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**Title: Endocrine treatment of high grade serous ovarian carcinoma; quantification of efficacy and identification of response predictors.**

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1 **ABSTRACT**

2 *Objectives.* The role of endocrine therapy (ET) in high grade serous ovarian carcinoma  
3 (HGSOC) is poorly defined due to the lack of phase III data and significant heterogeneity of  
4 clinical trials performed. In this study, we sought to identify predictive factors of endocrine  
5 sensitivity in HGSOC.

6 *Methods.* HGSOC patients who received at least four weeks of ET for relapsed disease  
7 following one line of chemotherapy at the Edinburgh Cancer Centre were  
8 identified. Exclusion criteria were use of endocrine therapy as maintenance therapy or of  
9 unknown duration. Duration of therapy and best CA125 response as per modified GCIG  
10 criteria were recorded. Oestrogen receptor (ER) histoscore, treatment free interval, prior lines  
11 of chemotherapy, and type of ET were evaluated as predictive factors.

12 *Results.* Of 431 patients identified, 269 were eligible (77.0 % letrozole, 18.6% tamoxifen,  
13 2.2% megestrol acetate, 2.2% other). The median duration of therapy was 126 days (range  
14 28-1427 days). 32.7% remained on ET for  $\geq 180$  days and 14.1% for  $\geq 365$  days. The CA125  
15 response and clinical benefit rates (response or stable disease) were 8.1% and 40.1%  
16 respectively. ER histoscore  $>200$  ( $P=0.0016$ ) and a treatment free interval of  $\geq 180$  days  
17 ( $P<0.0001$ ) were independent predictive factors upon multivariable analysis.

18 *Conclusions.* ET should be considered as a viable strategy to defer subsequent chemotherapy  
19 for relapsed HGSOC. Patients with an ER histoscore  $>200$  and a treatment free interval of  
20  $\geq 180$  days are most likely to derive benefit.

21

22

## 23 1. INTRODUCTION

24 The majority of patients with advanced stage high grade serous ovarian carcinoma (HGSOC)  
25 will unfortunately relapse despite optimal cytoreductive surgery and platinum based  
26 chemotherapy. Symptomatic relapses are treated with further systemic chemotherapy which  
27 can be effective for some patients. However, with time, the intervals between each treatment  
28 get progressively shorter with reduced efficacy and cumulative toxicity.

29 Endocrine therapy (ET) in relapsed HGSOC is easy to administer, has a low toxicity profile  
30 and is low cost. There is good pre-clinical evidence to support the role of oestrogen in  
31 regulating the growth of oestrogen receptor (ER) positive EOC [1, 2]. To date, more than 50  
32 phase II trials of ET in EOC have been performed with response rates between 10-15% and  
33 disease stabilisation rates of 30-40% as described in systematic reviews and meta-analyses [3,  
34 4]. Only one phase III randomised trial of tamoxifen against standard chemotherapy in the  
35 platinum resistant setting has been performed which did not demonstrate differences in  
36 overall survival [5]. As such, ET is not considered a standard of care and its use is  
37 inconsistent and variable worldwide.

38 However, most of these trials were conducted in heavily pre-treated populations of mixed ER  
39 positive and ER negative patients [3]. In studies which pre-selected for ER status, different  
40 thresholds of ER positivity and methods of measurements were used [3]. In addition, these  
41 trials did not account for EOC comprising at least five histological subtypes which are  
42 biologically and clinically distinct [6].

43 The Ovarian Cancer Tissue Consortium Study found HGSOC, endometrioid and low grade  
44 serous ovarian carcinomas (LGSOC) to express the highest levels of ER ( $\geq 50\%$  tumour  
45 nuclear staining) of 60%, 60% and 71% respectively [7]. These histologies likely represent  
46 the most endocrine sensitive subtypes with emerging retrospective data to support this.

47 Gershenson et al demonstrated the role of ET both as treatment for relapsed disease [8] and as  
48 first line maintenance in LGSOC [9]. Patients with LGSOC who received first line  
49 maintenance ET had a superior progression free survival of 64.9 months compared to 26.4  
50 months in those who underwent observation ( $p<0.001$ ). Another retrospective study by  
51 Heinzelmann-Schwarz et al showed improvement in recurrence free survival in patients with  
52 HGSOC who received first line maintenance letrozole versus observation ( $p=0.035$ )[10].  
53 Together, these studies illustrate the importance of performing histological subtype-specific  
54 clinical trials to derive an accurate assessment of endocrine sensitivity.

55 Two sequential phase II studies (Bowman et al [11] and Smyth et al [12]) identified an  
56 endocrine sensitive group of ovarian cancer patients with mixed histology as those with an  
57 ER histoscore  $\geq 150$ . This weighted scoring method accounts for percentage(%) tumour cells  
58 stained and stain intensity (0 no staining, 1+ weak staining, 2+ moderate staining, 3+ strong  
59 staining). It derives a score between 0 to 300 using the formula:  $[1 \times (\% \text{ cells } 1+) + 2 \times (\%$   
60  $\text{cells } 2+) + 3 \times (\% \text{ cells } 3+)]$  [13]. On the basis of these data, ET has been routinely used in  
61 our centre in patients with relapsed EOC with an ER histoscore of  $\geq 150$ . We sought to  
62 characterise the endocrine sensitivity of relapsed HGSOC as well as identify predictive  
63 factors in a large retrospective study.

## 64 **2. METHODS**

### 65 *2.1 Patient Identification*

66 We identified patients with a historical diagnosis of grade 2 or 3 serous carcinomas [14] who  
67 received at least one line of ET from the Edinburgh Ovarian Cancer Database between  
68 January 1974 and December 2015. This database contains detailed histopathological and  
69 clinical details of patients entered prospectively as part of routine care. Communication with  
70 the Lothian Research Ethics Committee determined that retrospective analysis of outcome

71 using the contents of the database were deemed audit by their definition and formal ethical  
72 approval was therefore not required.

### 73 *2.2 Inclusion and Exclusion Criteria*

74 Patients were included if they received at least four weeks of ET as treatment for relapsed  
75 disease as determined by the treating physician, following at least one line of previous  
76 chemotherapy, and with known duration of therapy. Those who received less than four weeks  
77 of ET were deemed to have had inadequate exposure to determine sensitivity. Patients who  
78 received ET as maintenance therapy were excluded.

### 79 *2.3 Recorded data*

80 All baseline and treatment demographics had been prospectively collected through the  
81 Edinburgh Ovarian Cancer Database as part of routine care. All available patient electronic  
82 and paper health records were also reviewed. Treatment characteristics were recorded for the  
83 first ET received. These included: duration of therapy, lines of ET, type of ET, prior lines of  
84 chemotherapy, and ER histoscore as recorded by the pathologist at diagnosis. The treatment  
85 free interval (TFI) was calculated from the last dose of chemotherapy to the date of ET  
86 initiation. The setting in which the last chemotherapy was received was recorded as 'platinum  
87 sensitive' or 'platinum resistant'. Patients who stopped ET due to toxicity or who were still  
88 on therapy at data cut-off were censored.

### 89 *2.4 Treatment efficacy*

90 Most clinicians used CA125 as a marker of response and did not perform radiological  
91 assessments of patients on ET until there was evidence of a significant rise in the CA125 or  
92 the patient developed symptoms. Therefore, in this non-trial setting, radiological PFS by  
93 RECIST could not be accurately defined. ET was continued until there was evidence of

94 symptomatic disease progression warranting further chemotherapy, or until death from  
95 ovarian cancer. In view of this, duration of therapy was recorded as an objective end-point  
96 for this study and surrogate measure of endocrine sensitivity. The best CA125 response  
97 across the duration of therapy was also recorded. Due to the variable frequency of CA125  
98 measurements, a modified GCIG criteria was adopted [15]. Patients were evaluable for  
99 CA125 response if they had an evaluable CA125 (>70U/ml) within four weeks of starting  
100 therapy, and at least three CA125 measures if they received ET for more than 12 weeks. At  
101 least two CA125 measures were required if ET was received for 12 weeks or less with the  
102 second CA125 within 4 weeks of cessation of ET. Patients were not evaluable for CA125  
103 response if they only had one CA125 measure, and if they received ET for 12 weeks or less  
104 with no CA125 progression. The 12 week threshold was adopted as the median time to  
105 CA125 response has been shown to be 12 weeks [11]. Patients treated for less than 12 weeks  
106 with clear CA125 progression were considered evaluable.

107 Definitions for CA125 complete response (CR), partial response (PR) and stable disease (SD)  
108 were as per GCIG criteria [15]. SD had to be maintained for at least 12 weeks from the start  
109 of therapy. Progressive disease (PD) was defined as doubling of CA125 from the baseline  
110 value. The CA125 overall response rate (ORR=CR+PR) and clinical benefit rate 1  
111 (CBR1=CR+PR+SD) were calculated and recorded.

112 A study by Hall et al showed that the change in rate of rise in CA125 can indicate activity of  
113 cytostatic agents such as tamoxifen [16]. In view of this, the characteristics of patients who  
114 had CA125 progression by GCIG criteria, followed by <50% rise of their CA125 for at least  
115 12 weeks were also explored (delayed SD).

116

117

118 *2.5 Statistical analysis*

119 Duration of therapy according to ER histoscore, TFI, prior lines of chemotherapy, best  
120 CA125 response and type of ET was evaluated using the Kaplan-Meier method and Cox  
121 regression models for univariable and multivariable analyses. Comparisons of CA125 ORR  
122 and CBR1 between groups were assessed using Chi-squared and Fisher's exact tests as  
123 appropriate. Statistical analyses were performed using R version 3.3.3.

124 **3. RESULTS**

125 431 patients received at least one line of ET. 162 patients were excluded (figure 1). 269  
126 received ET as treatment for relapsed disease. 143(53.2%) were confirmed as HGSOC  
127 through contemporary pathology review conducted through other research studies, and  
128 118(43.9%) and 8(3.0%) had a historical diagnosis of grade 3 and grade 2 serous carcinomas,  
129 respectively. The median age of diagnosis was 65 years (range 28-91 years).

130 *3.1 First endocrine therapy for relapse*

131 Of 269 patients, 209 (77.7%), 55 (20.4%) and five (1.9%) patients received one, two and  
132 three lines of ET, respectively. 207 (77.0%), 50 (18.6%) and six (2.2%) patients received  
133 letrozole, tamoxifen and megestrol acetate, respectively. 156 (58.0%) patients received ET  
134 after one prior line of chemotherapy, 87 (32.3%) after two lines, and 26 (9.7%) after three or  
135 more lines. 229 (85.1%) and 36 (13.4%) patients last received chemotherapy in the platinum  
136 sensitive and platinum resistant setting, respectively. ER histoscores were available in 225  
137 (83.6%) patients. The range of ER scores are illustrated in table 1. The majority of these  
138 histoscores (192, 85.3%) were from the primary chemotherapy naïve tumour.

