliver J, Mitchell D, Gist G, Rojas C, Blanton B, Robinson EL, Odunsi K, Sood AK, Casablanca Y, Darcy KM, Shriver CD, Petricoin EF,

Rao UNM, Maxwell GL, Conrads TP (2021) Extensive three-dimensional intratumor proteomic heterogeneity revealed by multiregion sampling in high-grade serous ovarian tumor specimens iScience 24:102757. doi: 10.1016/j.isci.2021.102757 [PubMed: 34278265]

23. Liu Y, Beyer A, Aebersold R (2016) On the Dependency of Cellular Protein Levels on mRNA Abundance Cell 165:535–550. doi: 10.1016/j.cell.2016.03.014 [PubMed: 27104977]
24. Jensen K, Krusenstjerna-Hafstrøm R, Lohse J, Petersen KH, Derand H (2017) A novel quantitative immunohistochemistry method for precise protein measurements directly in formalin-fixed, paraffin-embedded specimens: analytical performance measuring HER2 Mod. Pathol. 30:180–193. doi: 10.1038/modpathol.2016.176
25. Vathiotis IA, Yang Z, Reeves J, Toki M, Aung TN, Wong PF, Kluger H, Syrigos KN, Warren S, Rimm DL (2021) Models that combine transcriptomic with spatial protein information exceed the predictive value for either single modality NPJ precision oncology 5:45. doi: 10.1038/s41698-021-00184-1 [PubMed: 34050252]
26. Bromley AB, Altman AD, Chu P, Nation JG, Nelson GS, Ghatage P, Kalloger SE, Han G, Kobel M (2012) Architectural patterns of ovarian/pelvic high-grade serous carcinoma Int. J. Gynecol. Pathol 31:397–404. doi: 10.1097/PGP.0b013e31824c2372 [PubMed: 22833078]
27. Miller K, Price JH, Dobbs SP, McClelland RH, Kennedy K, McCluggage WG (2008) An immunohistochemical and morphological analysis of post-chemotherapy ovarian carcinoma J. Clin. Pathol 61:652–657. doi: 10.1136/jcp.2007.053793 [PubMed: 18006668]
28. Casey L, Köbel M, Ganesan R, Tam S, Prasad R, Böhm S, Lockley M, Jeyarajah AJ, Brockbank E, Faruqi A, Gilks CB, Singh N (2017) A comparison of p53 and WT1 immunohistochemical expression patterns in tubo-ovarian high-grade serous carcinoma before and after neoadjuvant chemotherapy Histopathology 71:736–742. doi: 10.1111/his.13272 [PubMed: 28570008]
29. Nielsen TO, Leung SCY, Rimm DL, Dodson A, Acs B, Badve S, Denkert C, Ellis MJ, Fineberg S, Flowers M, Kreipe HH, Laenkholm AV, Pan H, Penault-Llorca FM, Polley MY, Salgado R, Smith IE, Sugie T, Bartlett JMS, McShane LM, Dowsett M, Hayes DF (2020) Assessment of Ki67 in Breast Cancer: Updated Recommendations from the International Ki67 in Breast Cancer Working Group J. Natl. Cancer Inst doi: 10.1093/jnci/djaa201
30. Jaafari-Ashkavandi Z, Mehranmehr F, Roosta E (2019) MCM3 and Ki67 proliferation markers in odontogenic cysts and ameloblastoma Journal of oral biology and craniofacial research 9:47–50. doi: 10.1016/j.jobcr.2018.09.003 [PubMed: 30225187]
31. Lameira AG, Pontes FS, Guimarães DM, Alves AC, de Jesus AS, Pontes HA, Pinto Ddos S Jr., (2014) MCM3 could be a better marker than Ki-67 for evaluation of dysplastic oral lesions: an immunohistochemical study J. Oral Pathol. Med 43:427–434. doi: 10.1111/jop.12153 [PubMed: 24456424]
32. Li HT, Wei B, Li ZQ, Wang X, Jia WX, Xu YZ, Liu JY, Shao MN, Chen SX, Mo NF, Zhao D, Zuo WP, Qin J, Li P, Zhang QL, Yang XL (2020) Diagnostic and prognostic value of MCM3 and its interacting proteins in hepatocellular carcinoma Oncol. Lett 20:308. doi: 10.3892/ol.2020.12171
33. Nodin B, Fridberg M, Jonsson L, Bergman J, Uhlén M, Jirström K (2012) High MCM3 expression is an independent biomarker of poor prognosis and correlates with reduced RBM3 expression in a prospective cohort of malignant melanoma Diagn. Pathol. 7:82. doi: 10.1186/1746-1596-7-82
34. Raja R, Shetty DC, Chandrakanta, Juneja S, Tandon A, Gulati N (2021) MCM3 proliferative index is worthier over Ki-67 in the characterization of salivary gland tumors Indian J. Pathol. Microbiol 64:22–27. doi: 10.4103/ijpm.Ijpm_63_20
35. Nowinska K, Chmielewska M, Piotrowska A, Pula B, Pastuszewski W, Krecicki T, Podhorska-Okołow M, Zabel M, Dziegiel P (2016) Correlation between levels of expression of minichromosome maintenance proteins, Ki-67 proliferation antigen and metallothionein I/II in laryngeal squamous cell cancer Int. J. Oncol 48:635–645. doi: 10.3892/ijo.2015.3273 [PubMed: 26648405]
36. Jankowska-Konsur A, Kobierzycki C, Reich A, Grzegrzolka J, Maj J, Dziegiel P (2015) Expression of MCM-3 and MCM-7 in Primary Cutaneous T-cell Lymphomas Anticancer Res. 35:6017–6026 [PubMed: 26504025]
37. MacCallum DE, Hall PA (2000) The location of pKi67 in the outer dense fibrillary compartment of the nucleolus points to a role in ribosome biogenesis during the cell division cycle J.

