



## The mesenchymal transition subtype more responsive to dose dense taxane chemotherapy combined with carboplatin than to conventional taxane and carboplatin chemotherapy in high grade serous ovarian carcinoma: A survey of Japanese Gynecologic Oncology Group study (JGOG3016A1)

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### HIGHLIGHTS

- The morphologically defined mesenchymal transition type is highly representative of the mesenchymal transcriptome subtype.
- The mesenchymal transition type has the worst prognosis and shows an increased rate of suboptimal surgery.
- The mesenchymal transition type benefits from dose dense taxane and carboplatin chemotherapy compared to conventional TC.

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### ABSTRACT

**Objective.** Recently, we established new histopathological subtypes of high-grade serous ovarian cancer (HGSOC) that include the mesenchymal transition (MT) type, the immune reactive (IR) type, the solid and proliferative (SP) type and the papillo-glandular (PG) type. Furthermore, we identified that the mesenchymal transcriptome subtype might be sensitive to taxane. We investigated whether these different histopathological subtypes of HGSOC require individualized chemotherapy for optimal treatment.

**Methods.** We conducted the Japanese Gynecologic Oncology Group (JGOG) 3016A1 study, wherein we collected hematoxylin and eosin slides (total  $n = 201$ ) and performed a histopathological analysis of patients with HGSOC registered in the JGOG3016 study, which compared the efficacy of conventional paclitaxel and carboplatin (TC) and dose-dense TC (ddTC). We analyzed the differences in progression-free survival (PFS) and overall survival (OS) among the four histopathological subtypes. We then compared the PFS between the TC group and the ddTC group for each histopathological subtype.

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**Results.** There were significant differences in both PFS and OS among the four histopathological subtypes ( $p = 0.001$  and  $p < 0.001$ , respectively). Overall, the MT subtype had the shortest PFS (median 1.4 y) and OS (median 3.6 y). In addition, the MT subtype had a longer PFS in the ddTC group (median 1.8 y) than in the TC group (median 1.2 y) ( $p = 0.01$ ). Conversely, the other types had no significant difference in PFS when the two regimens were compared.

**Conclusions.** The MT type of HGSOC is sensitive to taxane; therefore, the ddTC regimen is recommended for this histopathological subtype.

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## 1. Introduction

Ovarian cancer is the leading cause of death among gynecologic malignancies [1]. High-grade serous ovarian carcinoma (HGSOC) is the most frequent histopathological type of ovarian cancer, accounting for approximately 70% of ovarian cancers in the US [2] and 33–36% in Japan [3][4]. The majority of HGSOCs are diagnosed at advanced stages; therefore, pharmacological treatment is necessary for this disease.

Paclitaxel and carboplatin combination (TC) therapy has been used as a standard first-line chemotherapy against ovarian cancer for two decades [5,6]. The Japanese Gynecologic Oncology Group (JGOG) conducted the JGOG3016 study, a randomized controlled trial that compared dose dense weekly paclitaxel combined with tri-weekly carboplatin (ddTC) and conventional tri-weekly paclitaxel and carboplatin (TC) treatment in patients with stage II–IV ovarian cancer, tubal cancer, and peritoneal cancer. That study demonstrated that progression-free survival (PFS) was significantly prolonged in patients taking ddTC than in those taking conventional TC as a first-line chemotherapy regimen [7]. However, the sensitivity to chemotherapy varies widely among cancer patients, and it is now considered necessary that individualized treatments should be administered in a clinical setting [8,9].

The Cancer Genome Atlas (TCGA) project revealed that HGSOCs are divided into four transcriptome subtypes: mesenchymal, immunoreactive, proliferative and differentiated [10,11]. Among these, the mesenchymal type has the worst prognoses, whereas the immunoreactive type has the best prognoses [9,11]. It is widely accepted that the gene expression profile of cancer is strongly related to the characteristics in its biology and its sensitivity to treatment. However, ovarian cancer subtypes with their unique gene expression have yet to be investigated for drug treatments in clinical settings [12]. Four obstacles to performing gene expression microarray analyses include cost, maintaining uniform conditions due to the variety of experimental procedures, inconsistent quality of nucleotides and the batch effect, which is due to systematic variation between groups of samples (batches) resulting from experimental features that are not of biological interest [13].

Previous studies showed that a remarkable desmoplastic reaction occurred in the mesenchymal subtypes and a marked T lymphocyte infiltration into the tumor was seen in the immunoreactive subtypes [14]. These reports suggested that in the HGSOC, the tumor microenvironment, specifically the interaction of the stroma and tumor, affected the gene expression profile and prognoses of the patients [14,15]. From this idea, we established a novel histopathological classification based on the tumor and stromal findings, corresponding to these four transcriptome subtypes of HGSOC. Of the four subtypes—mesenchymal transition (MT) type, immune reactive (IR) type, solid and proliferative (SP) type and papilloglandular (PG) type—the MT type has the worst prognoses and the IR type has the best prognoses [16].

