Metronomic oral cyclophosphamide in relapsed ovarian cancer

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Conflict of interest

Pavlina Spiliopoulou: no conflict of interest
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Abstract

Objectives: Our aim was to describe the clinical activity of metronomic cyclophosphamide in a population of patients with recurrent ovarian cancer and to identify predictors of clinical response.

Methods: We retrospectively reviewed all patients treated at our institution with oral metronomic cyclophosphamide for relapsed ovarian cancer between January 2012 and December 2016. These were identified from electronic chemotherapy prescription records. The primary endpoint was response rate by combined GCIG criteria. Data on patient demographics, previous therapies, platinum resistance, germline *BRCA1/2* (*gBRCA1/2)* status, disease response by radiological or CA125 criteria alone, adverse events secondary to metronomic cyclophosphamide treatment, progression-free survival and overall survival, were also evaluated.

Results: A total of fifty of 68 patients treated with oral metronomic cyclophosphamide, were evaluable for disease response. By combination criteria (radiological plus CA125), complete response was 0%, partial response 32%, stable disease 16% and progressive disease 52%. In the intention-to-treat population (ITT, n=68), progression-free survival and overall survival were 2.6 months and 6 months, respectively. Having a *gBRCA1*/2 mutation, reduced the risk of disease progression by radiological criteria (OR=0.07, 95% CI 0.008-0.67, p=0.02) and patients with *gBRCA1/2* mutations had improved progression-free survival (7.9 *vs* 2.5 months, HR 0.4 95%CI 0.23-0.74, p=0.003) and overall survival (15.5 *vs* 6 months, HR 0.49, 95% CI 0.28-0.85, p=0.02) with metronomic cyclophosphamide when compared with patients without *gBRCA1/2* mutations (or unknown *gBRCA1/2* status).

Conclusion: Oral metronomic cyclophosphamide showed a clinical benefit in 48% of patients with recurrent ovarian cancer and *gBRCA1/2* status can be an independent predictor of response.

Highlights

- Metronomic cyclophosphamide had an overall response rate of 32% in relapsed ovarian cancer
- Patients with germline *BRCA1/2* mutations have better progression-free survival and overall survival with metronomic cyclophosphamide, compared to patients without/unknown germline *BRCA1/2* status.
- Metronomic cyclophosphamide should be considered a therapeutic option in patients with germline *BRCA1/2* mutations.

Introduction

Despite recent advances in available treatments, ovarian cancer remains the most lethal gynaecologic malignancy, with a 5-year survival rate for stage IIIC/IV disease of 22-29% (1). The mainstay of systemic treatment is platinum chemotherapy and sensitivity to platinum-based chemotherapy is the major prognostic determinant (2, 3). Even patients who initially have platinum-sensitive disease, ultimately develop resistance. Once platinum resistance has developed, progression-free and overall survival are short and preservation of quality of life is of primary importance. Once platinum is no longer appropriate, treatment with cytotoxic agents such as weekly paclitaxel, liposomal doxorubicin, gemcitabine and topotecan, offer modest response rates, short progression-free survival and are associated with significant toxicity (4, 5). Weekly paclitaxel can offer a 20-40% response rate in platinum-resistant disease (6) and the addition of bevacizumab to chemotherapy improves progression-free survival and patient reported outcomes, albeit with no significant effect on overall survival (7).

There is still a need for well-tolerated treatment options for women with heavily pre-treated disease. A number of small non-randomised studies (Table S1) suggested that metronomic cyclophosphamide had activity in this setting (8-10), with acceptable toxicity, so we have adopted it as a treatment option in our clinical management guidelines. Germline *BRCA* mutation testing was introduced concurrently within the time frame of this study and we hypothesised that women with

multiple responses to prior lines might also carry a *BRCA* mutation and this might predict sensitivity to metronomic cyclophosphamide. In this retrospective study, we report the efficacy and toxicity of metronomic cyclophosphamide and we investigate potential predictive markers of response.