Pathol. 190:537–544. doi: 10.1002/(sici)1096-9896(200004)190:5<537::Aid-path577>3.0.Co;2-w [PubMed: 10727979]

38. Doger FK, Dikicioglu E, Ergin F, Unal E, Sendur N, Uslu M (2007) Nature of cell kinetics in psoriatic epidermis J. Cutan. Pathol 34:257–263. doi: 10.1111/j.1600-0560.2006.00719.x [PubMed: 17302610]
39. Madine MA, Swietlik M, Pelizon C, Romanowski P, Mills AD, Laskey RA (2000) The roles of the MCM, ORC, and Cdc6 proteins in determining the replication competence of chromatin in quiescent cells J. Struct. Biol 129:198–210. doi: 10.1006/jsbi.2000.4218 [PubMed: 10806069]
40. Lee YS, Ha SA, Kim HJ, Shin SM, Kim HK, Kim S, Kang CS, Lee KY, Hong OK, Lee SH, Kwon HS, Cha BY, Kim JW (2010) Minichromosome maintenance protein 3 is a candidate proliferation marker in papillary thyroid carcinoma Exp. Mol. Pathol 88:138–142. doi: 10.1016/j.yexmp.2009.09.015 [PubMed: 19818763]
41. Lee LH, Yang H, Bigras G (2014) Current breast cancer proliferative markers correlate variably based on decoupled duration of cell cycle phases Sci. Rep 4:5122. doi: 10.1038/srep05122 [PubMed: 24874299]
42. Amadori D, Volpi A, Maltoni R, Nanni O, Amaducci L, Amadori A, Giunchi DC, Vio A, Saragoni A, Silvestrini R (1997) Cell proliferation as a predictor of response to chemotherapy in metastatic breast cancer: a prospective study Breast Cancer Res. Treat. 43:7–14. doi: 10.1023/a:1005780107879
43. Rambau PF, Vierkant RA, Intermaggio MP, Kelemen LE, Goodman MT, Herpel E, Pharoah PD, Kommoss S, Jimenez-Linan M, Karlan BY, Gentry-Maharaj A, Menon U, Polo SH, Candido Dos Reis FJ, Doherty JA, Gayther SA, Sharma R, Larson MC, Harnett PR, Hatfield E, de Andrade JM, Nelson GS, Steed H, Schildkraut JM, Carney ME, Høgdall E, Whittemore AS, Widschwendter M, Kennedy CJ, Wang F, Wang Q, Wang C, Armasu SM, Daley F, Coulson P, Jones ME, Anglesio MS, Chow C, de Fazio A, García-Closas M, Brucker SY, Cybulski C, Harris HR, Hartkopf AD, Huzarski T, Jensen A, Lubiński J, Oszurek O, Benitez J, Mina F, Staebler A, Taran FA, Pasternak J, Talhouk A, Rossing MA, Hendley J, Edwards RP, Fereday S, Modugno F, Ness RB, Sieh W, El-Bahrawy MA, Winham SJ, Lester J, Kjaer SK, Gronwald J, Sinn P, Fasching PA, Chang-Claude J, Moysich KB, Bowtell DD, Hernandez BY, Luk H, Behrens S, Shah M, Jung A, Ghatage P, Alsop J, Alsop K, García-Donas J, Thompson PJ, Swerdlow AJ, Karpinskyj C, Cazorla-Jiménez A, García MJ, Deen S, Wilkens LR, Palacios J, Berchuck A, Koziak JM, Brenton JD, Cook LS, Goode EL, Huntsman DG, Ramus SJ, Köbel M (2018) Association of p16 expression with prognosis varies across ovarian carcinoma histotypes: an Ovarian Tumor Tissue Analysis consortium study The journal of pathology. Clinical research 4:250–261. doi: 10.1002/cjp2.109 [PubMed: 30062862]
44. Gorski JW, Ueland FR, Kolesar JM (2020) CCNE1 Amplification as a Predictive Biomarker of Chemotherapy Resistance in Epithelial Ovarian Cancer Diagnostics (Basel, Switzerland) 10. doi: 10.3390/diagnostics10050279