Furthermore, we discovered that the mesenchymal transcriptome subtype could be sensitive to taxane. Therefore, we hypothesized that the mesenchymal transition (MT) type which represents the “mesenchymal” transcriptome subtype might be sensitive to taxane. [16,17] If this histopathological classification can be used to assess a subtype's drug sensitivity, individualized treatments can be developed based on hematoxylin and eosin (HE) slide analyses. This would offer a low cost

and feasible approach to treatment. Subsequently, we conducted the JGOG3016A1 study, in which HGSOC cases registered in the JGOG3016 study were classified into histopathological subtypes. Consequently, a subtype more responsive to the ddTC regimen than to the TC regimen was identified. This study would be helpful to develop an individualized treatment of ovarian cancer.

## 2. Materials and methods

### 2.1. Gene expression microarray analysis

The detailed methods of gene expression microarray analysis are described in the supplementary text. Briefly, we calculated prediction scores for taxane sensitivity (T-scores) [17] and the mesenchymal gene expression subtype score (CLOVAR\_MES\_UP\_score) [11] within the three HGSOC gene expression microarray datasets; TCGA [10], GSE9891 [14], and KOV, which are our previously reported data set [16,18,19].

### 2.2. JGOG3016A1 study

Following the microarray-based prediction, we performed a study referred to as JGOG3016A1. The original sample size in the JGOG3016 study was 631, of which 355 cases were HGSOC [7,20]. After obtaining approval from the ethics committee at each institute, HE slides of HGSOC cases before treatment registered for the JGOG3016 study [7,20] were sent to the Kitasato Clinical Research Center (KCRC), where all the personal information on patients was anonymized. For each case, all the HE slides containing tumor tissue samples were collected and reviewed. The average number of slides was 13 (range; 1–58). These slides were comprised of 207 cases total from 34 institutes and were subsequently sent to Kyoto University, while their clinical information was not sent to Kyoto University. The data collection period was from January 2015 to September 2016. The final analysis of this study was made at the end of February 2017.

The HGSOC histopathological classification [16] was performed by two authors R.M and I.K. Briefly, a mesenchymal transition (MT) feature was assigned on the basis of the MT pattern or labyrinthine pattern with a remarkable desmoplastic reaction in >10% of the tumor area. If the MT feature was positive, the tumor was defined as being the MT subtype. In tumors other than the MT subtype, an immune reactive (IR) feature was assigned when numerous lymphocytes surrounded cancer nests (>100/× 400 visual field) and infiltrating cancer nests (>50/× 400 visual fields) and when this was associated with a smooth invasive front. If the IR feature was positive, the tumor was defined as being the IR subtype. The remaining tumors were classified into the solid and proliferative (SP) subtype or the papilloglandular (PG) subtype, depending on the presence of absence of these features. Because this classification procedure was conducted in a stepwise manner, it is applicable to heterogeneous tumors. This classification was performed using all the slides regardless of tumor site because we already found consistency between primary and tumor sites in our previous study [16].

We reviewed the HE slides independently, and when the histopathological diagnoses varied or were ambiguous, decisions regarding the final diagnoses and subtype were made by discussion. Of the 207

cases, six cases could not be classified due to tumors of insufficient volume for three cases, low-grade serous carcinoma for two cases, and the widely dispersed isolated tumor cells in one case, which did not meet our criteria. The remaining 201 cases were used for our final analysis. Of the 201 cases, 140 (70%) obtained immediate agreement without discussion, while 61 (30%) required discussion. Finally, we reached a consensus for all 201 cases. The results of the final histopathological classification were sent to the KCRC.

Data regarding the patients' characteristics and survival were managed at the KCRC. Clinical parameters were age at registration, post-surgical stage (II, III and IV) and size of surgical residual tumor (optimal;  $\leq 1$  cm residual tumor, suboptimal;  $> 1$  cm residual tumor). The data were recorded according to the criteria when the JGOG3016 study was initiated in 2003.

The primary goal was to test the hypothesis that the MT type is exclusively more responsive to ddTC than TC by analyzing the PFS in each subtype. Subsequently the OS was analyzed as well. For our secondary goal, we also evaluated all four histopathological subtypes of HGSOC, to determine if the MT subtype has the worst prognosis and the IR subtype has the best prognosis in terms of PFS and OS.

### 2.3. Statistical analysis

The primary goal of this study was to investigate whether the MT type is exclusively more responsive to ddTC than TC. The clinical question corresponded to the following four null hypotheses to be tested: in each subtype, the PFS functions between the two regimens were identical. In order to avoid multiplicity for all the four tests the Bonferroni correction was used by dividing the significance level (0.05) by the number of comparisons ( $n = 4$ ). Therefore, the new significance level for each comparison was 0.0125 (0.05/4). PFS curves were estimated by the Kaplan-Meier method. A two-sided log-rank test of each null hypothesis was performed at the significance level 0.0125. A one-way ANOVA assessed the age of patients and a Chi-squared test was used to assess the different stages and the state of residual tumors which were categorized into optimal and suboptimal groups. Furthermore, univariate and multivariate Cox proportional hazard regression model analyses were used to assess treatment, stage, and size of residual tumor (optimal versus suboptimal). The hazard ratios and the 95% confidential interval were calculated for each subtype in terms of treatment (TC vs ddTC), stage (II vs III/IV) and size of residual tumor (optimal vs suboptimal). Statistical significance was set at  $p$  values  $\leq 0.05$ . All statistical analyses for this JGOG3016A1 study were performed by KCRC collaborators.