Methods

Design

We retrospectively reviewed the records of patients with ovarian cancer who started treatment with metronomic cyclophosphamide between January 2012 and December 2016 in a large regional cancer centre in the UK. All women with epithelial ovarian cancer who were prescribed at least one cycle of metronomic cyclophosphamide for recurrent disease were identified from our chemotherapy prescribing database. Age, performance status, histology, the number of lines of previous therapy, platinum resistance, acquired versus primary platinum resistance and germline *BRCA* (*gBRCA)* status, where available, were recorded. Platinum resistance was defined as disease progression ≤6 months following last platinum dose. Primary resistance was defined as progression ≤6 months after first line, platinum-based chemotherapy and acquired resistance was defined as progression ≤6 months after second or subsequent lines of platinum-based chemotherapy. Patient selection criteria for treatment are described on supplementary Table S2. The dose of metronomic cyclophosphamide was either 50 mg or 100 mg orally/day for a 28-day cycle. The dose was decided at the discretion of the clinician, based on patient's performance status or body surface area. Patients were assessed clinically and had CA125 sampling on day one of every cycle. Radiological assessment was undertaken every 2 cycles, or earlier if there was evidence of clinical or CA125 progression.

Study endpoints

Primary study endpoint was response rate by combined radiological and CA125 criteria as defined by Gynaecologic Cancer Intergroup (GCIG) (11). Evaluable patients needed to have at least one radiological assessment and/or two CA125 measurements on metronomic cyclophosphamide. CA125 response was defined as a reduction of baseline CA125 level by ≥50%, that was maintained for 28 days and confirmed with a subsequent measurement (12). Radiological response was assessed by an independent radiologist at the time of treatment and RECIST 1.1 was applied retrospectively (13).

Secondary endpoints were response rate by either RECIST 1.1 or CA125 criteria in evaluable patients, duration of response, as well as progression-free survival, overall survival and treatment toxicity. Progression-free survival was defined as the time interval between the start of treatment and either CA125 and/or radiological progression, whichever occurred first. Overall survival was defined as the time interval between the start of treatment and death. CTCAE version 4.0 criteria were used for toxicity grading. Patients evaluable for toxicity had at least one cycle of treatment.

Statistical analysis and ethics

SPSS and PRISM software were used for statistical analysis. Fisher's test was used when comparisons were done with contingency table analysis. Log-rank test was used to compare Kaplan-Meier survival curves and to calculate hazard ratios for progression-free survival/overall survival. Binary logistic regression was used to identify markers of disease response to metronomic cyclophosphamide. The study was granted Caldicott Guardian approval by the R&D department of NHS, Greater Glasgow and Clyde, in November 2018.

Results

Patient characteristics

A total of 68 patients with recurrent epithelial ovarian cancer received at least one dose of metronomic cyclophosphamide (Figure 1 and Table S2). The median age was 69 years (range; 25-85). Starting dose was 100 mg/day in 4 patients (6%). The remainder (n=62, 91%) were treated with 50 mg/day. Two out of the 62 patients (3%) had dose escalation to 100 mg/day when adequate tolerance was established. The majority of patients (n=59, 87%) had platinum-resistant disease; of these, 18 (31%) patients had primary platinum resistance and 41 (69%) patients had acquired resistance to platinum. For patients with platinum-sensitive disease, the decision to offer metronomic cyclophosphamide chemotherapy was made either because of poor performance status, co-morbidities or previous treatment toxicity precluding intravenous chemotherapy (such as allergy to platinum). Other characteristics are shown in Table S3.

Response to treatment

Fifty (n=50; 73.5%) of 68 patients had at least one radiological assessment or two CA125 measurements on metronomic cyclophosphamide therapy and were included in the analysis of response. At the time of analysis, all patients had experienced disease progression or death. In all evaluable patients (n=50), disease response rates were complete response 0% (0/50), partial response 32% (16/50), stable disease 16% (8/50) and progressive disease 52% (26/50) by combined criteria. By RECIST alone, response rates were complete response=0% (0/43), partial response=7% (3/43), stable disease=28% (12/43) and progressive disease=65% (28/43), whereas by CA125 criteria responses were response=35% (17/48), non-response/non-progressive disease=50% (24/48) and progressive disease=15% (7/48) (Table 1).

The intention-to-treat population (n=68) included evaluable patients plus patients who were not evaluable due to the reasons illustrated in the Consort diagram (Figure 1). In the intention-to-treat population, the response rate was partial response=24% (16/68), stable disease=12% (8/68) and progressive disease=38% (26/68) by combined criteria. By RECIST alone, response rates were partial response =4% (3/68), stable disease =18% (12/68) and progressive disease =41% (28/68), whereas by CA125 criteria responses were response=25% (17/68), non-response/non- progressive disease =35% (24/68) and progressive disease =9% (6/68) (Table S4). Partial responses (by combined criteria) were more common in patients with platinum-sensitive disease compared to those with platinum-resistant disease (67% *vs* 24%, p=0.02). With regards to the platinum-resistant disease, patients with acquired resistance had more frequent responses compared to those with primary resistance (30% *vs* 9%, p=0.005) (Table S5).