139 *3.2 Overall CA125 response rate and duration of therapy*

140 Of 269 patients, 257 (95.5%) stopped ET due to disease progression, 11 (4.1%) were still on ET at  
141 the time of analysis, and one (0.4%) stopped ET due to toxicity. 172 (63.9%) patients were evaluable



142 for CA125 response. The median number of CA125s was three (range 2-8) and six (range 3-44) in  
143 those who received 12 weeks or less of ET, and more than 12 weeks of ET, respectively. The CA125  
144 response rate was 8.1% (GCIG CR 2.9%, PR 5.2%) and the CBR was 40.1%. The pattern of CA125  
145 responses for CR and PR are shown in supplemental figure S1A and S1B, respectively. The overall  
146 median duration of therapy was 126 days (range 28-1427 days).

### 147 *3.3 Delayed SD patients*

148 16 patients demonstrated delayed stabilisation of their CA125 (supplemental figure S1C).  
149 The median time to first CA125 progression was 42 days (21-114 days). The delayed SD  
150 group (patients whose CA125 rose then stabilised according to the criteria outlined above)  
151 had a significantly longer median duration of therapy than those whose disease progressed on  
152 CA125 criteria without subsequent stabilisation (196 days versus 84 days,  $P<0.0001$ ). The  
153 median duration of therapy between the GCIG-defined SD group and delayed SD group were  
154 comparable ( $P=0.288$ ) (figure 2C). In view of this, a second CBR (CBR2) which included the  
155 delayed SD patients as part of the GCIG-defined SD cohort was calculated and compared for  
156 each variable as an exploratory analysis.

### 157 *3.4 ER Histoscore*

158 148 patients with known ER histoscores had evaluable CA125 responses. There was an  
159 increasing trend in CA125 response rate of 5.8%, 8.6%, 9.1% and 14.7% in ER0-150,  
160 ER151-200, ER201-250 and ER251-300 groups, respectively. Similarly, there was an  
161 increasing trend of CBR1 with ER. These differences were not significant (table 3, figure  
162 2B). When the delayed SD patients were accounted for as part of the GCIG defined SD  
163 cohort, the CBR in the ER251-300 group was significantly higher than the ER0-150 group  
164 (CBR2 64.7% versus 37.1%;  $P=0.04$ ) (table 3, figure 2D).

165 The median duration of therapy was significantly longer at 140 days and 161 days in the  
166 ER201-250 (multivariable: HR 0.62, 95% CI 0.42-0.91,  $P=0.016$ ) and ER251-300 groups  
167 (multivariable: HR 0.63, 95% CI 0.41-0.96,  $P=0.032$ ) when compared to 88.5 days in those  
168 with ER  $\leq 150$  (table 2, figure 2A). There were no significant differences in median duration  
169 of therapy between the ER151-200 and ER  $\leq 150$  groups.

### 170 *3.5 Treatment free interval*

171 Of 269 patients, 259 (96.2%) received chemotherapy as their last treatment. 8(3.0%) patients  
172 received maintenance therapy and 2(0.7%) patients received secondary debulking as their last  
173 treatment and were thus excluded from this analysis. Of 259 patients, 164 (63.3%) were  
174 evaluable for CA125 responses. Patients who had a TFI 180-365 days and TFI>365 days had  
175 a significantly higher CA125 CBR1 of 50.0% ( $P =0.01$ ) and 60.6% ( $P=0.002$ ) when  
176 compared to 21.0 % in those with TFI<90 days (table 3, figure 2F). There were no significant  
177 differences between TFI <90days and 90-179 days.

178 The median duration of therapy was significantly longer at 161 days and 209 days in those  
179 with TFI 180-365 days (multivariable: HR 0.32, 95% CI 0.21-0.48,  $P <0.0001$ ) and TFI>365  
180 days (multivariable: HR 0.28, 95% CI 0.17-0.45,  $P <0.0001$ ) compared to 84 days in those  
181 with TFI<90days (table 2, figure 2E). There were no significant differences in duration of  
182 therapy between those with TFI<90days and 90-179 days.

### 183 *3.6 Prior lines of chemotherapy*

184 There was no significant difference in CA125 ORR, CBR1 or CBR2 between patients  
185 treated with different numbers of prior chemotherapy lines (table 3). The median duration of  
186 therapy was significantly longer at 142 days and 111 days in those who received ET after one  
187 (univariable: HR 0.46, 95% CI 0.30-0.70,  $p<0.001$ ) and two (univariable: HR 0.61, 95% CI  
188 0.39-0.95,  $p=0.03$ ) lines of chemotherapy compared to 88.5 days after 3 lines or more (table

189 2, supplemental figure S2). However, these differences were not significant upon  
190 multivariable analyses ( $P = 0.67$  and  $P = 0.406$ , respectively).

### 191 3.7 Type of ET

192 There was no significant difference in CA125 ORR, CBR1 or CBR2 between those treated  
193 with letrozole and tamoxifen (table 3). Compared to tamoxifen, patients who received  
194 letrozole had a significantly longer median duration of therapy (126 versus 98 days,  
195 univariable: HR=0.64, 95% CI 0.47-0.88,  $P=0.006$ ), but the difference was not significant  
196 upon multivariable analysis ( $P=0.255$ ) (table 2, supplemental figure S3). The number of  
197 patients who received megestrol acetate was too small for meaningful analysis.

### 198 3.8 Characteristics of patients deriving greatest benefit from ET

199 88 (32.7%) patients remained on ET for  $\geq 180$  days, and 38 (14.1%) for  $\geq 365$  days. Of the 38  
200 patients, 29 (76.3%) received ET after one line of chemotherapy and median TFI was 286  
201 days (range 42 – 4256 days). 34 patients had known ER histoscores, all of which were  $>200$   
202 (25, 73.5% 201-250, 9, 26.5%  $>250$ ). 33 (86.8%) patients were treated with letrozole. 28  
203 patients were evaluable for CA125 response, of which 9 (32.1%) achieved CA125 response  
204 (4 GCIG CR, 5 PR) and 16 (57.1%) achieved SD. The remaining 3 patients demonstrated  
205 delayed SD.

206 The median duration of therapy in the 14 patients who achieved CR or PR was significantly  
207 longer at 878 days (range 180-3981 days) compared to 241 days in the 49 patients who  
208 achieved GCIG defined SD (HR = 0.30 [0.15-0.60]  $P < 0.001$ ) (figure 2C).

209 **4. DISCUSSION**

210 The main strengths of this study is in its large size with known ER status in more than 80% of  
211 the cohort. This provided sufficient power to perform comprehensive multivariable analysis  
212 in order to identify independent predictors of endocrine sensitivity. Patient and treatment  
213 demographics were also all recorded prospectively on the Edinburgh Ovarian Cancer  
214 Database thus minimising information bias.

215 The main weaknesses were the lack of radiology response data and the use of surrogates in  
216 the form of CA125 responses and duration of therapy, thus limiting the interpretation of some  
217 of our results. As this study was not conducted within a trial setting, the CA125 time points  
218 were also heterogeneous which may have underestimated the response or stabilisation rates to  
219 ET. Whilst all physicians started ET as treatment for relapse, the timing of treatment  
220 initiation and cessation is likely to have been inconsistent. Most ER histoscores were also  
221 recorded at the time of diagnosis by different pathologists which may have introduced  
222 interobserver variation.

223 Although this study took place approximately over 25 years, more than half the samples were  
224 confirmed as HGSOC following contemporary pathology review. The remaining patients  
225 were almost ubiquitously diagnosed as grade 3 serous EOC which has shown to be largely  
226 concordant with HGSOC[14], with less than 3% of the analysis cohort comprising grade 2  
227 serous EOC. This provides confidence that this cohort was largely homogenous. To our  
228 knowledge, this is the biggest study performed that has attempted to quantify the efficacy of  
229 ET in relapsed HGSOC.

230 Most prospective and retrospective studies performed to date have been performed in mixed  
231 histological subtypes [11, 17, 18]. There is clear evidence that each EOC subtype is a discrete  
232 disease with unique molecular profiles, treatment responses and patient outcomes [19].

233 Recent retrospective studies have suggested a particular role for ET in the management of  
234 low grade serous ovarian cancer [8, 9]. As such, responses to ET in low grade serous ovarian  
235 cancer may have contributed to signals of efficacy in previous studies of mixed histological  
236 types and the exact sensitivity in HGSOV was unclear.

237 Our study illustrates that the degree of ER expression is proportional to endocrine sensitivity  
238 in HGSOV. Duration of therapy increases significantly in those with ER251-300 compared to  
239 those with ER0-150 with an increasing trend in the proportion of patients demonstrating a  
240 response or stabilisation of their CA125 with increasing ER histoscores. Our study also  
241 identified a third of patients who remained on ET for more than 6 months, and nearly 15% for  
242 more than a year. Interestingly, we found that those who sustained a complete or partial  
243 CA125 response remained on endocrine therapy for much longer than those who achieved  
244 CA125 stabilisation. Although these results are expected, it argues against indolent tumour  
245 biology as being solely responsible for these apparent long responders to ET.

246 We also describe a small group of patients who had PD according to GCIG criteria, but who  
247 subsequently demonstrated slowing in the rate of rise in CA125. This delayed SD group  
248 behaved very similarly to the GCIG defined SD group, likely representing a cytostatic effect  
249 of ET in line with the study by Hall et al [16]. Whilst acknowledging the exploratory nature  
250 of this observation, it may suggest that CA125 stabilisation from ET can be delayed and that  
251 stopping therapy as soon as it doubles may deprive some patients of potential benefit. It also  
252 generates the hypothesis that using response as a measure of ET efficacy is less representative  
253 than that of disease stabilisation.

254 When we accounted for the delayed SD group as part of the GCIG defined SD group, the  
255 CA125 CBR in the ER 251-300 group was significantly higher than those with ER 0-150. In  
256 this study, the differences in both duration of therapy and CA125 CBR only become apparent

257 in those with ER>200, although a gradient of response is likely to exist with increasing levels  
258 of ER.

259 These findings are largely concordant with the results of previous studies conducted in  
260 patients who were unselected according to histology. Bowman et al was an open label phase  
261 II study of letrozole in 60 patients with relapsed EOC who were unselected for ER [11]. 72%  
262 had serous histology (grade unspecified). The overall CA125 ORR was 8% and CBR was  
263 32%. ER and PR expression levels were retrospectively analysed and patients with ER  
264 histoscore  $\geq 150$  and PR histoscore  $\geq 70$  were found to have a 64% disease stabilisation rate  
265 compared to 3% in those with ER histoscore  $< 150$  [11]. This prompted the study by Smyth et  
266 al which only included patients with an ER histoscore  $\geq 150$  [12]. 52% of patients in this  
267 study had serous histology. The CA125 ORR doubled to 17% with a corresponding increase  
268 in CBR of 43%. When restricted to patients with an ER 250-300, the CA125 response rate  
269 once again doubled to 33%. Notably, the radiological objective response rate increased from  
270 0% to 16% and disease stabilisation rate from 16% to 42% in this trial by Smyth et al as  
271 compared to that in Bowman et al.