### 3. Results

First, we examined the correlation between T-scores representing taxane predictive scores [17] and CLOVER\_MES\_UP-scores [11] using the mesenchymal gene expression subtype in the HGSOC gene expression microarray datasets. In the three HGSOC datasets, TCGA, GSE9891 and KOV, these scores showed a strong positive correlation (Supplementary Fig. S1A;  $r = 0.87$ ,  $p < 0.001$ , Supplementary Fig. S1B;  $r = 0.88$ ,  $p < 0.001$ , Supplementary Fig. S1C;  $r = 0.92$ ,  $p < 0.001$ ,

respectively). We found that T-scores were more significantly elevated in the MT subtype than in the non-MT subtypes. (Supplementary Fig. S1D;  $p < 0.001$ ). Therefore, consistent with our previous report [16,17], these data again suggested that the MT subtype in HGSOC was sensitive to taxane chemotherapy.

In addition, we examined the association between clinical variables and the four subtypes (Table 1). There was no significant difference in age distribution among the patients with the four subtypes. For the proportion of those with stage III, the MT, IR, SP and PG types accounted for 88%, 81%, 67% and 60%, respectively. A skewering in the distribution of stages among the four groups can be observed ( $p = 0.008$ ). Consequently, statistical significance cannot be inferred due to the small sample size of stage II and IV groups. The residual tumor distribution was also significantly varied among the four subtypes ( $p$  value  $< 0.001$ ). The rate of suboptimal residual tumors found in the MT, IR, SP and PG subtypes was 82%, 41%, 51% and 50%, respectively. When we investigated the association between prognoses and the four subtypes, the IR type had the best prognosis, whereas, the MT type had the worst prognosis in both PFS and OS (Fig. 1, PFS;  $p = 0.001$ , OS;  $p < 0.001$ ). The median PFS was 5.8 years (y), 1.4 y, 2.1 y and 1.6 y in the IR, MT, SP and PG types, respectively. The median OS was not reached in the IR and SP types, whereas it was 3.6 y and 5.1 y in the MT type and PG types, respectively.

The clinical variables across the four subtypes of MT, IR, SP and PG types were compared between the TC group and the ddTC group. For age, stage and residual tumor size, there was no statistically significant difference in distribution among the four subtypes when the two treatment groups were compared (Table 2). However, in the SP type, the proportion of those with stage II cancer in the ddTC group, 4/26 (25%), tended to be more than that in the TC group, 2/27 (7%) ( $p = 0.12$ ). In the IR subtype, suboptimal residual tumor sizes tended to be more prevalent in the ddTC group, 9/16 (56%), than in the TC group, 4/16 (25%) ( $p = 0.15$ ).

Next, the PFS was compared between the ddTC and TC groups among the four subtypes. In the MT subtype, PFS was significantly longer in the ddTC group than in the TC group; the median PFS was 1.8 y in the ddTC group and 1.2 y in the TC group (Fig. 2A;  $p = 0.01$ ). In the MT cases with measurable diseases, the overall response rate (ORR) for the ddTC was 63% (17/27), which was similar to the ORR 67% (24/36) for the TC. Complete response was observed in 33% of the ddTC cases, which was higher than 17% of the TC cases ( $p = 0.15$ , Fisher's exact test). PFS between the ddTC and TC groups in the IR and PG subtypes was not significantly different (Fig. 2B, D). In the SP subtype, PFS tended to be longer in the ddTC group than in the TC group (Fig. 2C;  $p = 0.07$ ), which was probably influenced by the relatively higher proportion of stage II cases in the ddTC group than in the TC group as described above (Table 2). For the OS curves, there was no significant difference among the four subtypes when the ddTC and TC groups were compared (Supplementary Fig. S2). However, in the MT and SP subtypes, the ddTC group had a tendency for a longer OS than the TC group ( $p = 0.08$  and 0.18, respectively).

Finally, a Cox proportional hazard regression analysis was performed for the treatment regimen (ddTC vs. TC), stage (II vs. III-IV), and postoperative residual tumor (optimal vs. suboptimal) (Table 3).

**Table 1**  
Clinical parameters for age, stage and residual tumor among the four histological subtypes.

		MT		IR		SP		PG		p value
		No. of patients	%							
Age	Average (min-max)	59 (39-77)		57.5 (43-73)		59 (28-71)		57 (25-78)		0.3
Stage	II	2	3%	3	9%	8	16%	11	23%	0.008
	III	63	88%	26	81%	33	67%	29	60%	
	IV	7	10%	3	9%	8	16%	8	17%	
Residual tumor	Optimal	13	18%	19	59%	24	49%	24	50%	<0.0001
	Suboptimal	59	82%	13	41%	25	51%	24	50%	