Survival analysis

In the intention-to-treat population, progression-free survival and overall survival were 2.6 months and 6 months, respectively (Figure 2). Progression-free survival and overall survival were both greater in patients with platinum sensitive disease compared to those with platinum-resistant disease. Progression-free survival for platinum-sensitive versus platinum resistant patients was 8.2 *vs* 2.4 months (HR=0.4, 95%CI 0.2-0.7, p=0.01) and overall survival was 16.1 *vs* 5.5 months (HR=0.5, 95%CI 0.3-0.9, p=0.01), respectively. For patients whose disease either responded or remained stable through metronomic cyclophosphamide, there is a significant advantage in progression-free

187 survival, which translates into a significant increase in survival (10.4 *vs* 3.3 months, HR=0.15, 95%CI
188 0.02-0.8, p<0.0001), compared to patients whose disease did not respond to metronomic
189 cyclophosphamide (Figure 2).
190
191 *Toxicity*
192 Median number of delivered cycles for the patients included in the toxicity analysis was 3 (range; 1-
193 14). Two patients stopped treatment prematurely due to poor tolerability (grade 2 nausea) without
194 completing one full cycle (3%); these were not included in the toxicity analysis. Drug related adverse
195 effects were mainly of grade 1 or 2; the most common ones were neutropenia (18%), nausea (16%),
196 anaemia (7%), diarrhoea (5%), mucositis (5%) and fatigue (5%). There was one case of grade 3
197 neutropenia and one case of grade 3 transaminitis, both of which resolved after drug discontinuation.
198 Toxicity was the reason for discontinuation in 3 (5%) of 56 patients who received more than one cycle
199 of metronomic cyclophosphamide, although in two of these patients, the reason for discontinuation
200 was a combination of toxicity and disease progression. There were no treatment-related deaths
201 (Table S6).
202
203
204 *Markers of response to metronomic cyclophosphamide*
205 In order to identify markers of response to metronomic cyclophosphamide chemotherapy, we
206 performed multivariate analysis estimating the odds ratio (OR) of disease progression for the following
207 variables relating to patient characteristics: age (>70 years old), performance status (>1), *gBRCA*
208 status (mutant or wild type), platinum resistance and prior lines of platinum treatment (≥3 lines). We
209 assessed the risk of disease progression based on a) combined criteria (radiological and CA125
210 criteria), b) RECIST 1.1 and c) CA125 criteria alone. Disease progression on treatment was the
211 dependent variable (progression=1 and stable disease/partial response=0).
212 By univariate logistic regression, age and performance status did not have an effect on
213 disease progression with metronomic cyclophosphamide. *gBRCA* status had a statistically significant
214 effect on disease progression, when assessed by combined (OR=0.05, 95% CI 0.006-0.51, p=0.01)
215 and radiological criteria (OR=0.06, 95% CI 0.01-0.39, p=0.003). Number of prior lines of platinum
216 treatment (≥3 lines) was strongly associated with a reduced risk of disease progression with

metronomic cyclophosphamide, by combination criteria (OR=0.10, 95% CI 0.02-0.44, p=0.002) and radiological criteria (OR=0.14, 95% CI 0.03-0.59, p=0.007) (Table S7).

Multivariate analysis encompassing all the above factors showed that *gBRCA* mutation was associated with reduced risk for disease progression by radiological criteria (OR=0.07, 95% CI 0.008-0.67, p=0.02). Lines of prior platinum treatments reduced the probability of disease progression with metronomic cyclophosphamide by combination (OR=0.11, 95% CI 0.01-0.81, p=0.03), but not by radiological criteria (OR=0.3, 95% CI 0.04-1.87, p=0.2) (Table S8).