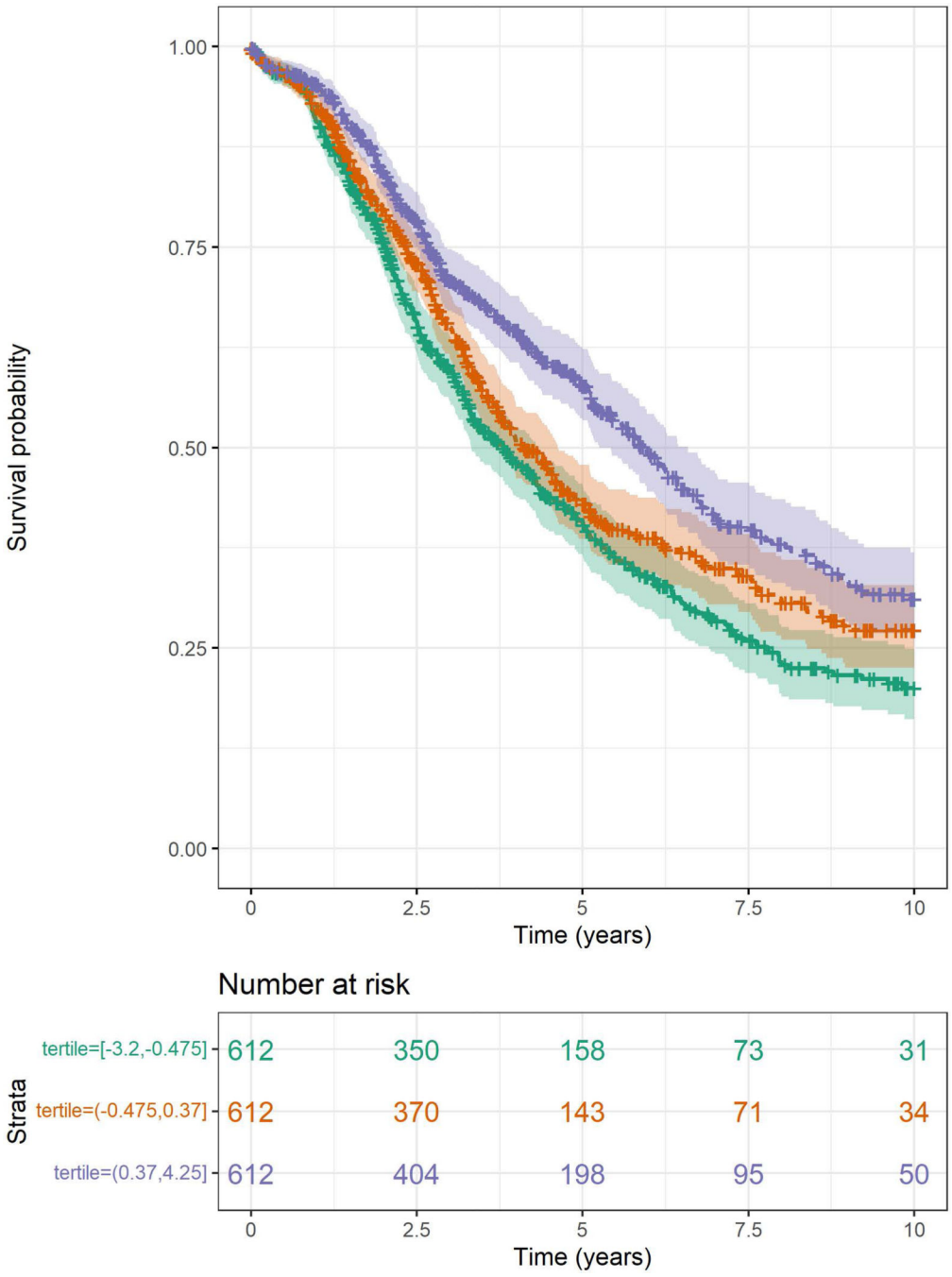

Figure 1.

Kaplan-Meier curves showing the 10-year overall survival of chemotherapy-naïve high-grade serous carcinoma patients from the Ovarian Tumor Tissue Analysis consortium grouped into tertiles of *MCM3* mRNA expression. Shaded areas indicate 95% confidence intervals.