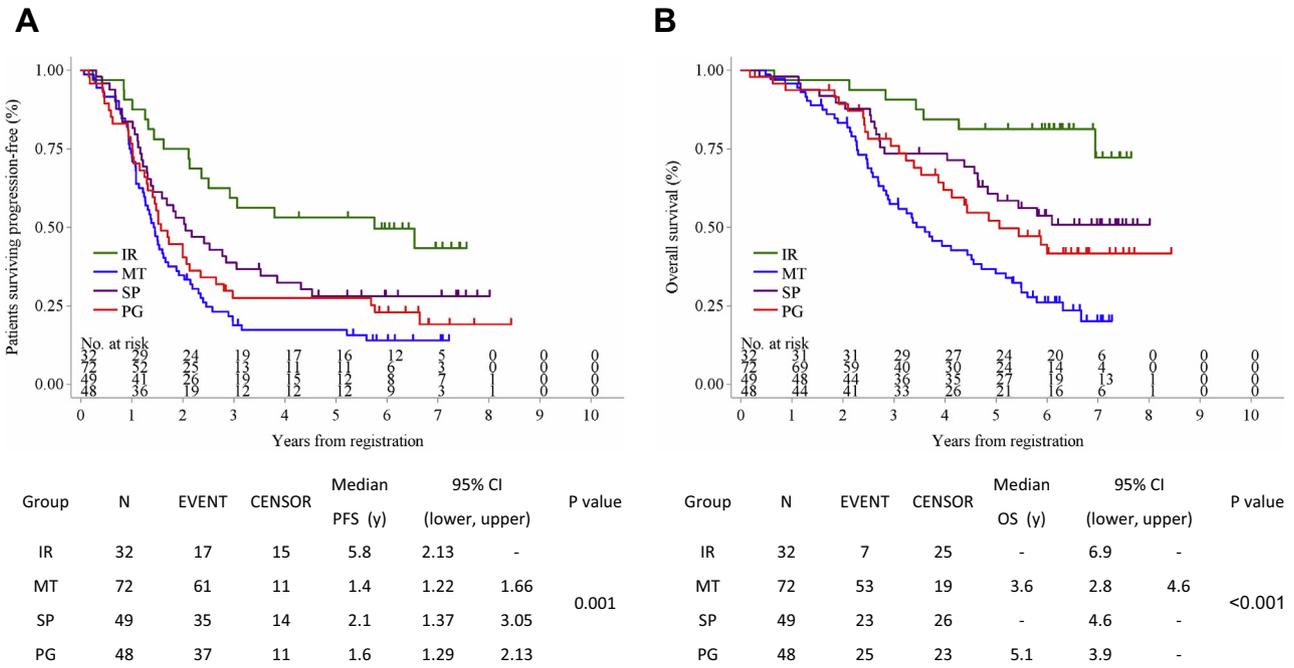


Fig. 1. Survival curves were assessed among the patients categorized into four subtypes (MT, IR, SP and PG). A) PFS. B) OS.

The univariate PFS analysis indicated a significantly reduced risk of recurrence for the ddTC regimen than for the TC regimen in the MT subtype (HR 0.51, 95% CI; 0.30–0.86). The multivariate PFS analysis also revealed a significantly reduced risk of recurrence for the ddTC regimen than for the TC one, for all cases and the MT type (HR 0.69, 95% CI; 0.50–0.95, and HR 0.51, 95% CI; 0.30–0.86, respectively). In the analysis of stage, a significantly higher risk of recurrence and survival was observed for stage III–IV than for stage II for all cases (univariate/multivariate PFS/OS) and in the SP subtype (univariate PFS). In the analysis of residual tumor, a significantly higher risk of recurrence and survival was observed for suboptimal surgery than for optimal surgery for all cases (univariate/multivariate PFS/OS), in the SP subtype (univariate PFS and univariate/multivariate OS) and in the PG subtype (univariate/multivariate OS). The representative images of the four high-grade serous ovarian carcinoma morphological subtypes are shown in Fig. 3.

#### 4. Discussion

For HGSO, the CLOVER\_MES\_UP score that represents the Mesenchymal gene expression subtype [11] and T-score that represents taxane sensitivity [17] showed strong positive correlation (Supplementary Fig. S1A, 1B, 1C). As expected from these data, the MT histopathological type, that correlated with the mesenchymal gene expression subtype [16], had higher T-scores than other histopathological subtypes (Supplementary Fig. S1D), indicating that the MT type may be taxane

sensitive. So far, researchers from Singapore have also reported that when universal epithelial-mesenchymal transition (EMT) scoring from cancer-specific transcriptomic EMT signatures of various cancers was applied, “mesenchymal-like” ovarian cancer correlated with taxane sensitivity [21].