Due to collinearity between *gBRCA* status and the variables of platinum resistance (Pearson chi-square p=0.007) and prior lines of platinum treatments (Pearson chi square p=0.001), we ran the analysis again, consecutively omitting the latter variables. When these variables were excluded, *gBRCA* status remained a statistically significant prognostic marker, both by combination and radiological criteria (Table S9). When the platinum resistance factor was omitted, *gBRCA* was associated with an odds risk for progression of 0.07 (95% CI 0.01-0.84, p=0.04) by combination and OR= 0.07 by radiological criteria (95% CI 0.01-0.55, p=0.01). When the factor of prior lines of platinum treatment was removed, *gBRCA* was associated with an odds risk for progression of 0.07 (95% CI 0.01-0.68, p=0.02) by combination and OR= 0.05 by radiological criteria (95% CI 0.01-0.44, p=0.01). Conversely, when *gBRCA* status was omitted, only the lines of prior lines of platinum treatment emerged as a statistically significant prognostic factor (Table S10).

Response rates in evaluable patients were significantly higher in patients with *gBRCA1/2* mutations by all methods of assessment (Table 2). Moreover, patients with *gBRCA1/2* mutations had an improved progression-free survival (7.9 *vs* 2.5 months, HR 0.4 95%CI 0.23-0.74, p=0.003) and a longer overall survival (15.5 *vs* 6 months, HR 0.49 95% CI 0.28-0.85, p=0.02) compared with patients without *gBRCA1/2* mutations (or unknown *gBRCA1/2* status) (Figure 3a). The swimmer's plot on Figure 3b shows duration of response for evaluable patients whose disease progressed based on either radiological or CA125 criteria, stratified by the presence of *gBRCA1/2* mutation. Progression-free rate at 6 months was higher in patients with *gBRCA* mutations compared to patients without *gBRCA* mutations or unknown *gBRCA* mutation status (65% *vs* 38.4%, p<0.0001, Figure 3b).

Discussion

Summary of main results

This retrospective non-randomised study showed that metronomic cyclophosphamide is an active and safe therapeutic in a heavily pre-treated population with recurrent ovarian cancer. Overall, 40% of patients were performance status of 2 and 33% had been treated with 3 or more prior lines but despite this, only two experienced grade 3 adverse events. Patients whose disease responded to metronomic cyclophosphamide achieved a 10-month survival, without the need for intravenous chemotherapy.

In a multivariate analysis we observed that *gBRCA1/2* mutation independently predicts radiological response to metronomic cyclophosphamide and is associated with a reduction in the risk of progression. In a similar way, having received more than 3 previous lines of platinum treatment was associated with reduced risk of progression. Of note, we also found that patients with acquired platinum resistance had a higher response rate than those with primary resistance suggesting different mechanisms of resistance in these groups. The finding that the number of prior lines is predictive, independent of the presence of a *gBRCA* mutation, could be the result of selecting a *BRCA* wild-type (*BRCAwt*) population with a homologous-recombination deficient phenotype. It may be that both *BRCA* mutant (*BRCAm*) and homologous-recombination deficient tumours are more likely to respond to an alkylating agent despite the metronomic dosing (15). Thus, though we have found that patients had higher response rates after ≥3 lines of treatment, it is possible that metronomic cyclophosphamide could be a useful option earlier in the treatment history if other markers than repeated clinical response, such as *BRCAm* or HRD were used to select patients.

Results in the context of published literature

In the intention-to-treat population, progression-free survival and overall survival are consistent with results from a recent randomised phase 2 study with metronomic cyclophosphamide with or without the anti-angiogenic agent nintedanib (14) where progression-free survival was 2.6 months and overall survival was 6.4 months, adding confidence to our outcomes. This study adds to the experience of others who have investigated metronomic cyclophosphamide, either as monotherapy or combination with other treatments (Table S1).

There is recent preclinical evidence suggesting that *BRCA*-deficient ovarian tumours are more likely to be infiltrated by anti-tumour lymphocytes and are more responsive to immunotherapy (16). Metronomic cyclophosphamide has an immunostimulatory effect (17-21), that is perhaps more potent than its cytotoxic effect, and this could also explain the improved outcomes in the *BRCA1/2* deficient population. There is also growing evidence that platinum-based chemotherapy can result in immune stimulation in ovarian cancer with T cell activation, enhanced T cell receptor clonality and increased IFNγ production in the tumour microenvironment (22, 23). Therefore, multiple lines of platinum-based treatment could prime the tumour microenvironment in a way that is conducive to the effect of metronomic cyclophosphamide.