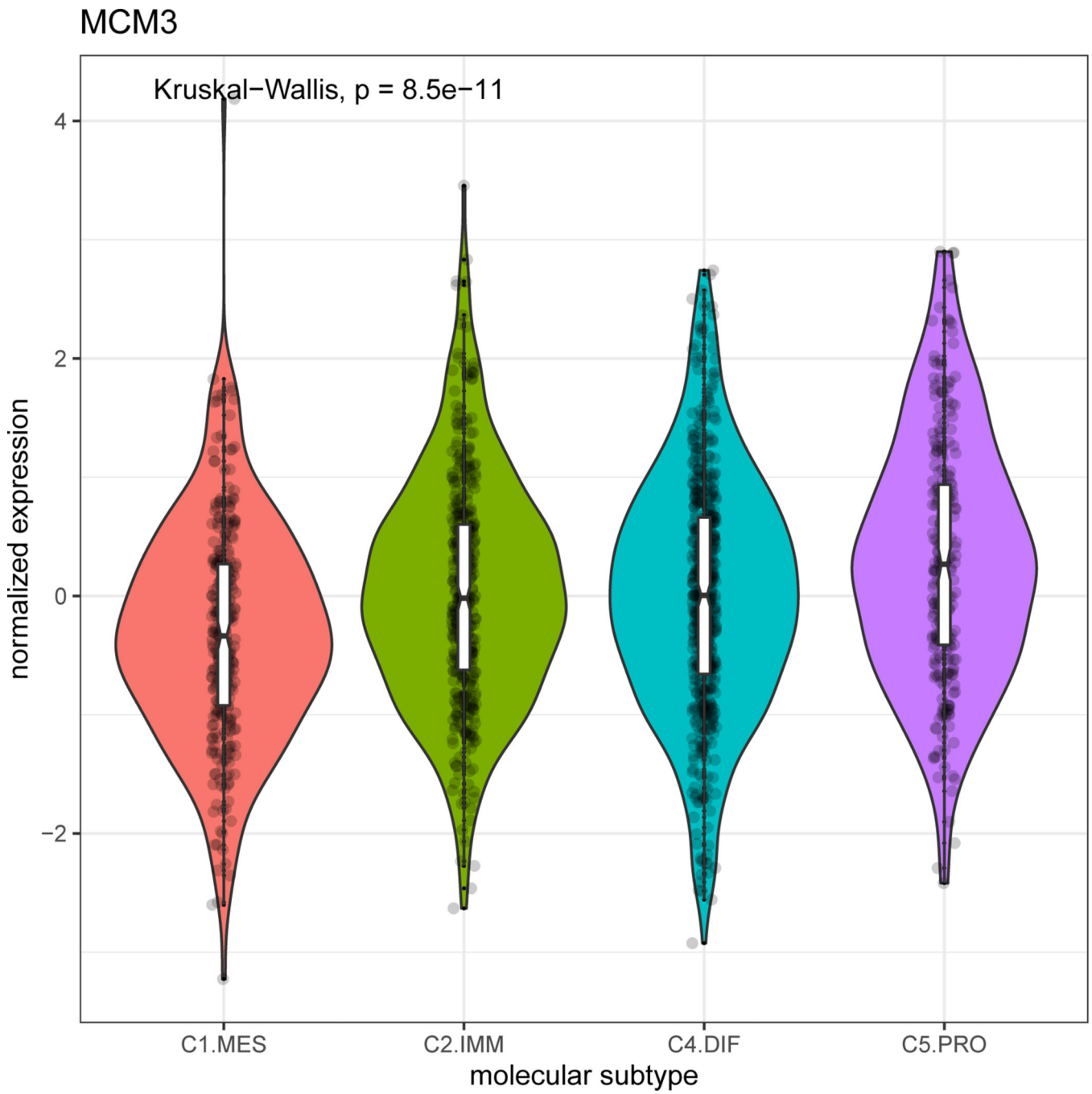

Figure 2.

A. Violin plots of *MCM3* mRNA expression stratified by molecular subtype of tubo-ovarian high-grade serous carcinoma (HGSC). The boxplots within the violin plots correspond to quartiles. The upper and lower whiskers extend no further than 1.5 interquartile range from the hinge to the furthest value. The displayed p-value corresponds to the Kruskal-Wallis non-parametric test of differences between the molecular subtype-specific gene expression distributions.