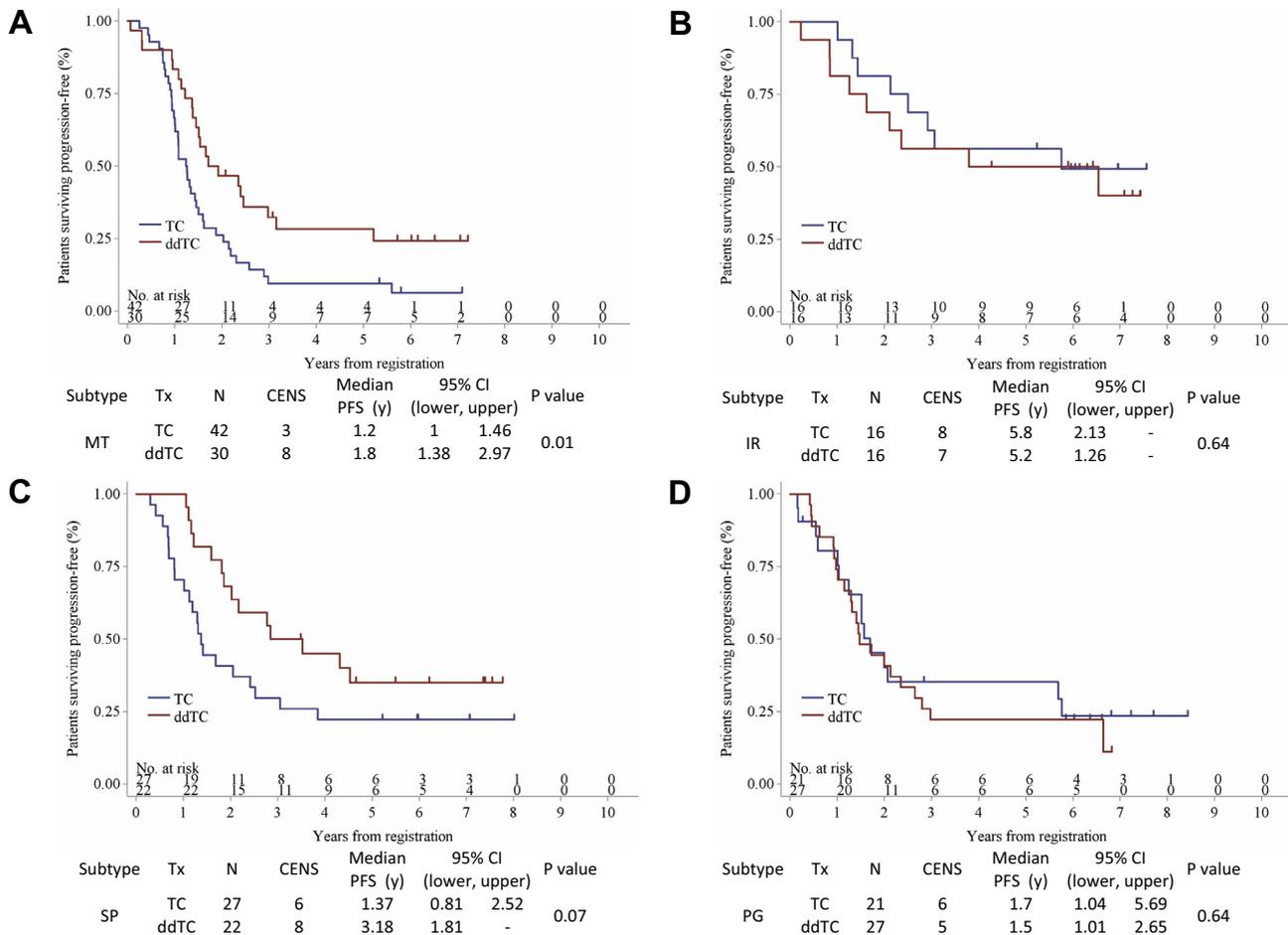
In order to prove the hypothesis that the MT subtype has a taxane sensitivity, a new clinical trial to compare taxane monotherapy to a placebo treatment would be ideal. However, because the taxane and platinum combination regimen has been a standard strategy for treating ovarian cancer patients, such clinical trial is ethically not feasible. In the JGOG3016 study, tri-weekly administration of carboplatin AUC6 was the same, whereas paclitaxel regime differed. For the ddTC regimen, paclitaxel was given weekly at 80 mg/m<sup>2</sup> (240 mg/m<sup>2</sup> per three weeks) and for the TC regimen paclitaxel was given tri-weekly at 180 mg/m<sup>2</sup>. Therefore, in the JGOG 3016A1 study, PFS of the MT subtype was compared between the ddTC and TC groups as a primary endpoint.

We classified the HGSO cases into the four histopathological subtypes [16] without knowing any clinical information. For postoperative residual tumor, the MT subtype showed an increased rate of suboptimal surgery, whereas, the IR subtype showed an increased rate of optimal surgery (Table 1). For both PFS and OS, the MT subtype had the worst prognosis and the IR subtype had the best prognosis (Fig. 1). These results were consistent with our previous reports [16,22]. It has been reported that the mesenchymal transcriptome subtype was associated with a higher rate of suboptimal surgery [23] and was more likely to have

Table 2

The characteristic of the patients between two regimens are observed for age, stage and surgical residual tumor.

		MT			IR			SP			PG										
		TC		ddTC	p	TC		ddTC	p	TC		ddTC	p								
		No of pts	%	No of pts	%	No of pts	%	No of pts	%	No of pts	%	No of pts	%								
Age	Average (min–max)	59.5 (39–77)		58 (42–76)	0.23	58.5 (47–70)		54.5 (43–73)	0.41	61 (32–71)		58.5 (28–69)	0.80	56 (25–78)		58 (36–76)	0.56				
Stage	II	1	2%	1	3%	1	3	19%	0	0%	0.23	2	7%	6	27%	0.12	4	19%	7	26%	0.73
	III	38	90%	25	83%		11	69%	15	94%		20	74%	13	59%		14	67%	15	56%	
	IV	3	7%	4	13%		2	13%	1	6%		5	19%	3	14%		3	14%	5	19%	
Residual tumor	Optimal	6	14%	7	23%	0.36	12	75%	7	44%	0.15	13	48%	11	50%	1	11	52%	13	48%	1
	Suboptimal	36	86%	23	77%		4	25%	9	56%		14	52%	11	50%		10	48%	14	52%	



**Fig. 2.** PFS curves were assessed between the TC group and the ddTC group among the four histopathological subtypes. A) The MT type. B) The SP type. C) The IR type D) The PG type.