The majority of our patients had platinum-resistant disease. More responses were observed in platinum-sensitive compared to platinum-resistant disease. We did not see radiological responses in *BRCAwt* platinum-resistant tumours and median progression-free survival was short in both the *BRCAwt* and platinum-resistant, intention-to-treat populations. However, progression-free survival results with metronomic cyclophosphamide in our platinum-resistant cohort are not dissimilar to results observed with weekly paclitaxel in the BAROCCO phase 2 study (24) and 6 of 24 women with *BRCAwt* and platinum-resistant disease had progression-free survival of ≥ 5 months. CA125 response rates were higher compared to reported real world response rates for endocrine therapy which offers another well-tolerated oral option in this setting (25, 26).

Novel therapeutics such as anti-PD1 immune checkpoint inhibitors, may have some activity in combination with inhibitors of poly-ADP ribose polymerase (PARPi) in platinum-resistant disease (27), but these treatments are both toxic and expensive. Given the potential immunostimulatory properties of metronomic cyclophosphamide, it would be interesting to test the combination of metronomic cyclophosphamide and immune checkpoint inhibitors in a randomised trial. This may offer a more cost effective and better tolerated option than the combination with PARP inhibitors. It has also been hypothesised that immune modulation with metronomic cyclophosphamide and normalisation of the vasculature with anti-angiogenesis agents achieve effector immune cell homing in the tumour microenvironment (28). This was recently tested in a phase 2 randomised study with metronomic cyclophosphamide with or without nintedanib but a benefit of adding the antiangiogenic inhibitor was not observed (14).

Strengths and weaknesses

Although our study has one of the largest sample sizes in the literature, it is retrospective, non-randomised and has several limitations. Our intention-to-treat population included patients who did not complete a full cycle of metronomic cyclophosphamide due to rapidly progressive disease and/or declining performance status and who, in retrospect, should have received best supportive care. This group would not have been included in clinical trials and so we feel it is reasonable to compare the response rate in our evaluable population to those of published clinical trials. In our study, apart from radiological criteria, we also used combination response criteria (radiological plus CA125) to assess the efficacy of metronomic cyclophosphamide. Lindemann et al recently showed that, in platinum-resistant disease, CA125 lacks concordance with radiological results (29). Therefore, our results could perhaps be skewed towards better response rate. The retrospective nature of both radiological and toxicity assessment adds to the risk of bias. In the present study, only 5 (73.5%) of 68 patients had received prior treatment with a PARPi, none of whom responded to metronomic cyclophosphamide. Given that the majority of patients requiring treatment for recurrent ovarian cancer now will have been already exposed to PARPi, the applicability of our findings to the contemporary recurrent ovarian cancer population is not clear.

Implications for Practice and Future Research

As a result, we regard metronomic cyclophosphamide as a viable option, particularly for patients who are not medically fit to withstand the side-effects of intravenous chemotherapy with either platinum sensitive or resistant disease. Similarly, metronomic cyclophosphamide can be an option for patients with platinum-resistant disease who have progressed through non-platinum chemotherapy with or without bevacizumab. Future research could focus on identifying the ideal sequencing of metronomic cyclophosphamide treatment, as well as potentially enhanced activity when given in combination with other treatments (such as immunotherapy).

Conclusion

Overall, metronomic cyclophosphamide presents a low-toxicity option that should be considered in the recurrent ovarian cancer setting. Patients with *gBRCA* mutations and/or those that have responded to multiple prior platinum treatments, have a particularly high rate of response. This clinical predictive

factor may be particularly relevant in countries where *gBRCA* testing is not feasible or PARPi too costly.