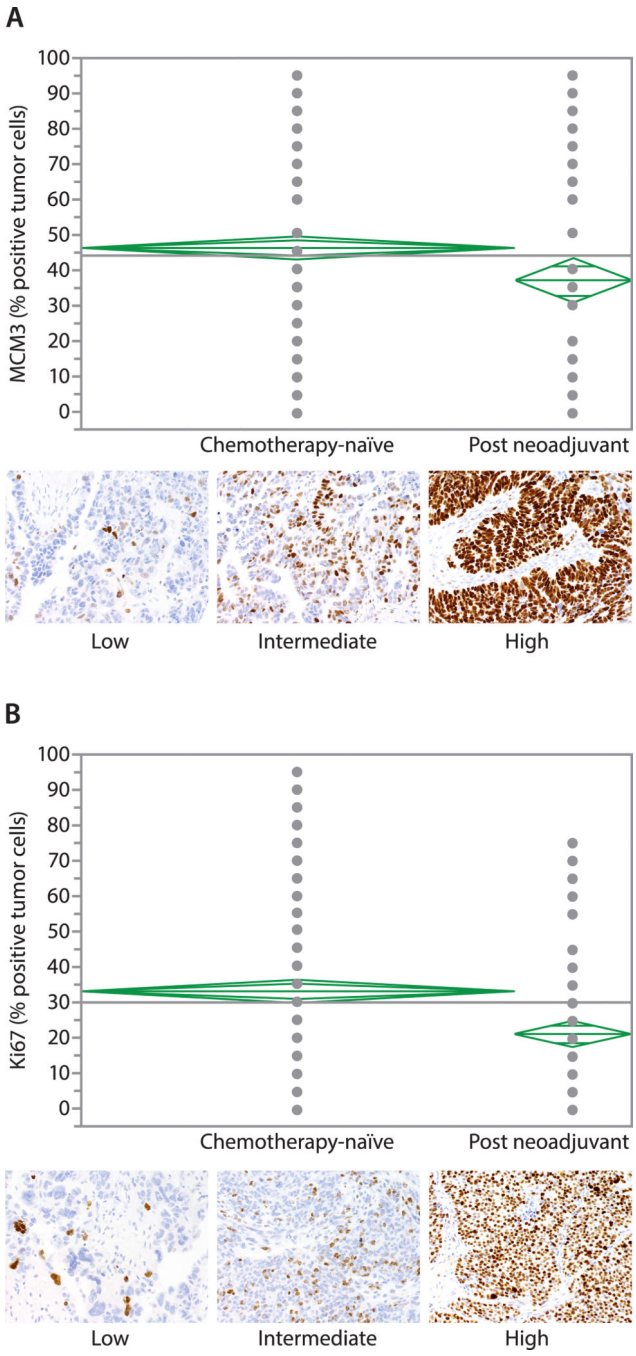

Figure 3.

A. Differences in MCM3 protein expression by immunohistochemistry (IHC) for chemotherapy-naïve versus post neoadjuvant chemotherapy samples of ovarian carcinomas. Representative images of low, intermediate, and high MCM3 expression by IHC are also shown (200x). **B.** Differences in Ki-67 protein expression by IHC for chemotherapy-naïve versus post neoadjuvant chemotherapy samples of ovarian carcinomas. Representative images of low, intermediate, and high Ki-67 expression by IHC are also shown (200x).

Green diamonds show mean with confidence interval. Horizontal grey line is grant mean across all samples.