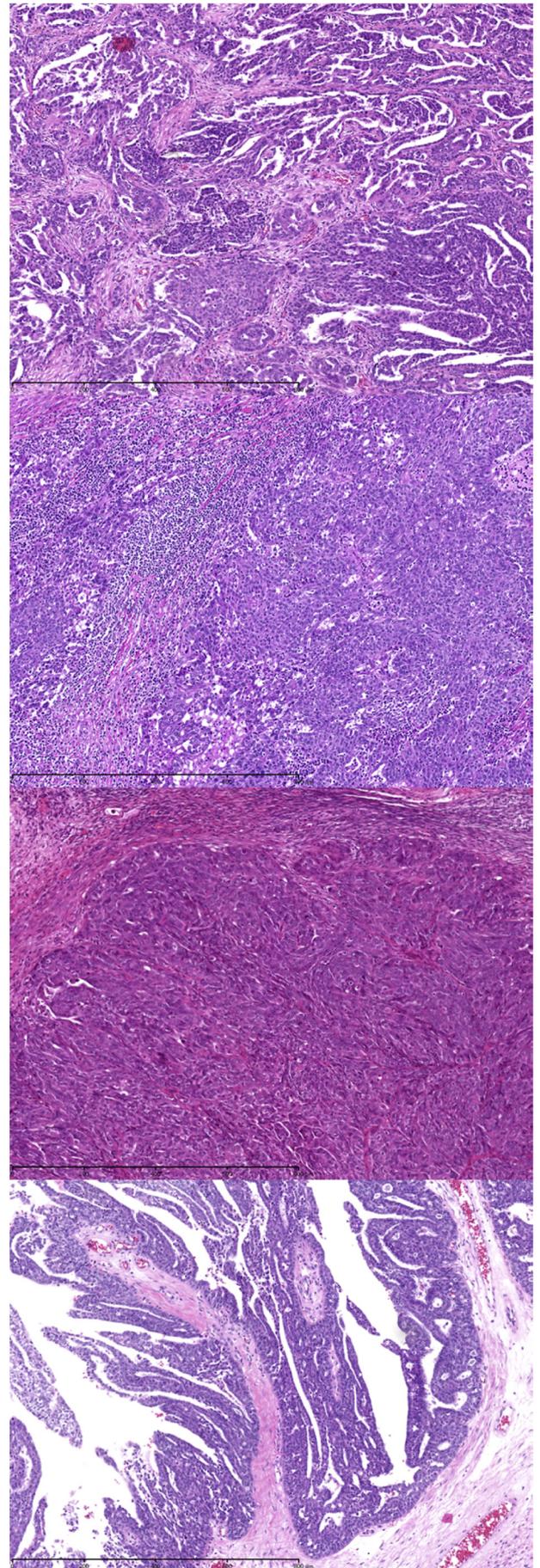
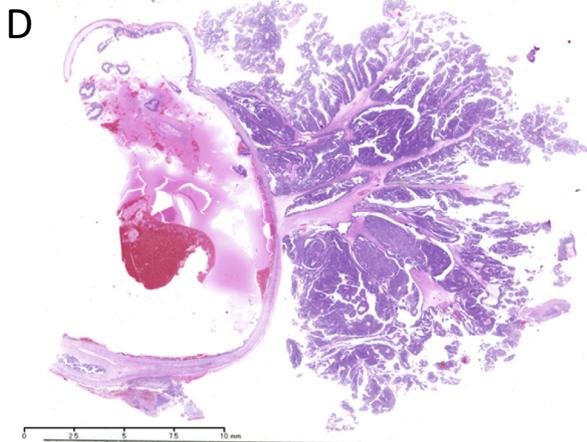
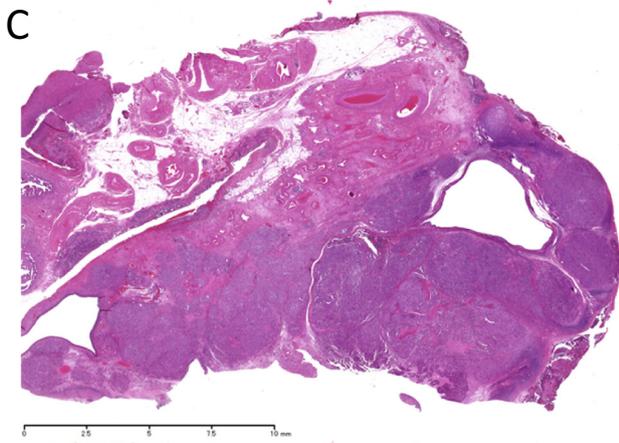
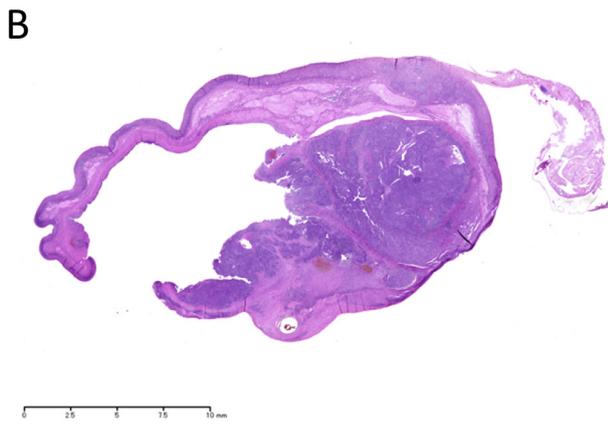
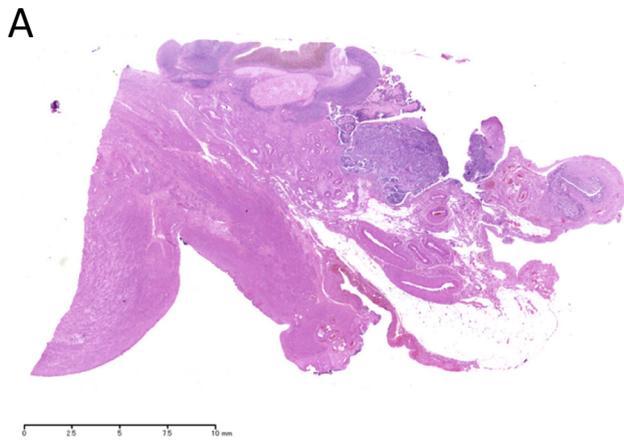
upper abdominal or miliary dissemination patterns [24]. A meta-analysis that assessed 1528 cases of gene expression microarray data reported that EMT pathway and TGF-beta pathway were enriched in tumors resulting in suboptimal surgery [25]. These pathways were also characterized by the MT subtype [16]. For advanced ovarian cancer with bulky or upper abdominal peritoneal dissemination, it is important to make a decision whether to perform a primary debulking surgery or to administer neoadjuvant chemotherapy followed by interval debulking surgery in order to achieve complete tumor resection [26,27]. When we biopsy the tumor