272 A more recent phase II umbrella study evaluated anastrozole in ER positive and/or PR  
273 positive ( $>10\%$  nuclear staining) platinum resistant or refractory ovarian cancer [18]. The  
274 majority of patients in this study had HGSOE though the exact proportion was unspecified. It  
275 found that patients with an ER histoscore of 200-300 had a longer median progression free  
276 survival compared to those with histoscores  $< 200$ . Although the difference was not  
277 statistically significant due to the small numbers of patients analysed, these findings are in  
278 line with those presented here from our centre.

279 The data presented here are particularly pertinent as not all studies have concurred with the  
280 association between degree of ER expression and endocrine responsiveness in ovarian cancer

281 [17, 18, 20, 21]. It has also highlighted the use of the ER histoscore (range 0-300), a weighted  
282 score which accounts for percentage tumour cells stained and stain intensity, as an important  
283 method of determining ER positivity. The majority of aromatase inhibitor trials used a  
284 minimum threshold of more than 1% nuclear staining in order to confirm ER positivity. It is  
285 possible that the greater granularity provided by the histoscore at high levels of ER is  
286 required to discriminate patients who are most likely to benefit from ET.

287 A few studies have attempted to establish the relationship between platinum sensitivity and  
288 endocrine sensitivity however no significant correlation has been demonstrated [4, 8, 17]. A  
289 meta-analysis of over fifty trials of ET found a lower CBR in those with platinum resistant  
290 disease compared to platinum sensitive disease although the result of was not significant[4].

291 In our study, the platinum sensitivity of tumours at the time of ET initiation was unable to be  
292 determined. However, we found that endocrine sensitivity increased with longer treatment  
293 free intervals prior to ET initiation. The differences in duration of ET and CA125 CBR only  
294 became apparent in those with a TFI $\geq$  180 days when compared to TFI<90 days, a time frame  
295 which mirror the definitions used when describing platinum sensitivity. Furthermore, the  
296 majority of patients received chemotherapy for platinum sensitive disease before embarking  
297 on ET for their subsequent relapse.

298 Although line of therapy was not an independent predictor in our study, the close association  
299 described between line of therapy and TFI in the literature[22] may suggest that patients with  
300 HGSOC are most likely to benefit from early introduction of ET for relapsed disease (i.e.  
301 when patients are more likely to have the longest TFI). This is supported by several studies of  
302 ET in patients with mixed histology [4, 23, 24]. Notably, an analysis of several tamoxifen  
303 trials compared those which had more than 50% of patients receiving only one prior line of

304 treatment to those with heavily pre-treated patients. The ORR in the less-treated group was  
305 25.8% compared to 4.1% in the heavily-treated group [24].

306 There is minimal data to demonstrate superiority of aromatase inhibitors over anti-oestrogens  
307 in ovarian cancer [17]. Although our study found no differences between letrozole and  
308 tamoxifen upon multivariable analysis, the majority of long term responders ( $\geq 365$  days) in  
309 our study received letrozole contributing to the growing pool of evidence supporting letrozole  
310 as a good choice of ET in this disease. This is in keeping with the superiority of letrozole  
311 over tamoxifen demonstrated in post-menopausal women with ER positive breast cancer, in  
312 both the adjuvant and metastatic settings [25, 26]

## 313 **5. CONCLUSION**

314 Our data provide evidence that ET has a role to play in the management of ER positive  
315 relapsed HGSOC and quantifies the extent of benefit in this type of ovarian cancer. It  
316 supports the use of ET as a means of delaying subsequent chemotherapy. Patients with an ER  
317 histoscore  $>200$  and a treatment free interval of 180 days or more are likely to derive the  
318 greatest benefit.

## 319 **ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

320 Communication with the Lothian Research Ethics Committee 2 determined that retrospective  
321 analysis of outcome using the contents of the Edinburgh Ovarian Cancer Database were  
322 deemed audit by their definition and formal ethical approval was not required.

## 323 **CONSENT FOR PUBLICATION**

324 Not applicable.

## 325 **AVAILABILITY OF DATA AND MATERIAL**



326 All patient data was extracted from the Edinburgh Ovarian Cancer Database. These were  
327 prospectively entered between January 1974 and December 2015 as part of routine care.

### 328 **CONFLICT OF INTEREST**

329 CG reports grants and personal fees from Astrazeneca, personal fees from Roche, personal  
330 fees from Clovis, grants and personal fees from Tesaro, personal fees from Roche, grants and  
331 personal fees from Nucana, grants from Aprea, grants from Novartis, personal fees from  
332 Foundation One, outside the submitted work. In addition, CG has a patent Molecular  
333 Diagnostic Test for Cancer issued. CD reports personal fees and non-financial support from  
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### 341 **AUTHORS' CONTRIBUTIONS**

- 342 • BS contributed to the design of the study, data collection, data interpretation, and  
343 drafting the manuscript.
- 344 • RLH contributed to the design of the study, data analysis and interpretation, and  
345 critical revision of the manuscript.
- 346 • HN contributed to the design of the study and data collection.
- 347 • JDT and XY contributed to the data collection.

- 348 • TR prospectively collected the data as part of the Edinburgh Ovarian Cancer  
349 Database.
- 350 • CD contributed to the data collection.
- 351 • MJM and FN contributed to the data collection and critical review of the manuscript.
- 352 • MC contributed to the data collection and the critical review of the manuscript.
- 353 • CSH contributed to the design of the study, data collection, data interpretation, and  
354 critical review of the manuscript.
- 355 • CG contributed to the design of the study, data interpretation, critical revision of the  
356 manuscript and overall supervision of this study.

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## 361 **SUPPLEMENTARY MATERIAL**

362 Supplementary information is available at the Journal of Gynaecologic Oncology website.

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446

## 447 **TABLE AND FIGURE LEGENDS**

448 *Table 1: Characteristics of patients treated with 1<sup>st</sup> ET.* Rx=treatment; ER=oestrogen  
449 receptor; ET=endocrine therapy; N=number; chemo=chemotherapy; NA=not applicable.

450 *Table 2: Predictive factors of duration of endocrine therapy: univariable and multivariable*  
451 *analysis (n=269).* N=numbers; DOT=duration of therapy; CI=confidence intervals;  
452 HR=hazard ratio; ER=oestrogen receptor; TFI=treatment free interval; ref=reference value;  
453 NE=non evaluable; Megace=megesterol acetate.

454 *Table 3: Predictive factors of CA125 response (n=172).* N=numbers; CR=complete response;  
455 PR=partial response; SD=stable disease; ORR=objective response rate; CBR1=clinical  
456 benefit rate 1 (CR+PR+SD); CBR2=clinical benefit rate 2 (CR+PR+SD + 2nd SD);  
457 ER=oestrogen receptor; TFI=treatment free interval; Megace=megesterol acetate.

458 *Figure 1. Characteristics of patients treated with endocrine therapy.* Rx=treatment;  
459 HGSOC= high grade serous ovarian carcinoma; G=grade.

460 *Figure 2. Duration of endocrine therapy and CA125 response rate based on ER histoscore*  
461 *and treatment free interval (TFI).* (A) Duration of therapy versus ER histoscore, (B) CA125  
462 response rate versus ER histoscore, (C) CA125 response versus duration of therapy, (D)  
463 CA125 response rate (including SD<sup>2</sup> patients as part of CBR) versus ER histoscore. (E)  
464 Duration of therapy versus TFI (F) CA125 response rate versus TFI. CR=complete response;

465 PR=partial response; SD=stable disease; SD<sup>2</sup>=delayed SD patients; CBR=clinical benefit rate  
466 (CR+PR+SD), PD=progressive disease.

467 *S1. Pattern of CA125 response in patients. (A) complete response (CR), (B) partial response*  
468 *(PR), (C) delayed stable disease (SD).*

469 *S2. Prior lines of chemotherapy versus duration of endocrine therapy.*

470 *S3. Type of endocrine therapy versus duration of endocrine therapy.*

471

472

**Title: Endocrine treatment of high grade serous ovarian carcinoma; quantification of efficacy and identification of response predictors.**

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1 **ABSTRACT**

2 *Objectives.* The role of endocrine therapy (ET) in high grade serous ovarian carcinoma  
3 (HGSOC) is poorly defined due to the lack of phase III data and significant heterogeneity of  
4 clinical trials performed. In this study, we sought to identify predictive factors of endocrine  
5 sensitivity in HGSOC.

6 *Methods.* HGSOC patients who received at least four weeks of ET for relapsed disease  
7 following one line of chemotherapy at the Edinburgh Cancer Centre were  
8 identified. Exclusion criteria were use of endocrine therapy as maintenance therapy or of  
9 unknown duration. Duration of therapy and best CA125 response as per modified GCIG  
10 criteria were recorded. Oestrogen receptor (ER) histoscore, treatment free interval, prior lines  
11 of chemotherapy, and type of ET were evaluated as predictive factors.

12 *Results.* Of 431 patients identified, 269 were eligible (77.0 % letrozole, 18.6% tamoxifen,  
13 2.2% megestrol acetate, 2.2% other). The median duration of therapy was 126 days (range  
14 28-1427 days). 32.7% remained on ET for  $\geq 180$  days and 14.1% for  $\geq 365$  days. The CA125  
15 response and clinical benefit rates (response or stable disease) were 8.1% and 40.1%  
16 respectively. ER histoscore  $>200$  ( $P=0.0016$ ) and a treatment free interval of  $\geq 180$  days  
17 ( $P<0.0001$ ) were independent predictive factors upon multivariable analysis.

18 *Conclusions.* ET should be considered as a viable strategy to defer subsequent chemotherapy  
19 for relapsed HGSOC. Patients with an ER histoscore  $>200$  and a treatment free interval of  
20  $\geq 180$  days are most likely to derive benefit.

21

22



23 **1. INTRODUCTION**

24 The majority of patients with advanced stage high grade serous ovarian carcinoma (HGSOC)  
25 will unfortunately relapse despite optimal cytoreductive surgery and platinum based  
26 chemotherapy. Symptomatic relapses are treated with further systemic chemotherapy which  
27 can be effective for some patients. However, with time, the intervals between each treatment  
28 get progressively shorter with reduced efficacy and cumulative toxicity.

29 Endocrine therapy (ET) in relapsed HGSOC is easy to administer, has a low toxicity profile  
30 and is low cost. There is good pre-clinical evidence to support the role of oestrogen in  
31 regulating the growth of oestrogen receptor (ER) positive EOC [1, 2]. To date, more than 50  
32 phase II trials of ET in EOC have been performed with response rates between 10-15% and  
33 disease stabilisation rates of 30-40% as described in systematic reviews and meta-analyses [3,  
34 4]. Only one phase III randomised trial of tamoxifen against standard chemotherapy in the  
35 platinum resistant setting has been performed which did not demonstrate differences in  
36 overall survival [5]. As such, ET is not considered a standard of care and its use is  
37 inconsistent and variable worldwide.