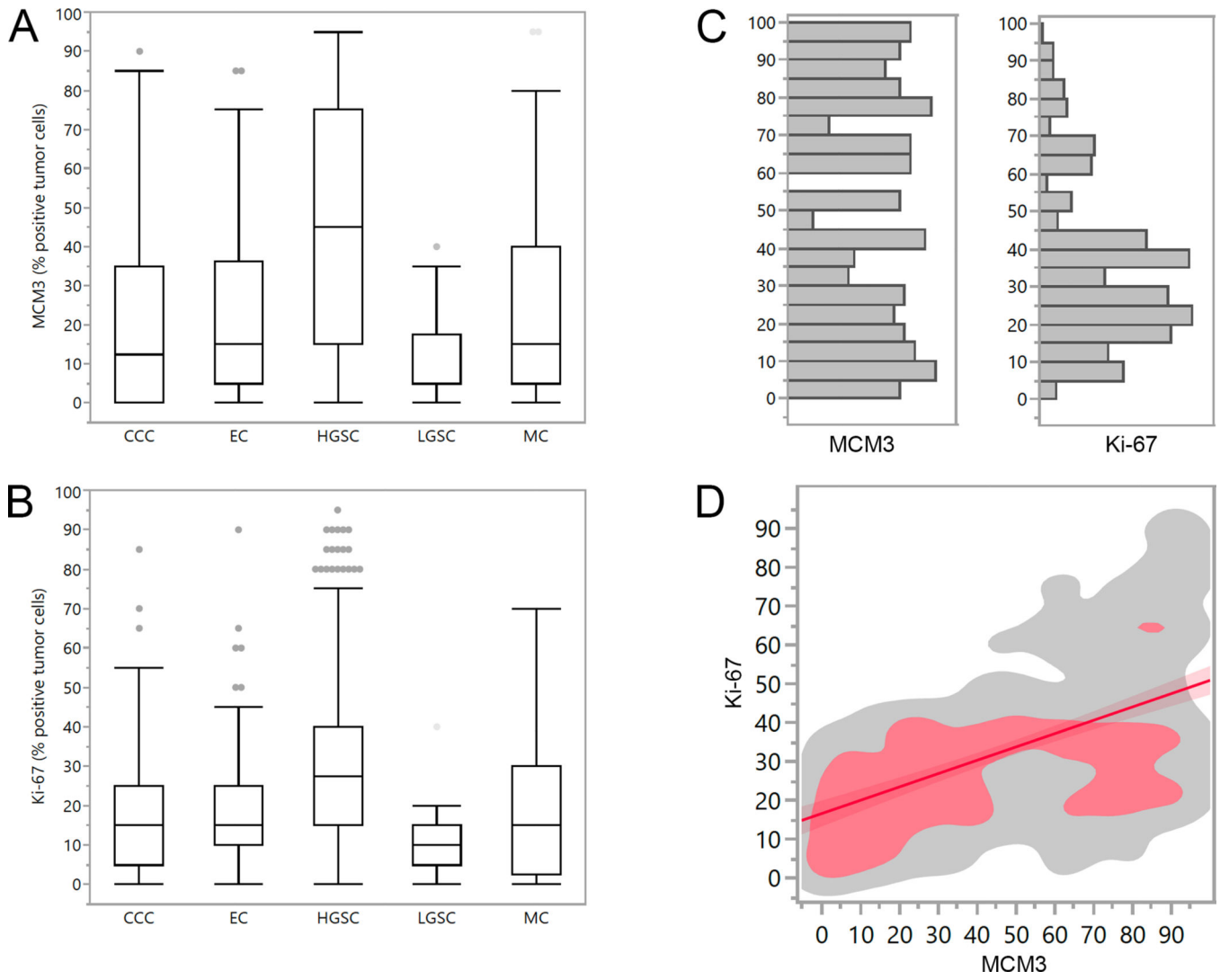

Figure 4.

A. Distribution of MCM3 protein expression by immunohistochemistry (IHC) across ovarian carcinoma histotypes. **B.** Distribution of Ki-67 protein expression by IHC across ovarian carcinoma histotypes. **C.** Distribution of MCM3 and Ki-67 expression by IHC within high-grade serous carcinomas (HGSC). **D.** Nonparametric density plot displaying nonparametric densities of MCM3 with Ki-67 on a scatter plot matrix. Red encompasses 50% of the data points, grey 90%. Red regression line with confidence interval. A.-D. restricted to chemotherapy-naïve cases.