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Tables and Figures

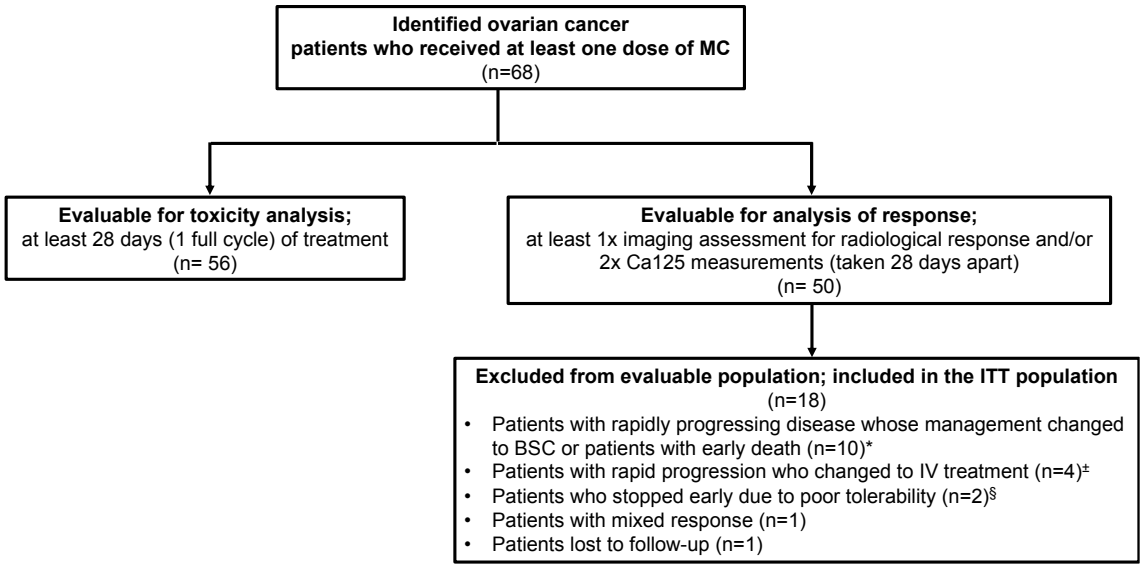

Figure 1: Patient Consort diagram. *Seven out of ten patients had less than 1 cycle of metronomic cyclophosphamide. ±Three out of four patients had less than 1 cycle of metronomic cyclophosphamide. § Both patients had less than 1 cycle of metronomic cyclophosphamide. ITT= intention to treat, BSC= best supportive care, IV= intravenous.

Response rate criteria		**All (n=68)**
Combined criteria*	**Evaluable patients**	**n=50**
number (%)	Complete response	0 (0%)
	Partial response	16 (32%)
	Stable disease	8 (16%)
	Progressive disease	26 (52%)
Radiological[$]	**Evaluable patients**	**n=43**
number (%)	Complete response	0 (0%)
	Partial response	3 (7%)
	Stable disease	12 (28%)
	Progressive disease	28 (65%)
CA125 response^	**Evaluable patients**	**n= 48**
number (%)	Response	17 (35%)
	Non-response/non-PD	24 (50%)
	Progressive disease	7 (15%)

Table 1: Response rates in the evaluable population.

*at least 1 tumour assessment and/or 2 x Ca125 measurements. [$]At least 1 radiological tumour assessment. ^At least 2xCa125 measurements