38 However, most of these trials were conducted in heavily pre-treated populations of mixed ER  
39 positive and ER negative patients [3]. In studies which pre-selected for ER status, different  
40 thresholds of ER positivity and methods of measurements were used [3]. In addition, these  
41 trials did not account for EOC comprising at least five histological subtypes which are  
42 biologically and clinically distinct [6].

43 The Ovarian Cancer Tissue Consortium Study found HGSOC, endometrioid and low grade  
44 serous ovarian carcinomas (LGSOC) to express the highest levels of ER ( $\geq 50\%$  tumour  
45 nuclear staining) of 60%, 60% and 71% respectively [7]. These histologies likely represent  
46 the most endocrine sensitive subtypes with emerging retrospective data to support this.

47 Gershenson et al demonstrated the role of ET both as treatment for relapsed disease [8] and as  
48 first line maintenance in LGSOC [9]. Patients with LGSOC who received first line  
49 maintenance ET had a superior progression free survival of 64.9 months compared to 26.4  
50 months in those who underwent observation ( $p<0.001$ ). Another retrospective study by  
51 Heinzelmann-Schwarz et al showed improvement in recurrence free survival in patients with  
52 HGSOC who received first line maintenance letrozole versus observation ( $p=0.035$ )[10].  
53 Together, these studies illustrate the importance of performing histological subtype-specific  
54 clinical trials to derive an accurate assessment of endocrine sensitivity.

55 Two sequential phase II studies (Bowman et al [11] and Smyth et al [12]) identified an  
56 endocrine sensitive group of ovarian cancer patients with mixed histology as those with an  
57 ER histoscore  $\geq 150$ . This weighted scoring method accounts for percentage(%) tumour cells  
58 stained and stain intensity (0 no staining, 1+ weak staining, 2+ moderate staining, 3+ strong  
59 staining). It derives a score between 0 to 300 using the formula:  $[1 \times (\% \text{ cells } 1+) + 2 \times (\% \text{ cells } 2+) + 3 \times (\% \text{ cells } 3+)]$  [13]. On the basis of these data, ET has been routinely used in  
61 our centre in patients with relapsed EOC with an ER histoscore of  $\geq 150$ . We sought to  
62 characterise the endocrine sensitivity of relapsed HGSOC as well as identify predictive  
63 factors in a large retrospective study.

## 64 **2. METHODS**

### 65 *2.1 Patient Identification*

66 We identified patients with a historical diagnosis of grade 2 or 3 serous carcinomas [14] who  
67 received at least one line of ET from the Edinburgh Ovarian Cancer Database between  
68 January 1974 and December 2015. This database contains detailed histopathological and  
69 clinical details of patients entered prospectively as part of routine care. Communication with  
70 the Lothian Research Ethics Committee determined that retrospective analysis of outcome

71 using the contents of the database were deemed audit by their definition and formal ethical  
72 approval was therefore not required.

### 73 *2.2 Inclusion and Exclusion Criteria*

74 Patients were included if they received at least four weeks of ET as treatment for relapsed  
75 disease as determined by the treating physician, following at least one line of previous  
76 chemotherapy, and with known duration of therapy. Those who received less than four weeks  
77 of ET were deemed to have had inadequate exposure to determine sensitivity. Patients who  
78 received ET as maintenance therapy were excluded.

### 79 *2.3 Recorded data*

80 All baseline and treatment demographics had been prospectively collected through the  
81 Edinburgh Ovarian Cancer Database as part of routine care. All available patient electronic  
82 and paper health records were also reviewed. Treatment characteristics were recorded for the  
83 first ET received. These included: duration of therapy, lines of ET, type of ET, prior lines of  
84 chemotherapy, and ER histoscore as recorded by the pathologist at diagnosis. The treatment  
85 free interval (TFI) was calculated from the last dose of chemotherapy to the date of ET  
86 initiation. The setting in which the last chemotherapy was received was recorded as 'platinum  
87 sensitive' or 'platinum resistant '. Patients who stopped ET due to toxicity or who were still  
88 on therapy at data cut-off were censored.

### 89 *2.4 Treatment efficacy*

90 Most clinicians used CA125 as a marker of response and did not perform radiological  
91 assessments of patients on ET until there was evidence of a significant rise in the CA125 or  
92 the patient developed symptoms. Therefore, in this non-trial setting, radiological PFS by  
93 RECIST could not be accurately defined. ET was continued until there was evidence of

94 symptomatic disease progression warranting further chemotherapy, or until death from  
95 ovarian cancer. In view of this, duration of therapy was recorded as an objective end-point  
96 for this study and surrogate measure of endocrine sensitivity. The best CA125 response  
97 across the duration of therapy was also recorded. Due to the variable frequency of CA125  
98 measurements, a modified GCIG criteria was adopted [15]. Patients were evaluable for  
99 CA125 response if they had an evaluable CA125 (>70U/ml) within four weeks of starting  
100 therapy, and at least three CA125 measures if they received ET for more than 12 weeks. At  
101 least two CA125 measures were required if ET was received for 12 weeks or less with the  
102 second CA125 within 4 weeks of cessation of ET. Patients were not evaluable for CA125  
103 response if they only had one CA125 measure, and if they received ET for 12 weeks or less  
104 with no CA125 progression. The 12 week threshold was adopted as the median time to  
105 CA125 response has been shown to be 12 weeks [11]. Patients treated for less than 12 weeks  
106 with clear CA125 progression were considered evaluable.

107 Definitions for CA125 complete response (CR), partial response (PR) and stable disease (SD)  
108 were as per GCIG criteria [15]. SD had to be maintained for at least 12 weeks from the start  
109 of therapy. Progressive disease (PD) was defined as doubling of CA125 from the baseline  
110 value. The CA125 overall response rate (ORR=CR+PR) and clinical benefit rate 1  
111 (CBR1=CR+PR+SD) were calculated and recorded.

112 A study by Hall et al showed that the change in rate of rise in CA125 can indicate activity of  
113 cytostatic agents such as tamoxifen [16]. In view of this, the characteristics of patients who  
114 had CA125 progression by GCIG criteria, followed by <50% rise of their CA125 for at least  
115 12 weeks were also explored (delayed SD).

116

117

118 *2.5 Statistical analysis*

119 Duration of therapy according to ER histoscore, TFI, prior lines of chemotherapy, best  
120 CA125 response and type of ET was evaluated using the Kaplan-Meier method and Cox  
121 regression models for univariable and multivariable analyses. Comparisons of CA125 ORR  
122 and CBR1 between groups were assessed using Chi-squared and Fisher's exact tests as  
123 appropriate. Statistical analyses were performed using R version 3.3.3.

124 **3. RESULTS**

125 | 431 patients received at least one line of ET. 162 patients were excluded (Figure 1). 269  
126 received ET as treatment for relapsed disease. 143(53.2%) were confirmed as HGSOE  
127 through contemporary pathology review conducted through other research studies, and  
128 118(43.9%) and 8(3.0%) had a historical diagnosis of grade 3 and grade 2 serous carcinomas,  
129 respectively. The median age of diagnosis was 65 years (range 28-91 years).

130 *3.1 First endocrine therapy for relapse*

131 Of 269 patients, 209 (77.7%), 55 (20.4%) and five (1.9%) patients received one, two and  
132 three lines of ET, respectively. 207 (77.0%), 50 (18.6%) and six (2.2%) patients received  
133 letrozole, tamoxifen and megestrol acetate, respectively. 156 (58.0%) patients received ET  
134 after one prior line of chemotherapy, 87 (32.3%) after two lines, and 26 (9.7%) after three or  
135 more lines. 229 (85.1%) and 36 (13.4%) patients last received chemotherapy in the platinum  
136 sensitive and platinum resistant setting, respectively. ER histoscores were available in 225  
137 (83.6%) patients. The range of ER scores are illustrated in table 1. The majority of these  
138 histoscores (192, 85.3%) were from the primary chemotherapy naïve tumour.

139 *3.2 Overall CA125 response rate and duration of therapy*

140 Of 269 patients, 257 (95.5%) stopped ET due to disease progression, 11 (4.1%) were still on ET at  
141 the time of analysis, and one (0.4%) stopped ET due to toxicity. 172 (63.9%) patients were evaluable

142 for CA125 response. The median number of CA125s was three (range 2-8) and six (range 3-44) in  
143 those who received 12 weeks or less of ET, and more than 12 weeks of ET, respectively. The CA125  
144 response rate was 8.1% (GCIG CR 2.9%, PR 5.2%) and the CBR was 40.1%. The pattern of CA125  
145 responses for CR and PR are shown in [supplemental figure S1A and S1B](#) ~~Figure 2A and 2B~~,  
146 respectively. The overall median duration of therapy was 126 days (range 28-1427 days).

### 147 3.3 Delayed SD patients

148 16 patients demonstrated delayed stabilisation of their CA125 ([supplemental figure S1](#) ~~Figure~~  
149 ~~2C~~). The median time to first CA125 progression was 42 days (21-114 days). The delayed  
150 SD group (patients whose CA125 rose then stabilised according to the criteria outlined  
151 above) had a significantly longer median duration of therapy than those whose disease  
152 progressed on CA125 criteria without subsequent stabilisation (196 days versus 84 days,  
153  $P < 0.0001$ ). The median duration of therapy between the GCIG-defined SD group and  
154 delayed SD group were comparable ( $P = 0.288$ ) ([Figure 23C](#)). In view of this, a second CBR  
155 (CBR2) which included the delayed SD patients as part of the GCIG-defined SD cohort was  
156 calculated and compared for each variable as an exploratory analysis.

### 157 3.4 ER HistoScore

158 148 patients with known ER histoscores had evaluable CA125 responses. There was an  
159 increasing trend in CA125 response rate of 5.8%, 8.6%, 9.1% and 14.7% in ER0-150,  
160 ER151-200, ER201-250 and ER251-300 groups, respectively. Similarly, there was an  
161 increasing trend of CBR1 with ER. These differences were not significant (table 3, figure  
162 [23B](#)). When the delayed SD patients were accounted for as part of the GCIG defined SD  
163 cohort, the CBR in the ER251-300 group was significantly higher than the ER0-150 group  
164 (CBR2 64.7% versus 37.1%;  $P = 0.04$ ) (table 3, figure [23D](#)).

165 The median duration of therapy was significantly longer at 140 days and 161 days in the  
166 ER201-250 (multivariable: HR 0.62, 95% CI 0.42-0.91,  $P=0.016$ ) and ER251-300 groups  
167 (multivariable: HR 0.63, 95% CI 0.41-0.96,  $P=0.032$ ) when compared to 88.5 days in those  
168 with ER  $\leq 150$  (table 2, figure 23A). There were no significant differences in median duration  
169 of therapy between the ER151-200 and ER  $\leq 150$  groups.

### 170 3.5 Treatment free interval

171 Of 269 patients, 259 (96.2%) received chemotherapy as their last treatment. 8(3.0%) patients  
172 received maintenance therapy and 2(0.7%) patients received secondary debulking as their last  
173 treatment and were thus excluded from this analysis. Of 259 patients, 164 (63.3%) were  
174 evaluable for CA125 responses. Patients who had a TFI 180-365 days and TFI>365 days had  
175 a significantly higher CA125 CBR1 of 50.0% ( $P =0.01$ ) and 60.6% ( $P=0.002$ ) when  
176 compared to 21.0 % in those with TFI<90 days (table 3, figure 23F). There were no  
177 significant differences between TFI <90days and 90-179 days.