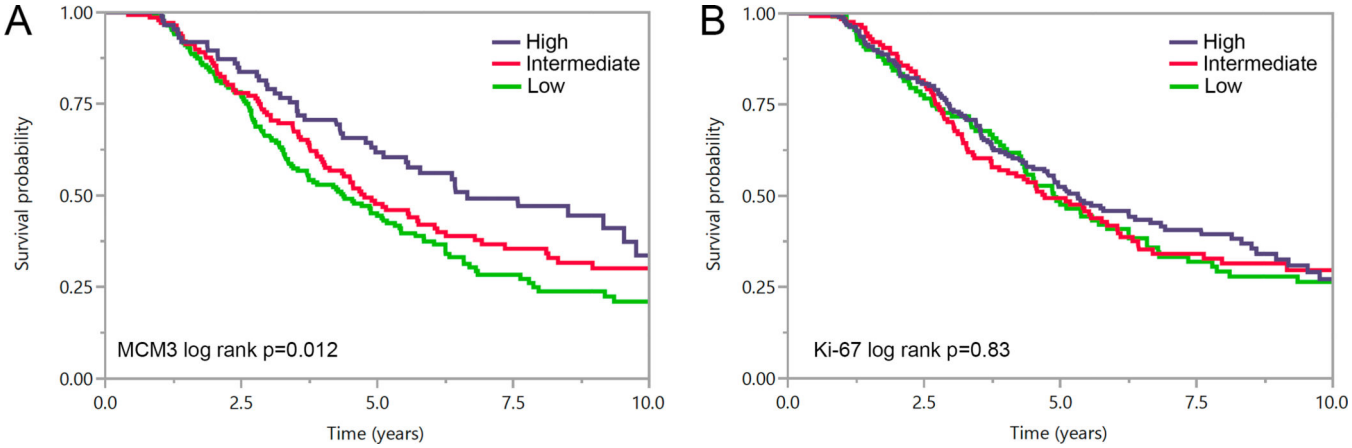

Figure 5.

A. Kaplan-Meier curves illustrating the 10-year disease-specific survival for patients with chemotherapy-naïve high-grade serous carcinomas (HGSC) for 3 groups regarding the MCM3 protein level by immunohistochemistry. B. Kaplan-Meier curves illustrating the 10-year disease-specific survival for patients with chemotherapy-naïve high-grade serous carcinomas (HGSC) for 3 groups regarding Ki-67 protein expression by immunohistochemistry.