tissues before treatment, the histopathological examination would allow us to identify the MT subtype, which may help us choose a more appropriate treatment strategy.

The main objective of this study was to assess whether the PFS in each HGSOC histopathological subtype differed according to the treat group. As we expected, the MT subtype showed a significantly longer PFS in the ddTC group than in the TC group with 49% reduction in the progression of disease (Fig. 2A, Table 3). Although not statistically significant, the SP subtype showed longer PFS in the ddTC group (Fig. 2C). This result may

**Table 3**  
Cox regression hazard analysis for treatment by the ddTC compared to the TC regimen, stages III–IV compared to stage II, and suboptimal residual tumors compared to optimal ones.

PFS		MT			IR			SP			PG			All		
Univariate	Covariates	HR	95% HR CI		HR	95% HR CI		HR	95% HR CI		HR	95% HR CI		HR	95% HR CI	
	Treatment	0.51	0.30	0.86	1.26	0.48	3.27	0.54	0.28	1.07	1.17	0.60	2.26	0.73	0.53	1.009
	Stage	-	-	-	-	-	-	6.10	1.46	25.56	2.40	0.99	5.80	4.14	2.02	8.457
Multivariate	Residual tumor	1.70	0.84	3.45	2.46	0.94	6.43	2.43	1.22	4.84	1.63	0.84	3.13	2.25	1.59	3.183
	Covariates	HR	95% HR CI		HR	95% HR CI		HR	95% HR CI		HR	95% HR CI		HR	95% HR CI	
	Treatment	0.51	0.30	0.86	1.00	0.37	2.68	0.61	0.31	1.22	1.12	0.58	2.17	0.69	0.5	0.95
OS	Stage	-	-	-	-	-	-	3.93	0.86	17.96	2.17	0.87	5.38	3.02	1.44	6.335
	Residual tumor	1.68	0.83	3.41	2.46	0.91	6.67	1.76	0.85	3.64	1.36	0.69	2.68	1.86	1.29	2.669
	Covariates	HR	95% HR CI		HR	95% HR CI		HR	95% HR CI		HR	95% HR CI		HR	95% HR CI	
Univariate	Treatment	0.60	0.34	1.08	2.55	0.49	13.18	0.56	0.24	1.33	1.03	0.46	2.30	0.74	0.5	1.08
	Stage	-	-	-	-	-	-	-	-	-	2.23	0.76	6.54	4.97	1.83	13.5
	Residual tumor	1.47	0.71	3.02	4.44	0.86	22.93	3.45	1.35	8.80	2.80	1.20	6.54	3.2	2.04	5.027
Multivariate	Covariates	HR	95% HR CI		HR	95% HR CI		HR	95% HR CI		HR	95% HR CI		HR	95% HR CI	
	Treatment	0.60	0.34	1.07	1.67	0.30	9.29	0.57	0.24	1.34	0.88	0.39	1.99	0.69	0.47	1.013
	Stage	-	-	-	-	-	-	-	-	-	1.68	0.55	5.11	2.89	1.03	8.109
OS	Residual tumor	1.48	0.72	3.05	3.78	0.68	21.09	3.44	1.35	8.78	2.52	1.04	6.10	2.73	1.71	4.354



come from the disproportionate number of patients in stage II who received ddTC, 6/25 (27%) versus the TC group, 2/27 (7%) (Table 2). The IR and PG subtypes displayed no significant difference between the ddTC group and the TC group (Fig. 1, Table 3). In any case, as predicted, the MT subtype truly benefited from the ddTC regimen compared to the TC regimen.