178 The median duration of therapy was significantly longer at 161 days and 209 days in those  
179 with TFI 180-365 days (multivariable: HR 0.32, 95% CI 0.21-0.48,  $P <0.0001$ ) and TFI>365  
180 days (multivariable: HR 0.28, 95% CI 0.17-0.45,  $P <0.0001$ ) compared to 84 days in those  
181 with TFI<90days (table 2, figure 23E). There were no significant differences in duration of  
182 therapy between those with TFI<90days and 90-179 days.

### 183 3.6 Prior lines of chemotherapy

184 There was no significant difference in CA125 ORR, CBR1 or CBR2 between patients  
185 treated with different numbers of prior chemotherapy lines (table 3). The median duration of  
186 therapy was significantly longer at 142 days and 111 days in those who received ET after one  
187 (univariable: HR 0.46, 95% CI 0.30-0.70,  $p<0.001$ ) and two (univariable: HR 0.61, 95% CI  
188 0.39-0.95,  $p=0.03$ ) lines of chemotherapy compared to 88.5 days after 3 lines or more (table

189 | 2, [supplemental figure S24](#)). However, these differences were not significant upon  
190 | multivariable analyses ( $P = 0.67$  and  $P = 0.406$ , respectively).

### 191 | 3.7 Type of ET

192 | There was no significant difference in CA125 ORR, CBR1 or CBR2 between those treated  
193 | with letrozole and tamoxifen (table 3). Compared to tamoxifen, patients who received  
194 | letrozole had a significantly longer median duration of therapy (126 versus 98 days,  
195 | univariable: HR=0.64, 95% CI 0.47-0.88,  $P=0.006$ ), but the difference was not significant  
196 | upon multivariable analysis ( $P=0.255$ ) (table 2, [supplemental figure S32](#)). The number of  
197 | patients who received megestrol acetate was too small for meaningful analysis.

### 198 | 3.8 Characteristics of patients deriving greatest benefit from ET

199 | 88 (32.7%) patients remained on ET for  $\geq 180$  days, and 38 (14.1%) for  $\geq 365$  days. Of the 38  
200 | patients, 29 (76.3%) received ET after one line of chemotherapy and median TFI was 286  
201 | days (range 42 – 4256 days). 34 patients had known ER histoscores, all of which were  $>200$   
202 | (25, 73.5% 201-250, 9, 26.5%  $>250$ ). 33 (86.8%) patients were treated with letrozole. 28  
203 | patients were evaluable for CA125 response, of which 9 (32.1%) achieved CA125 response  
204 | (4 GCIG CR, 5 PR) and 16 (57.1%) achieved SD. The remaining 3 patients demonstrated  
205 | delayed SD.

206 | The median duration of therapy in the 14 patients who achieved CR or PR was significantly  
207 | longer at 878 days (range 180-3981 days) compared to 241 days in the 49 patients who  
208 | achieved GCIG defined SD (HR = 0.30 [0.15-0.60]  $P < 0.001$ ) (figure [23C](#)).



209 **4. DISCUSSION**

210 The main strengths of this study is in its large size with known ER status in more than 80% of  
211 the cohort. This provided sufficient power to perform comprehensive multivariable analysis  
212 in order to identify independent predictors of endocrine sensitivity. Patient and treatment  
213 demographics were also all recorded prospectively on the Edinburgh Ovarian Cancer  
214 Database thus minimising information bias.

215 The main weaknesses were the lack of radiology response data and the use of surrogates in  
216 the form of CA125 responses and duration of therapy, thus limiting the interpretation of some  
217 of our results. As this study was not conducted within a trial setting, the CA125 time points  
218 were also heterogeneous which may have underestimated the response or stabilisation rates to  
219 ET. Whilst all physicians started ET as treatment for relapse, the timing of treatment  
220 initiation and cessation is likely to have been inconsistent. Most ER histoscores were also  
221 recorded at the time of diagnosis by different pathologists which may have introduced  
222 interobserver variation.

223 Although this study took place approximately over 25 years, more than half the samples were  
224 confirmed as HGSOE following contemporary pathology review. The remaining patients  
225 were almost ubiquitously diagnosed as grade 3 serous EOC which has shown to be largely  
226 concordant with HGSOE[14], with less than 3% of the analysis cohort comprising grade 2  
227 serous EOC. This provides confidence that this cohort was largely homogenous. To our  
228 knowledge, this is the biggest study performed that has attempted to quantify the efficacy of  
229 ET in relapsed HGSOE.

230 Most prospective and retrospective studies performed to date have been performed in mixed  
231 histological subtypes [11, 17, 18]. There is clear evidence that each EOC subtype is a discrete  
232 disease with unique molecular profiles, treatment responses and patient outcomes [19].

233 Recent retrospective studies have suggested a particular role for ET in the management of  
234 low grade serous ovarian cancer [8, 9]. As such, responses to ET in low grade serous ovarian  
235 cancer may have contributed to signals of efficacy in previous studies of mixed histological  
236 types and the exact sensitivity in HGSOV was unclear.

237 Our study illustrates that the degree of ER expression is proportional to endocrine sensitivity  
238 in HGSOV. Duration of therapy increases significantly in those with ER251-300 compared to  
239 those with ER0-150 with an increasing trend in the proportion of patients demonstrating a  
240 response or stabilisation of their CA125 with increasing ER histoscores. Our study also  
241 identified a third of patients who remained on ET for more than 6 months, and nearly 15% for  
242 more than a year. Interestingly, we found that those who sustained a complete or partial  
243 CA125 response remained on endocrine therapy for much longer than those who achieved  
244 CA125 stabilisation. Although these results are expected, it argues against indolent tumour  
245 biology as being solely responsible for these apparent long responders to ET.

246 We also describe a small group of patients who had PD according to GCIG criteria, but who  
247 subsequently demonstrated slowing in the rate of rise in CA125. This delayed SD group  
248 behaved very similarly to the GCIG defined SD group, likely representing a cytostatic effect  
249 of ET in line with the study by Hall et al [16]. Whilst acknowledging the exploratory nature  
250 of this observation, it may suggest that CA125 stabilisation from ET can be delayed and that  
251 stopping therapy as soon as it doubles may deprive some patients of potential benefit. It also  
252 generates the hypothesis that using response as a measure of ET efficacy is less representative  
253 than that of disease stabilisation.

254 When we accounted for the delayed SD group as part of the GCIG defined SD group, the  
255 CA125 CBR in the ER 251-300 group was significantly higher than those with ER 0-150. In  
256 this study, the differences in both duration of therapy and CA125 CBR only become apparent

257 in those with ER>200, although a gradient of response is likely to exist with increasing levels  
258 of ER.

259 These findings are largely concordant with the results of previous studies conducted in  
260 patients who were unselected according to histology. Bowman et al was an open label phase  
261 II study of letrozole in 60 patients with relapsed EOC who were unselected for ER [11]. 72%  
262 had serous histology (grade unspecified). The overall CA125 ORR was 8% and CBR was  
263 32%. ER and PR expression levels were retrospectively analysed and patients with ER  
264 histoscore  $\geq 150$  and PR histoscore  $\geq 70$  were found to have a 64% disease stabilisation rate  
265 compared to 3% in those with ER histoscore  $< 150$  [11]. This prompted the study by Smyth et  
266 al which only included patients with an ER histoscore  $\geq 150$  [12]. 52% of patients in this  
267 study had serous histology. The CA125 ORR doubled to 17% with a corresponding increase  
268 in CBR of 43%. When restricted to patients with an ER 250-300, the CA125 response rate  
269 once again doubled to 33%. Notably, the radiological objective response rate increased from  
270 0% to 16% and disease stabilisation rate from 16% to 42% in this trial by Smyth et al as  
271 compared to that in Bowman et al.

272 A more recent phase II umbrella study evaluated anastrozole in ER positive and/or PR  
273 positive ( $>10\%$  nuclear staining) platinum resistant or refractory ovarian cancer [18]. The  
274 majority of patients in this study had HGSOE though the exact proportion was unspecified. It  
275 found that patients with an ER histoscore of 200-300 had a longer median progression free  
276 survival compared to those with histoscores  $< 200$ . Although the difference was not  
277 statistically significant due to the small numbers of patients analysed, these findings are in  
278 line with those presented here from our centre.

279 The data presented here are particularly pertinent as not all studies have concurred with the  
280 association between degree of ER expression and endocrine responsiveness in ovarian cancer

281 [17, 18, 20, 21]. It has also highlighted the use of the ER histoscore (range 0-300), a weighted  
282 score which accounts for percentage tumour cells stained and stain intensity, as an important  
283 method of determining ER positivity. The majority of aromatase inhibitor trials used a  
284 minimum threshold of more than 1% nuclear staining in order to confirm ER positivity. It is  
285 possible that the greater granularity provided by the histoscore at high levels of ER is  
286 required to discriminate patients who are most likely to benefit from ET.

287 A few studies have attempted to establish the relationship between platinum sensitivity and  
288 endocrine sensitivity however no significant correlation has been demonstrated [4, 8, 17]. A  
289 meta-analysis of over fifty trials of ET found a lower CBR in those with platinum resistant  
290 disease compared to platinum sensitive disease although the result of was not significant[4].

291 In our study, the platinum sensitivity of tumours at the time of ET initiation was unable to be  
292 determined. However, we found that endocrine sensitivity increased with longer treatment  
293 free intervals prior to ET initiation. The differences in duration of ET and CA125 CBR only  
294 became apparent in those with a TFI $\geq$  180 days when compared to TFI<90 days, a time frame  
295 which mirror the definitions used when describing platinum sensitivity. Furthermore, the  
296 majority of patients received chemotherapy for platinum sensitive disease before embarking  
297 on ET for their subsequent relapse.

298 Although line of therapy was not an independent predictor in our study, the close association  
299 described between line of therapy and TFI in the literature[22] may suggest that patients with  
300 HGSOC are most likely to benefit from early introduction of ET for relapsed disease (i.e.  
301 when patients are more likely to have the longest TFI). This is supported by several studies of  
302 ET in patients with mixed histology [4, 23, 24]. Notably, an analysis of several tamoxifen  
303 trials compared those which had more than 50% of patients receiving only one prior line of

304 treatment to those with heavily pre-treated patients. The ORR in the less-treated group was  
305 25.8% compared to 4.1% in the heavily-treated group [24].