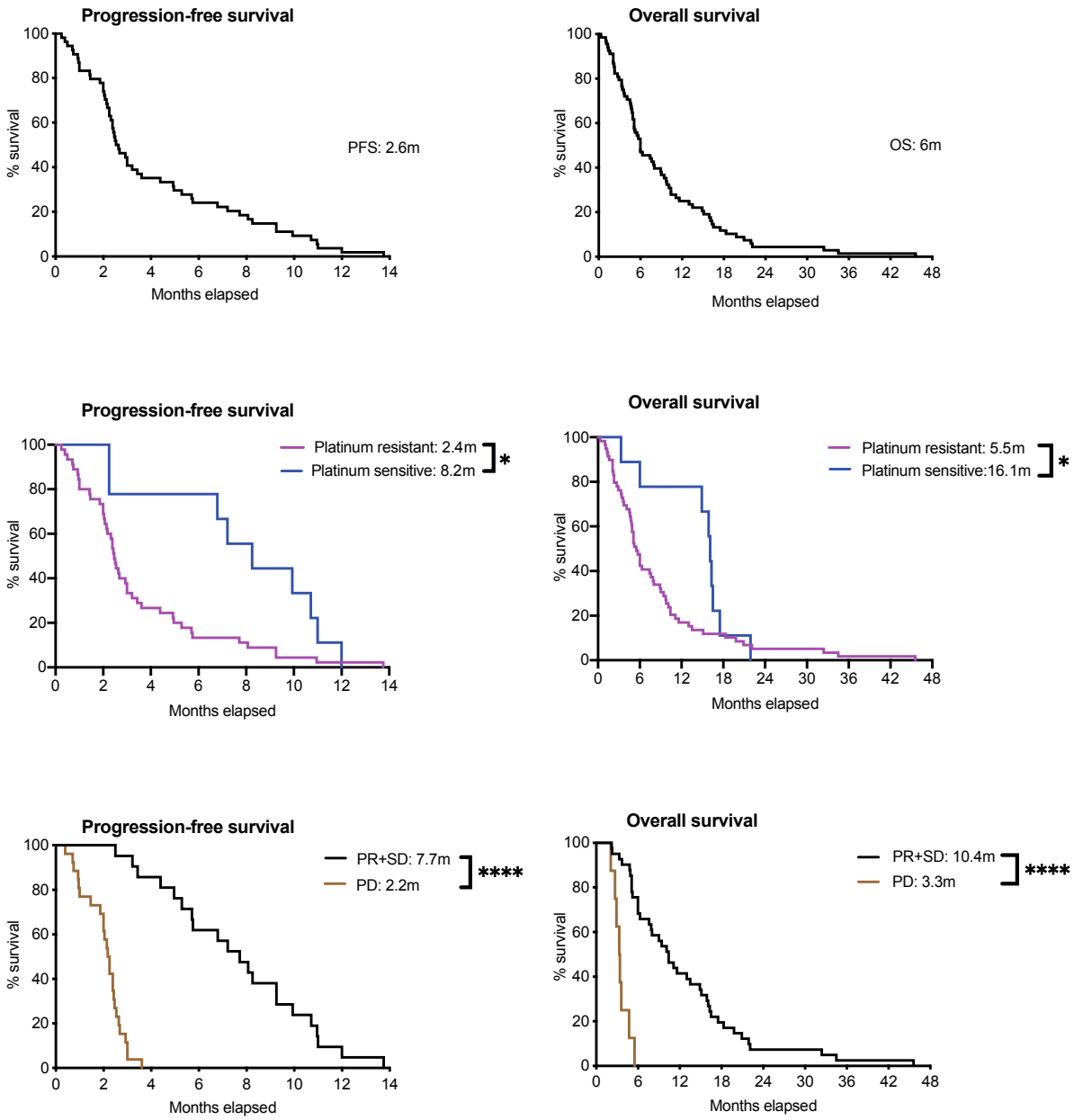

Figure 2: Kaplan-Meier curves for progression-free survival (PFS) and overall survival (OS) in the intention-to-treat (ITT) population. Kaplan-Meier curves for PFS and OS for patients with platinum-sensitive disease, platinum-resistant (acquired or primary resistance), as well as patients who had partial response/stable disease (PR+SD) versus patients who had progressive disease (PD) on metronomic cyclophosphamide. Log-rank (Mantel-Cox) test was used for curve comparisons.

Response rate criteria		All (n=68)	*Patients without gBRCA mutations**	*Patients with gBRCA mutations*	p value^
Combination criteria$	**Evaluable patients**	**n=50**	**n= 40**	**n= 10**	
	Complete response	0 (0%)	0 (0%)	0 (0%)	
	Partial response	16 (32%)	9 (22.5%)	7 (70%)	**p=0.01**
	Stable disease	8 (16%)	7 (17.5%)	1 (10%)	
	Progressive disease	26 (52%)	24 (60%)	2 (20%)	
Radiological criteria+	**Evaluable patients**	**n=43**	**n= 33**	**n= 10**	
	Complete response	0 (0%)	0 (0%)	0 (0%)	
	Partial response	3 (7%)	0 (0%)	3 (30%)	**p=0.0008**
	Stable disease	12 (28%)	7 (21%)	5 (50%)	
	Progressive disease	28 (65%)	26 (79%)	2 (20%)	
Ca125 response criteria±	**Evaluable patients**	**n= 48**	**n=39**	**n=9**	
	Response	17 (35%)	10 (26%)	7 (78%)	**p=0.03**
	Non-response/non-PD	24 (50%)	23 (59%)	1 (11%)	
	Progressive disease	7 (15%)	6 (15%)	1 (11%)	

Table 2: Response rates in the evaluable population comparing patients with *gBRCA* mutations to patients without *gBRCA* mutations. * This group includes patients with wild type *gBRCA* and patients with unknown status. Patients with *BRCA1/2* variants of unknown significance were included in the *BRCA1/2* wild-type cohort. ^Chi-square test to compare partial response to treatment versus non-response (i.e. stable disease plus progressive disease). $at least 1 tumour assessment and/or 2xCa125 measurements. +At least 1 radiological tumour assessment. ±At least 2xCa125 measurements.