Table 1.

Clinicopathological characteristics of patients with chemotherapy-naïve and post neoadjuvant chemotherapy tubo-ovarian high-grade serous carcinomas with *MCM3* mRNA expression data by NanoString assay from the Ovarian Tumor Tissue Analysis consortium.

		Chemotherapy-naïve cases N (%, column)	Post neoadjuvant chemotherapy cases N (%, column)
N		1836	519
Age, years (mean, range)		63 (29–93)	65 (31–90)
FIGO Stage (N, %)	**I,II**	297 (16.4)	11 (2.1)
	III,IV	1515 (83.6)	501 (97.9)
	Unknown	24	7
Tumor sample site	**Adnexal**	1447 (81.4)	363 (72.3)
	Omentum	199 (11.2)	88 (17.5)
	Peritoneum	34 (1.9)	39 (7.8)
	Other	98 (5.5)	12 (2.4)
	Unknown	58	17

Table 2.

Clinicopathological characteristics of patients in the OVCAL BC cohort with chemotherapy-naïve tubo-ovarian high-grade serous carcinomas with MCM3 immunohistochemistry data.

			MCM3 low N (%, column)	MCM3 intermediate N (%, column)	MCM3 high N (%, column)	Total	p-value
N			168	138	87	**393**	
Age, years (mean, 95% CI)			60.3 (59.1–61.6)	61.4 (59.9–62.9)	61.6 (59.6–63.5)	**60.9 (59.9–61.8)**	0.76
FIGO Stage (N, %)		**I**	9 (5.5)	21 (15.9)	6 (7.1)	**36 (9.5)**	
		II	21 (12.9)	18 (13.6)	13 (15.3)	**52 (13.7)**	
		III	115 (70.6)	86 (65.2)	60 (70.6)	**261 (68.7)**	
		IV	18 (11.0)	7 (5.3)	6 (7.1)	**31 (8.2)**	
		Unknown	5	6	2	**13**	0.05
Residual disease		**Absent**	55 (34.2)	56 (42.4)	29 (34.5)	**140 (37.1)**	
		≤ 1 cm	38 (23.6)	31 (23.5)	24 (28.6)	**93 (24.7)**	
		> 1 cm	68 (42.2)	45 (34.1)	31 (36.9)	**144 (38.2)**	
		Unknown	7	6	3	**16**	0.48
TP53		**Normal**	3 (1.9)	4 (2.9)	2 (2.3)	**9 (2.4)**	
		Abnormal OE	100 (63.3)	91 (66.9)	54 (62.8)	**245 (64.5)**	
		Abnormal CA	48 (30.4)	37 (27.2)	22 (25.6)	**107 (28.2)**	
		Abnormal CY	7 (4.4)	4 (2.9)	8 (9.3)	**19 (5.0)**	0.48
p16		**Normal**	58 (34.5)	41 (29.7)	11 (12.8)	**24 (6.1)**	
		Abnormal block positive	98 (58.3)	90 (65.2)	70 (81.4)	**258 (65.8)**	
		Abnormal CA	12 (7.1)	7 (5.1)	5 (5.8)	**110 (28.1)**	0.005
RB1		**Normal (retained)**	152 (92.1)	117 (86.0)	48 (58.5)	**317 (82.8)**	
		Abnormal (loss)	13 (7.9)	19 (14.0)	34 (41.5)	**66 (17.2)**	<0.0001
Cyclin E1	**CISH**	**Normal**	126 (89.4)	110 (87.3)	69 (92.0)	**305 (89.2)**	
		High-level amplification	15 (10.6)	16 (12.7)	6 (8.0)	**37 (10.8)**	0.58
	IHC	**Normal**	129 (76.8)	96 (70.6)	69 (80.2)	**294 (75.4)**	
		Overexpression	39 (23.2)	40 (29.4)	17 (19.8)	**96 (24.6)**	0.23

OE=overexpression, CA=complete absence, CY=cytoplasmic, CISH=chromogenic in situ hybridization, IHC=immunohistochemistry. Variable numbers of missing data for associations with biomarkers not shown.