306 There is minimal data to demonstrate superiority of aromatase inhibitors over anti-oestrogens  
307 in ovarian cancer [17]. Although our study found no differences between letrozole and  
308 tamoxifen upon multivariable analysis, the majority of long term responders ( $\geq 365$  days) in  
309 our study received letrozole contributing to the growing pool of evidence supporting letrozole  
310 as a good choice of ET in this disease. This is in keeping with the superiority of letrozole  
311 over tamoxifen demonstrated in post-menopausal women with ER positive breast cancer, in  
312 both the adjuvant and metastatic settings [25, 26]

## 313 **5. CONCLUSION**

314 Our data provide evidence that ET has a role to play in the management of ER positive  
315 relapsed HGSOc and quantifies the extent of benefit in this type of ovarian cancer. It  
316 supports the use of ET as a means of delaying subsequent chemotherapy. Patients with an ER  
317 histoscore  $>200$  and a treatment free interval of 180 days or more are likely to derive the  
318 greatest benefit.

## 319 **ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

320 Communication with the Lothian Research Ethics Committee 2 determined that retrospective  
321 analysis of outcome using the contents of the Edinburgh Ovarian Cancer Database were  
322 deemed audit by their definition and formal ethical approval was not required.

## 323 **CONSENT FOR PUBLICATION**

324 Not applicable.

## 325 **AVAILABILITY OF DATA AND MATERIAL**

326 All patient data was extracted from the Edinburgh Ovarian Cancer Database. These were  
327 prospectively entered between January 1974 and December 2015 as part of routine care.

### 328 **CONFLICT OF INTEREST**

329 CG reports grants and personal fees from Astrazeneca, personal fees from Roche, personal  
330 fees from Clovis, grants and personal fees from Tesaro, personal fees from Roche, grants and  
331 personal fees from Nucana, grants from Aprea, grants from Novartis, personal fees from  
332 Foundation One, outside the submitted work. In addition, CG has a patent Molecular  
333 Diagnostic Test for Cancer issued. CD reports personal fees and non-financial support from  
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- 340 • RLH was supported by an MRC PhD Studentship.

### 341 **AUTHORS' CONTRIBUTIONS**

- 342 • BS contributed to the design of the study, data collection, data interpretation, and  
343 drafting the manuscript.
- 344 • RLH contributed to the design of the study, data analysis and interpretation, and  
345 critical revision of the manuscript.
- 346 • HN contributed to the design of the study and data collection.
- 347 • JDT and XY contributed to the data collection.

- 348 • TR prospectively collected the data as part of the Edinburgh Ovarian Cancer  
349 Database.
- 350 • CD contributed to the data collection.
- 351 • MJM and FN contributed to the data collection and critical review of the manuscript.
- 352 • MC contributed to the data collection and the critical review of the manuscript.
- 353 • CSH contributed to the design of the study, data collection, data interpretation, and  
354 critical review of the manuscript.
- 355 • CG contributed to the design of the study, data interpretation, critical revision of the  
356 manuscript and overall supervision of this study.

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### 361 **SUPPLEMENTARY MATERIAL**

362 Supplementary information is available at the Journal of Gynaecologic Oncology website.

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#### 447 **TABLE AND FIGURE LEGENDS**

448 *Table 1: Characteristics of patients treated with 1<sup>st</sup> ET.* Rx=treatment; ER=oestrogen  
449 receptor; ET=endocrine therapy; N=number; chemo=chemotherapy; NA=not applicable.

450 *Table 2: Predictive factors of duration of endocrine therapy: univariable and multivariable*  
451 *analysis (n=269).* N=numbers; DOT=duration of therapy; CI=confidence intervals;  
452 HR=hazard ratio; ER=oestrogen receptor; TFI=treatment free interval; ref=reference value;  
453 NE=non evaluable; Megace=megesterol acetate.

454 *Table 3: Predictive factors of CA125 response (n=172).* N=numbers; CR=complete response;  
455 PR=partial response; SD=stable disease; ORR=objective response rate; CBR1=clinical  
456 benefit rate 1 (CR+PR+SD); CBR2=clinical benefit rate 2 (CR+PR+SD + 2nd SD);  
457 ER=oestrogen receptor; TFI=treatment free interval; Megace=megesterol acetate.

458 *Figure 1. Characteristics of patients treated with endocrine therapy.* Rx=treatment;  
459 HGSOC= high grade serous ovarian carcinoma; G=grade.

460 ~~*Figure 2. Pattern of CA125 response in patients. (A) complete response (CR), (B) partial*~~  
461 ~~*response (PR), (C) delayed stable disease (SD).*~~

462 *Figure 23. Duration of endocrine therapy and CA125 response rate based on ER histoscore*  
463 *and treatment free interval (TFI). (A) Duration of therapy versus ER histoscore, (B) CA125*  
464 *response rate versus ER histoscore, (C) CA125 response versus duration of therapy, (D)*

465 CA125 response rate (including SD<sup>2</sup> patients as part of CBR) versus ER histoscore. (E)  
466 Duration of therapy versus TFI (F) CA125 response rate versus TFI. CR=complete response;  
467 PR=partial response; SD=stable disease; SD<sup>2</sup>=delayed SD patients; CBR=clinical benefit rate  
468 (CR+PR+SD), PD=progressive disease.

469 *S1* Figure 2. Pattern of CA125 response in patients. (A) complete response (CR), (B) partial  
470 *response (PR), (C) delayed stable disease (SD).*

471

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472 *S2*. *Prior lines of chemotherapy versus duration of endocrine therapy.*

473 *S3*. *Type of endocrine therapy versus duration of endocrine therapy.*

474

475

4. Table 1 revised

[Click here to download 4. Table: GYN-18-1005R1\\_Table1\\_1st\\_ET\\_patient\\_characteristics\\_revised.xlsx](#)

Table 1: Characteristics of patients treated with 1 <sup>st</sup> ET	
Indication	Treatment for relapse (N=269) N (%)
No. of ET received	
<b>1</b>	209(77.7)
<b>2</b>	55(20.4)
<b>3</b>	5(1.9)
ER Histoscore	
<b>0-150</b>	50(18.6)
<b>151-200</b>	55(20.4)
<b>201-250</b>	69(25.7)
<b>251-300</b>	51(19.0)
<b>Unknown</b>	44(16.3)
Source of ER	
<b>Primary tumour</b>	192(85.3)
<b>Interval debulking or relapse disease</b>	27(12.0)
<b>Unknown</b>	6(2.7)
Type of ET	
<b>Letrozole</b>	207(77.0)
<b>Tamoxifen</b>	50(18.6)
<b>Megesterol Acetate</b>	6(2.2)
<b>NE<sup>a</sup></b>	6(2.2)
Prior lines of chemo	
<b>1</b>	156(58.0)
<b>2</b>	87(32.3)
<b>3+</b>	26(9.7)
Last regime received	
<b>Platinum sensitive</b>	229 (85.1)
<b>Platinum resistant</b>	36(13.4)
<b>Other</b>	4(1.5)

a-received 2 ET sequentially due to toxicity.  
Legend: Rx=treatment; ER=oestrogen receptor;  
ET=endocrine therapy; N=number;  
chemo=chemotherapy; NA=not applicable.

#### 4. Table 2

[Click here to download 4. Table: Table2\\_predictive\\_factors\\_ETduration.xlsx](#)

Table 2: Predictive factors of duration of endocrine therapy: univariate and multivariable analysis (n=269)									
ER	N	%	median DOT	univariate			multivariate		
			days	HR	95% CI	P	HR	95% CI	P
<b>≤150</b>	50	18.6	88.5	ref	ref	ref	ref	ref	ref
<b>151-200</b>	55	20.4	126	0.7	0.47 - 1.03	0.071	0.76	0.50-1.16	0.201
<b>201-250</b>	69	25.7	140	0.59	0.4 - 0.86	0.006	0.62	0.42-0.91	0.016
<b>251-300</b>	51	19.0	161	0.57	0.38 - 0.84	0.005	0.63	0.41-0.96	0.032
<b>UK</b>	44	16.4							
TFI/days									
<b>&lt;90</b>	63	23.4	84	ref	ref	ref	ref	ref	ref
<b>90-179</b>	66	24.5	93.5	0.87	0.61 - 1.24	0.436	0.79	0.52-1.22	0.292
<b>180-365</b>	82	30.5	161	0.35	0.24 - 0.50	<0.0001	0.32	0.21-0.48	<.0001
<b>&gt;365</b>	48	17.9	209	0.34	0.23 - 0.51	<0.0001	0.28	0.17-0.45	<.0001
<b>NE</b>	10	3.7							
Therapy									
<b>Letrozole</b>	207	77.0	126	0.64	0.47 - 0.88	0.006	0.8	0.54-1.18	0.255
<b>Megace</b>	6	2.2	317	0.45	0.19 - 1.06	0.068	0.17	0.17-1.99	0.391
<b>Tamoxifen</b>	50	18.6	98	ref	ref	ref	ref	ref	ref
<b>NE<sup>a</sup></b>	6	2.2							
Prior lines of chemotherapy									
<b>1</b>	156	58.0	142	0.46	0.30 - 0.70	<0.001	0.89	0.52-1.53	0.670
<b>2</b>	87	32.3	111	0.61	0.39 - 0.95	0.030	0.79	0.46-1.37	0.406
<b>3+</b>	26	9.7	88.5	ref	ref	ref	ref	ref	ref
a- patients received 2 ET sequentially due toxicity.									
Legend: N=numbers;DOT=duration of therapy; CI=confidence intervals; HR=hazard ratio;									
ER=oestrogen receptor; TFI=treatment free interval; ref=reference value; NE=non evaluable									
Megace=megesterol acetate.									

#### 4. Table 3

[Click here to download 4. Table: Table3\\_predictive\\_factors\\_CA125response.xlsx](#)