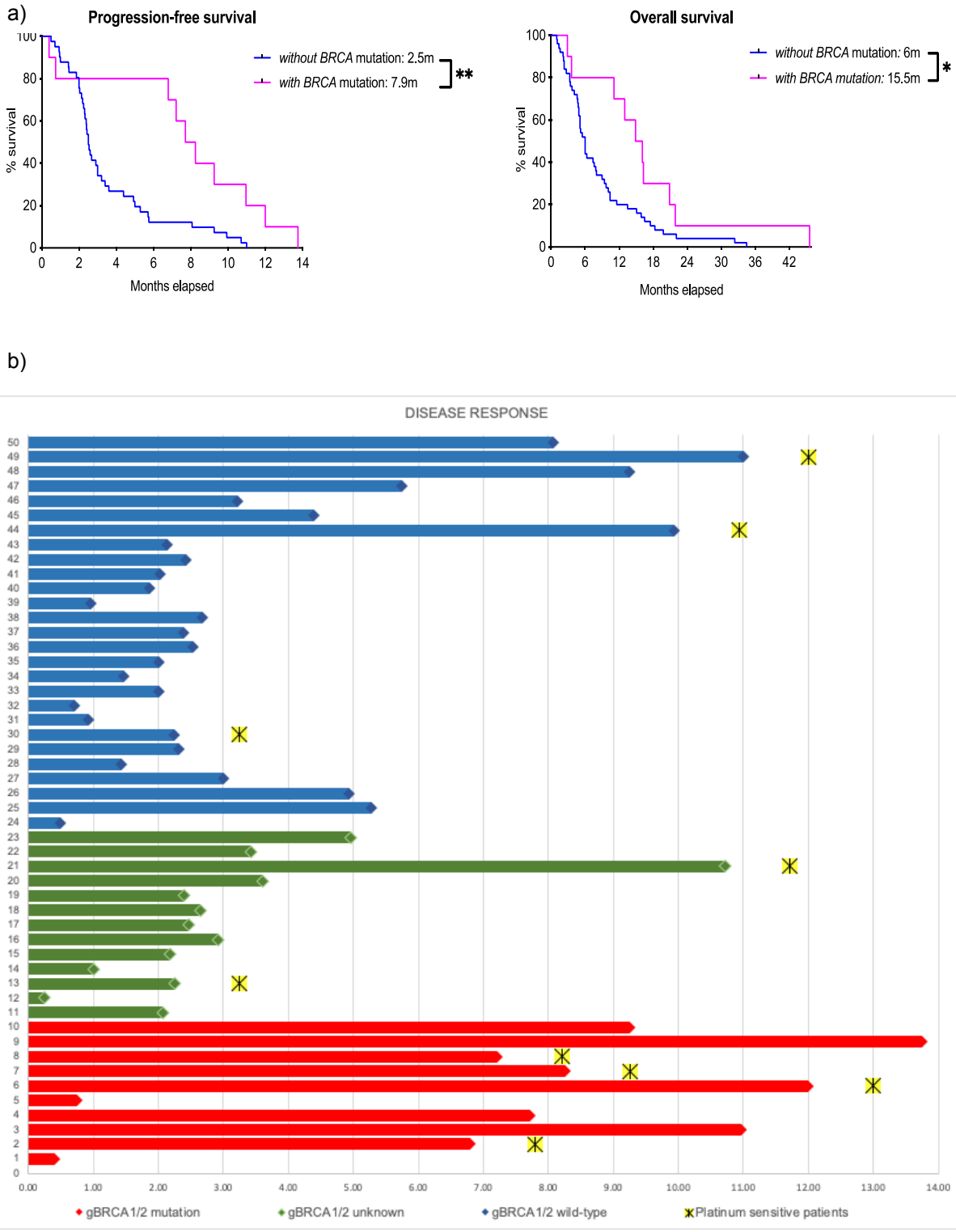

Figure 3: a) Kaplan-Meier curves for progression-free survival (PFS) and overall survival (OS) in the intention-to-treat (ITT) population with or without *gBRCA1/2* mutations. b) swimmers-plot depicting patients' treatment until disease progression. Disease progression was defined either by radiological or Ca125 criteria. Log-rank (Mantel-Cox) test was used for curve comparisons in (a).