The mechanism to explain why the mesenchymal-like phenotype correlates with taxane sensitivity is unknown. In ovarian cancer, TGFBI, which is a downstream gene of TGF- $\beta$ , was reported to contribute to stabilization of microtubules and to increase the paclitaxel sensitivity [28]. In lung cancer, EMT inhibits the expression of beta tubulin, which induces paclitaxel sensitivity [29]. In contrast, Snail [30] and ZEB1 [31] by inducing EMT were reported to contribute to paclitaxel resistance in ovarian cancer cell lines experiments. PI3K dependent EMT was also reported to contribute to paclitaxel resistance [32]. The discordance of previous reports could be explained by tumor microenvironment interaction, which would affect chemo response [33]. In ovarian cancer, it is known that paclitaxel targets VEGF-mediated angiogenesis [34]. The activated VEGF pathway in the mesenchymal transcriptome subtype of HGSOc [35] might contribute to paclitaxel sensitivity [21]. In any case, the mechanism by which the MT subtype responds well to the ddTC regimen should be further investigated.

One of the limitations of this study is interobserver variation of histopathological classification. In our previous study [16], the overall consistency rates of diagnosed MT, IR, SP, and PG by six observers had an average of 74% (61–89%). Similarly, in the current study, immediate agreement was obtained in 70% of the cases. The inconsistency for the other cases could be caused by the nature of subjective judgement for pathological diagnosis. Thus, further study should be performed to decrease interobserver variation. Additionally, the inconsistency could be caused by existence of heterogeneous tumors. A part of HGSOc tumors could not be classified by gene expression based TCGA molecular subtyping, even on a NanoString platform, due to mixed subtype signals [36].

Another limitation of this study is the small sample size, and therefore, a validation study is required before making practical applications. Recently, the ICON8 study analyzed >1500 ovarian cancer cases who were randomly assigned to three regimen groups of the TC (carboplatin AUC 5 once every 3 weeks and paclitaxel 175 mg/m<sup>2</sup> once every 3 weeks), ddTC (carboplatin AUC 5 once every 3 weeks and paclitaxel 80 mg/m<sup>2</sup> once every week), and ddTWC (carboplatin AUC 2 once every week and paclitaxel 80 mg/m<sup>2</sup> once every week). In the ICON8 study, although the ddTC regimen and the ddTWC regimen tended to prolong median survival time in PFS compared to the TC regimen by three months, the difference did not reach statistical significance [36,37]. One of the reasons the result of ICON8 was different from JGOG3016 study is considered to be a genetic factor among ethnic groups. In Asian population, ovarian cancer prognoses are relatively better [38]. Additionally, Japanese patients have a tendency of having neutropenia and higher response rates during the TC regimen compared to US patients in non-small-cell lung cancer [39]. Therefore, Japanese ovarian cancer patients generally respond well to the chemotherapy, which may contribute to significantly different survival between the ddTC regimen and the TC one in JGOG3016 study. However, given the results shown in the current study, histopathological classification of HGSOc cases in the ICON8 study could demonstrate a benefit of ddTC exclusively in the MT subtype. Through a validation study using a large cohort like ICON8, we might integrate the histopathological classification into the standard care for ovarian cancer.

## 5. Conclusion

The MT subtype, which tends to result in suboptimal surgery and has the worst prognoses of all the subtypes in HGSOc, benefits more greatly from the ddTC regimen compared to the conventional TC one. We believe that the pathological diagnosis for the MT subtype in HGSOc has the potential to lead to their greater individualization. Further clinical validation study to investigate the reproducibility and basic research to discover the mechanism will be required as a future plan.

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## Author contributions

Planning and execution of this survey: RM, NM, AO, IK, MM; Sample preparation: HT, MY, HI, MS, TN, OT, MT, TS, SI, KT, HN; Data analysis: RM, HM; Manuscript authoring: RM, NM, MM; Contextualization of findings of this work: RM, MN, HM, MM. All authors read and approved of the final manuscript.

## Conflict of interest statement

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The other authors have no conflict of interest to declare.

## Appendix A. Supplementary data

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**Fig. 3.** The representative images of the four high-grade serous ovarian carcinoma morphological subtypes. A) Mesenchymal transition type (MT type): large papillae infiltration and isolated spindle cancer cells with destructive stromal reaction. B) Immune reactive type (IR type): relatively round tumor invasive front with a large number of lymphocytes surrounding and infiltrating the tumor. C) Solid and proliferative type (SP type): solid growth architecture with less desmoplastic reaction and fewer lymphocytes. D) Papillo glandular type (PG type): papillary growth and glandular architecture with less desmoplastic reaction and fewer lymphocytes. The figures on the left are loupe images, and the images on the right are at 200 $\times$  magnification.

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