Table 3: Predictive factors of CA125 response (n=172)											
	N	CR	PR	SD	2 <sup>nd</sup> SD	ORR	p <sup>c</sup>	CBR1 <sup>a</sup>	p <sup>d</sup>	CBR2 <sup>b</sup>	p <sup>e</sup>
		N (%)	N (%)	N (%)		N (%)		N (%)			
<b>ER</b>											
<b>≤150</b>	35	1(2.9)	1(2.9)	10(28.6)	1(2.9)	2(5.7)	ref	12(34.4)	ref	13(37.1)	ref
<b>151-200</b>	35	1(2.9)	2(5.7)	11(31.4)	2(5.7)	3(8.6)	1.00	14(40.0)	0.805	16(45.7)	0.627
<b>201-250</b>	44	1(2.3)	3(6.8)	17(38.6)	4(9.1)	4(9.1)	0.688	21(47.7)	0.330	35(79.5)	0.131
<b>251-300</b>	34	2(5.9)	3(8.8)	11(32.4)	6(17.6)	5(14.7)	0.260	16(47.1)	0.404	22(64.7)	0.040
<b>UK</b>	27										
<b>TFI/days</b>											
<b>&lt;90</b>	38	0	1(2.6)	7(18.4)	5(13.2)	1(2.6)	ref	8(21.0)	ref	13(34.2)	ref
<b>90-179</b>	46	0	2(4.3)	11(23.9)	3(6.5)	2(4.3)	1.00	13(28.2)	0.613	16(34.7)	1
<b>180-365</b>	52	2(3.8)	4(7.7)	20(38.5)	6(11.5)	6(11.5)	0.231	26(50.0)	0.010	32(61.5)	0.019
<b>&gt;365</b>	28	2(7.1)	2(7.1)	13(46.4)	2(7.1)	4(14.2)	0.154	27(60.6)	0.002	19(67.7)	0.014
<b>NE</b>	11										
<b>Therapy</b>											
<b>Letrozole</b>	128	4(3.1)	6(4.7)	43(33.6)	12(9.4)	10(7.8)	0.510	53(41.4)	0.495	65(50.8)	0.437
<b>Megace</b>	4	0	0	2(50.0)	1(25.0)	0	1.00	2(50.0)	0.602	3(75.0)	0.310
<b>Tamoxifen</b>	36	1(2.8)	3(8.3)	8(22.2)	3(8.3)	4(11.1)	ref	12(33.3)	ref	15(41.6)	ref
<b>NE<sup>f</sup></b>	4										
<b>Prior lines of chemotherapy</b>											
<b>1</b>	97	4(4.1)	5(5.2)	29(30.0)	11(11.3)	9(9.3)	0.682	38(39.2)	0.838	49(50.5)	0.516
<b>2</b>	57	0	3(5.3)	22(38.6)	4(7.0)	3(5.3)	0.588	25(43.9)	0.606	29(50.9)	0.537
<b>3+</b>	18	1(5.6)	1(5.6)	4(22.2)	1(5.6)	2(11.1)	ref	6(33.3)	ref	7(39.0)	ref
a- Clinical benefit rate calculated using GCIG criteria											
b-Clinical benefit rate with delayed SD patients included in the SD cohort											
c-in relation to ORR											
d-in relation to CBR1											
e-in relation to CBR2											
f-received 2 ET sequentially due to toxicity											
Legend: N=numbers; CR=complete response; PR=partial response; SD=stable disease; ORR=objective response rate; CBR1=clinical benefit rate 1 (CR+PR+SD); CBR2=clinical benefit rate 2 (CR+PR+SD + 2nd SD); ER=oestrogen receptor; TFI=treatment free interval;Megace=megesterol acetate.											

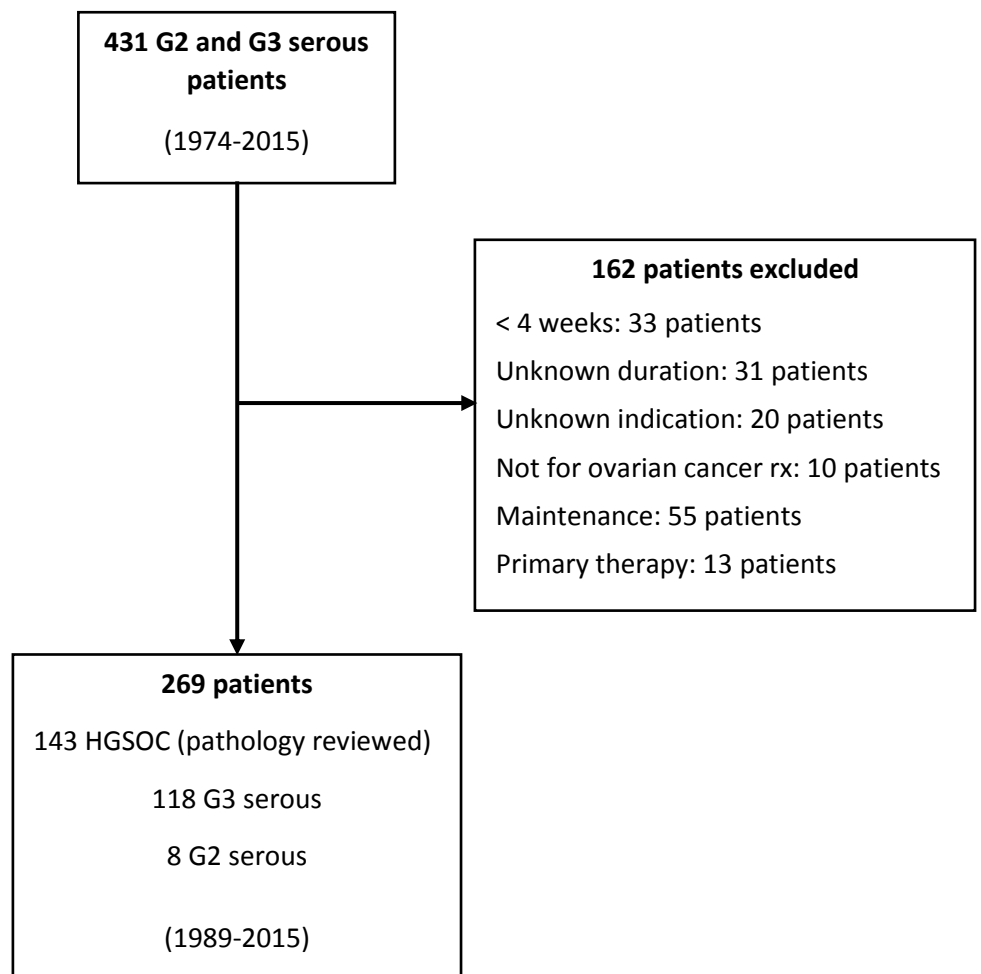


Figure 1: Characteristics of patients treated with endocrine therapy.

Rx=treatment; HGSOc= high grade serous ovarian carcinoma; G=grade.

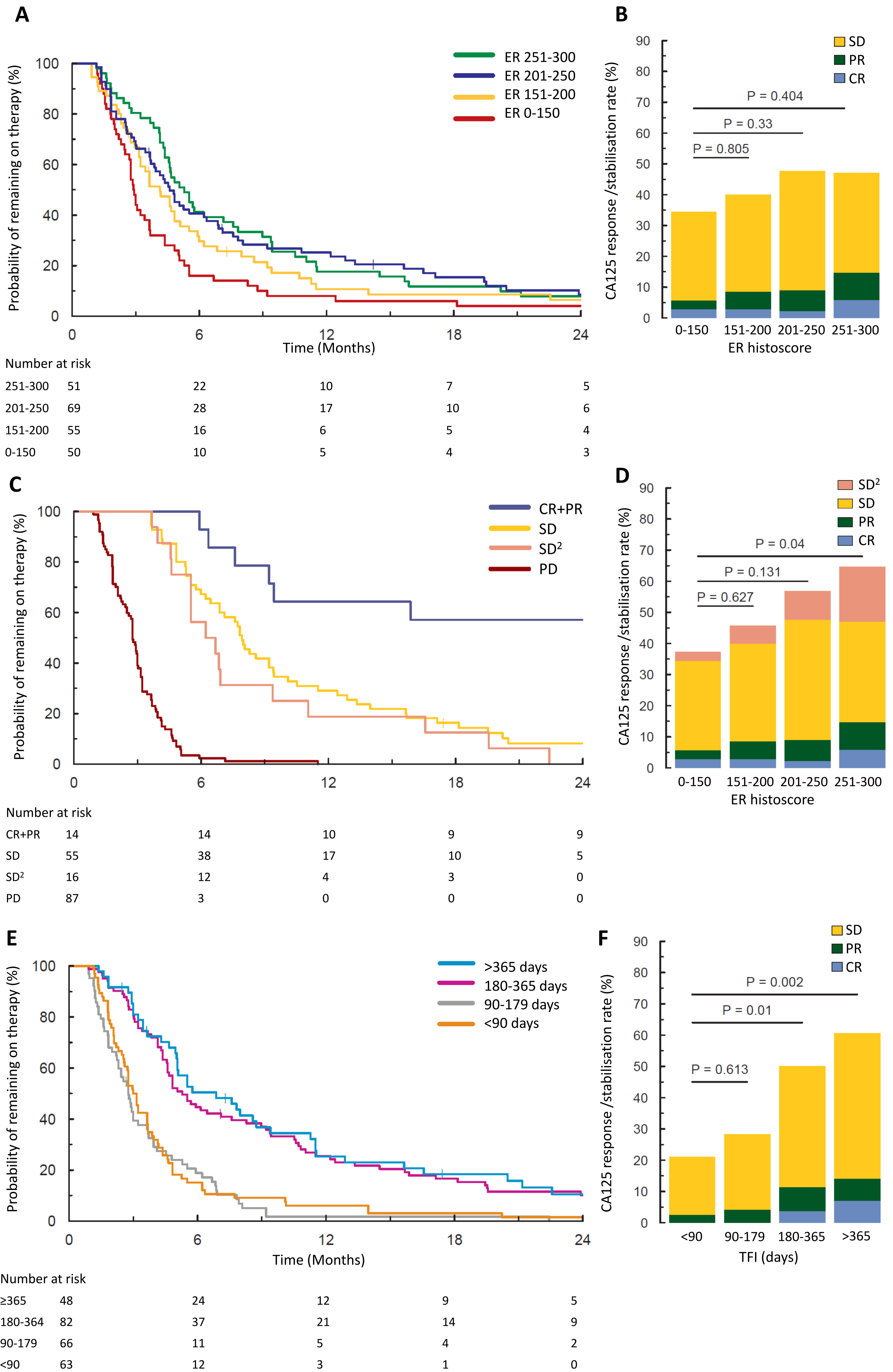
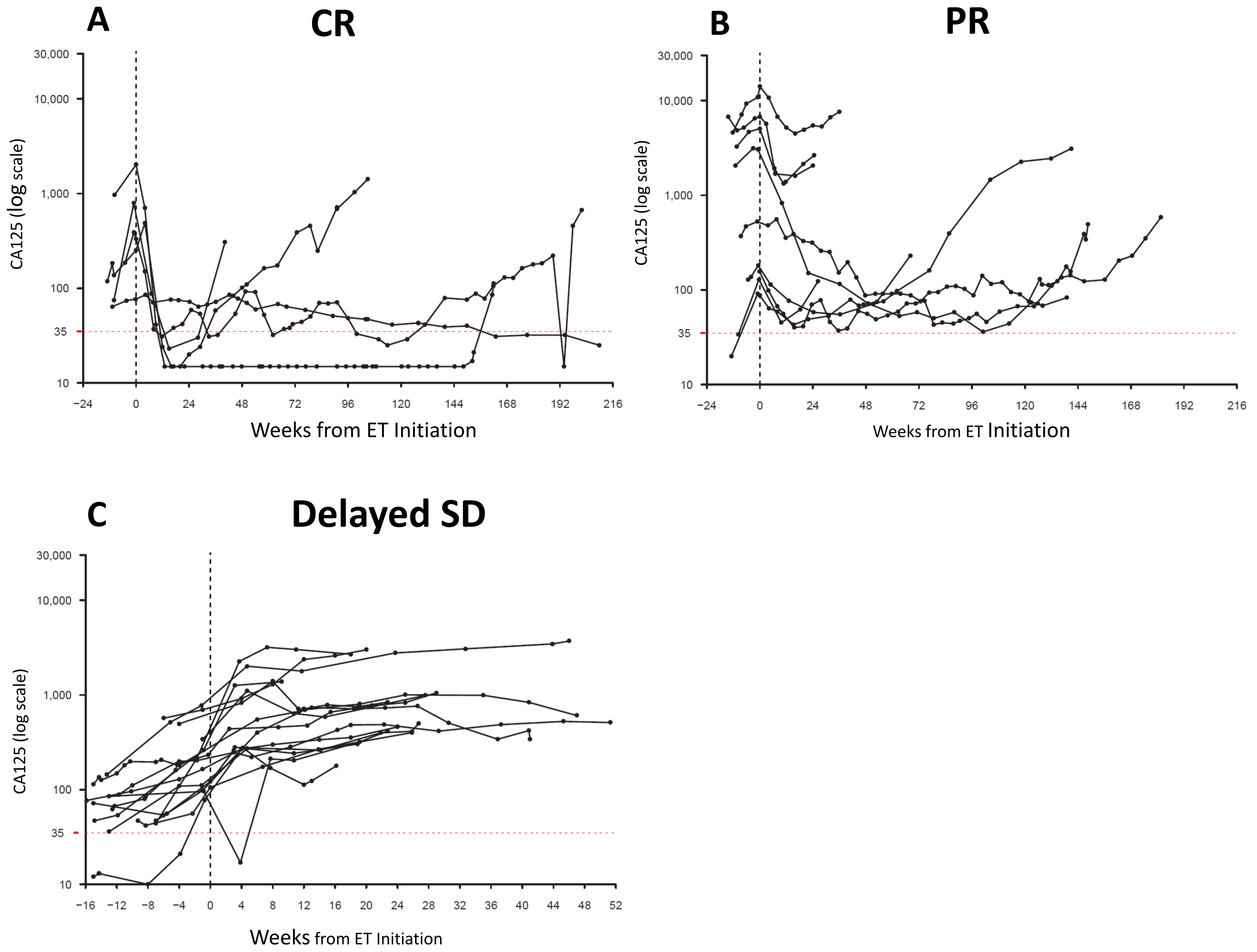
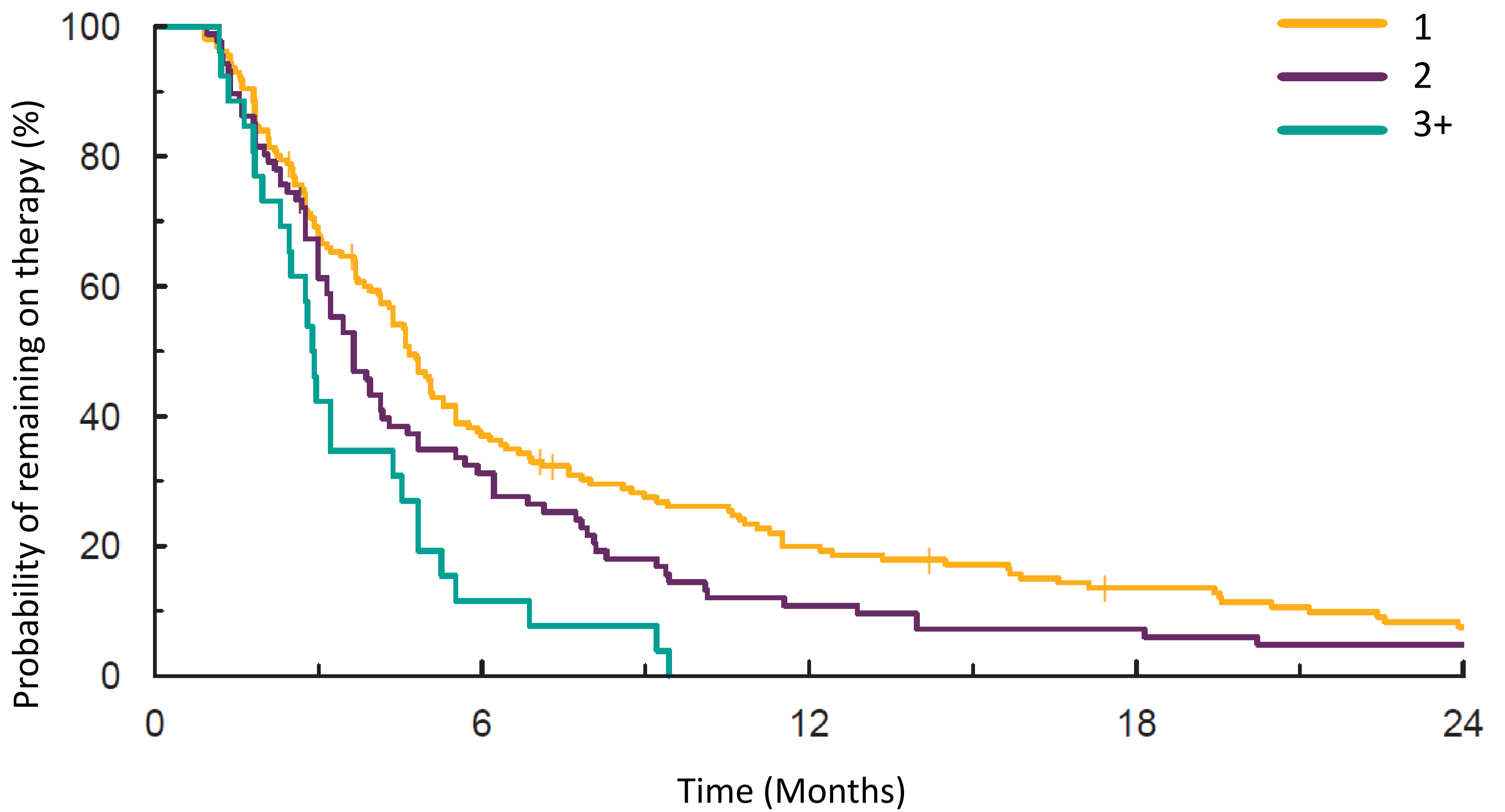


Figure 2. Duration of endocrine therapy and CA125 response rate based on ER histoscore and treatment free interval (TFI) (A) Duration of therapy versus ER histoscore, (B) CA125 response rate versus ER histoscore, (C) CA125 response versus duration of therapy, (D) CA125 response rate (including SD<sup>2</sup> patients as part of CBR) versus ER histoscore. (E) Duration of therapy versus TFI (F) CA125 response rate versus TFI. CR=complete response; PR=partial response; SD=stable disease; SD<sup>2</sup>=delayed SD patients; CBR=clinical benefit rate (CR+PR+SD), PD=progressive disease.





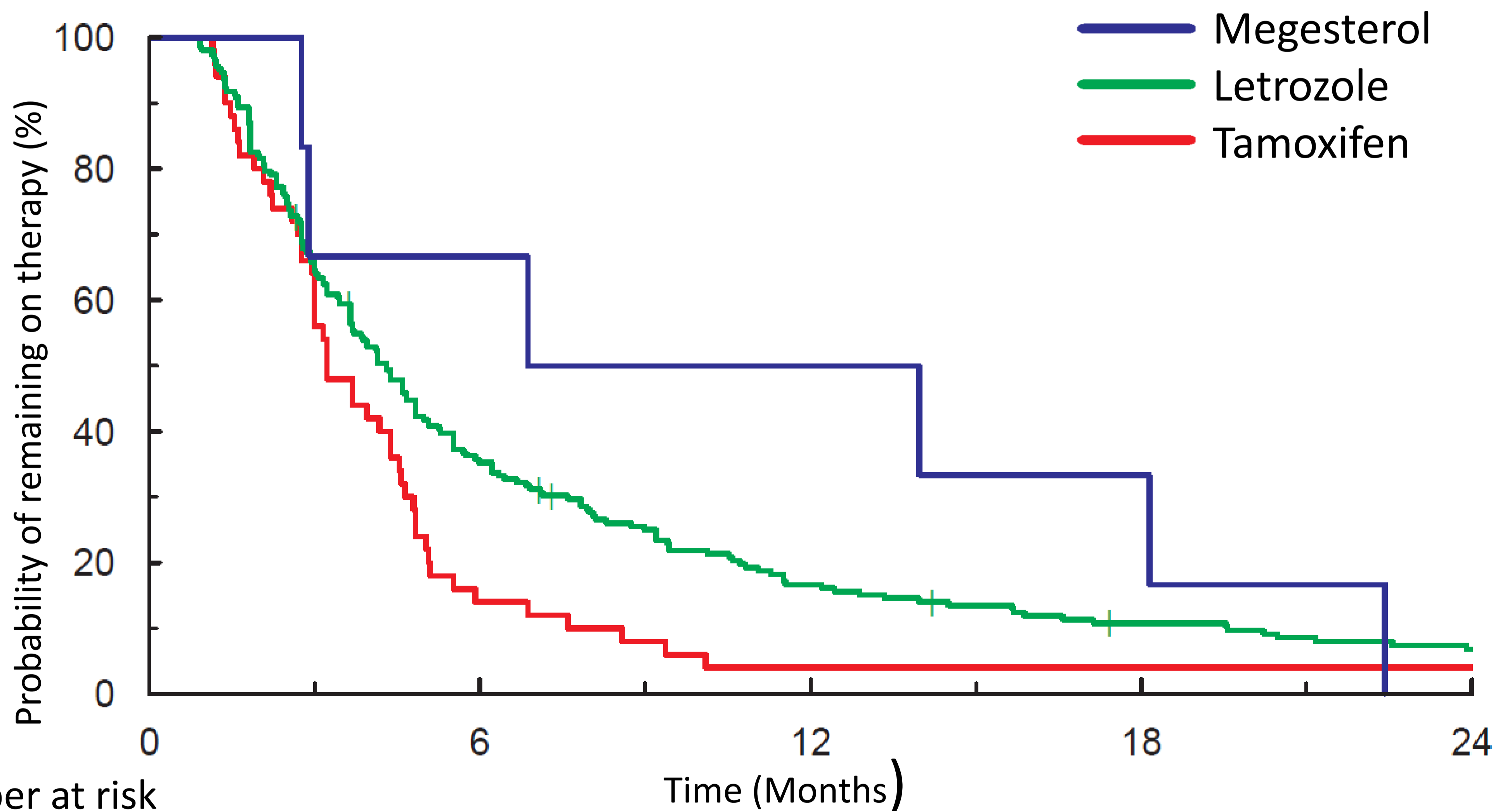
S1. Pattern of CA125 response in patients. (A) complete response (CR), (B) partial response (PR), (C) delayed stable disease (SD).



Number at risk

1	156	57	32	20	11
2	87	27	10	8	5
3+	26	4	0	0	0

S2. Prior lines of chemotherapy versus duration of endocrine therapy.



Number at risk

Megesterol	6	5	4	3	0
Letrozole	207	71	33	21	13
Tamoxifen	50	8	3	3	3

S3. Type of endocrine therapy versus duration of endocrine therapy.

**Highlights:**

- Endocrine therapy has efficacy in relapsed high grade serous ovarian cancer.
- It can be used to delay subsequent chemotherapy.
- Those with ER H-score  $> 200$  and treatment free interval  $> 180$  days are most likely to benefit.