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Endocrine treatment of high grade serous ovarian carcinoma; quantification of efficacy and identification of response predictors

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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% megesterol 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.

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1. INTRODUCTION

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

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

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

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

3

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 ≥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 x (% cells 1+) + 2 x (%

60   cells 2+) + 3 x (% 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 ≥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).

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2.5 Statistical analysis

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

3. RESULTS

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

3.1 First endocrine therapy for relapse

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

3.2 Overall CA125 response rate and duration of therapy

Of 269 patients, 257 (95.5%) stopped ET due to disease progression, 11 (4.1%) were still on ET at 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 ≤150 (table 2, figure 2A). There were no significant differences in median duration

169   of therapy between the ER151-200 and ER ≤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 megesterol 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 ≥180 days, and 38 (14.1%) for ≥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).

4. DISCUSSION

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

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

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

Most prospective and retrospective studies performed to date have been performed in mixed histological subtypes [11, 17, 18]. There is clear evidence that each EOC subtype is a discrete 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 HGSOC was unclear.

237 Our study illustrates that the degree of ER expression is proportional to endocrine sensitivity

238 in HGSOC. 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 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 ≥150 and PR histoscore ≥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 ≥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 anastrazole 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 HGSOC 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 (≥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**

337 **FUNDING**

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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TABLE AND FIGURE LEGENDS

Table 1: Characteristics of patients treated with 1st ET. Rx=treatment; ER=oestrogen receptor; ET=endocrine therapy; N=number; chemo=chemotherapy; NA=not applicable.

Table 2: Predictive factors of duration of endocrine therapy: univariable and multivariable analysis (n=269). 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.

Table 3: Predictive factors of CA125 response (n=172). 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^2 patients as part of CBR) versus ER histoscore. (E) Duration of therapy versus TFI (F) CA125 response rate versus TFI. CR=complete response;

465   PR=partial response; SD=stable disease; SD^2=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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ABSTRACT

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

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

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

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

1. INTRODUCTION

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

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

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

The Ovarian Cancer Tissue Consortium Study found HGSOC, endometrioid and low grade serous ovarian carcinomas (LGSOC) to express the highest levels of ER (≥50% tumour nuclear staining) of 60%, 60% and 71% respectively [7]. These histologies likely represent 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 ≥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 x (% cells 1+) + 2 x (%

60 cells 2+) + 3 x (% 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 ≥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

4

71 using the contents of the database were deemed audit by their definition and formal ethical

72 approval was therefore not required.

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.

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.

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.

3. RESULTS

124

125 431 patients received at least one line of ET. 162 patients were excluded (fFigure 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 megesterol 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

7

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 S1BFigure 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 S1Figure

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) (fFigure 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 ≤150 (table 2, figure 23A). There were no significant differences in median duration

169   of therapy between the ER151-200 and ER ≤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 S21). However, these differences were not significant upon

190 multivariable analyses (*P* = 0.67 and *P* = 0.406, respectively).

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 megesterol acetate was too small for meaningful analysis.

3.8 Characteristics of patients deriving greatest benefit from ET

199 88 (32.7%) patients remained on ET for ≥180 days, and 38 (14.1%) for ≥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).

4. DISCUSSION

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

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

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

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

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

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

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

When we accounted for the delayed SD group as part of the GCIG defined SD group, the CA125 CBR in the ER 251-300 group was significantly higher than those with ER 0-150. In 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 ≥150 and PR histoscore ≥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 ≥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 anastrazole 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 HGSOC 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 (≥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.

357 **ACKNOWLEDGEMENTS**

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 1ˢᵗ 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 2̲3̶. 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^2 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^2=delayed SD patients; CBR=clinical benefit rate

468 (CR+PR+SD), PD=progressive disease.

469 *S1Figure 2. Pattern of CA125 response in patients.* (A) complete response (CR), (B) partial

470 response (PR), (C) delayed stable disease (SD).

471

472 *S21. Prior lines of chemotherapy versus duration of endocrine therapy.*

473 *S32. Type of endocrine therapy versus duration of endocrine therapy.*

474

475

4. Table 1 revised

Table 1: Characteristics of patients treated with 1st ET

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

Table 2: Predictive factors of duration of endocrine therapy: univariate and multivariable analysis (n=269)

	N	%	median DOT days	univariate HR	univariate 95% CI	univariate P	multivariate HR	multivariate 95% CI	multivariate P
ER									
≤150	50	18.6	88.5	ref	ref	ref	ref	ref	ref
151-200	55	20.4	126	0.7	0.47 - 1.03	0.071	0.76	0.50-1.16	0.201
201-250	69	25.7	140	0.59	0.4 - 0.86	0.006	0.62	0.42-0.91	0.016
251-300	51	19.0	161	0.57	0.38 - 0.84	0.005	0.63	0.41-0.96	0.032
UK	44	16.4							
TFI/days									
<90	63	23.4	84	ref	ref	ref	ref	ref	ref
90-179	66	24.5	93.5	0.87	0.61 - 1.24	0.436	0.79	0.52-1.22	0.292
180-365	82	30.5	161	0.35	0.24 - 0.50	<0.0001	0.32	0.21-0.48	<.0001
>365	48	17.9	209	0.34	0.23 - 0.51	<0.0001	0.28	0.17-0.45	<.0001
NE	10	3.7							
Therapy									
Letrozole	207	77.0	126	0.64	0.47 - 0.88	0.006	0.8	0.54-1.18	0.255
Megace	6	2.2	317	0.45	0.19 - 1.06	0.068	0.17	0.17-1.99	0.391
Tamoxifen	50	18.6	98	ref	ref	ref	ref	ref	ref
NE[a]	6	2.2							
Prior lines of chemotherapy									
1	156	58.0	142	0.46	0.30 - 0.70	<0.001	0.89	0.52-1.53	0.670
2	87	32.3	111	0.61	0.39 - 0.95	0.030	0.79	0.46-1.37	0.406
3+	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	2nd SD	ORR	P^c	CBR1[a]	P^d	CBR2[b]	P^e
		N (%)	N (%)	N (%)		N (%)		N (%)			
ER											
≤150	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
151-200	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
201-250	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
251-300	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
UK	27										
TFI/days											
<90	38	0	1(2.6)	7(18.4)	5(13.2)	1(2.6)	ref	8(21.0)	ref	13(34.2)	ref
90-179	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
180-365	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
>365	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
NE	11										
Therapy											
Letrozole	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
Megace	4	0	0	2(50.0)	1(25.0)	0	1.00	2(50.0)	0.602	3(75.0)	0.310
Tamoxifen	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
NE[f]	4										
Prior lines of chemotherapy											
1	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
2	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
3+	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);

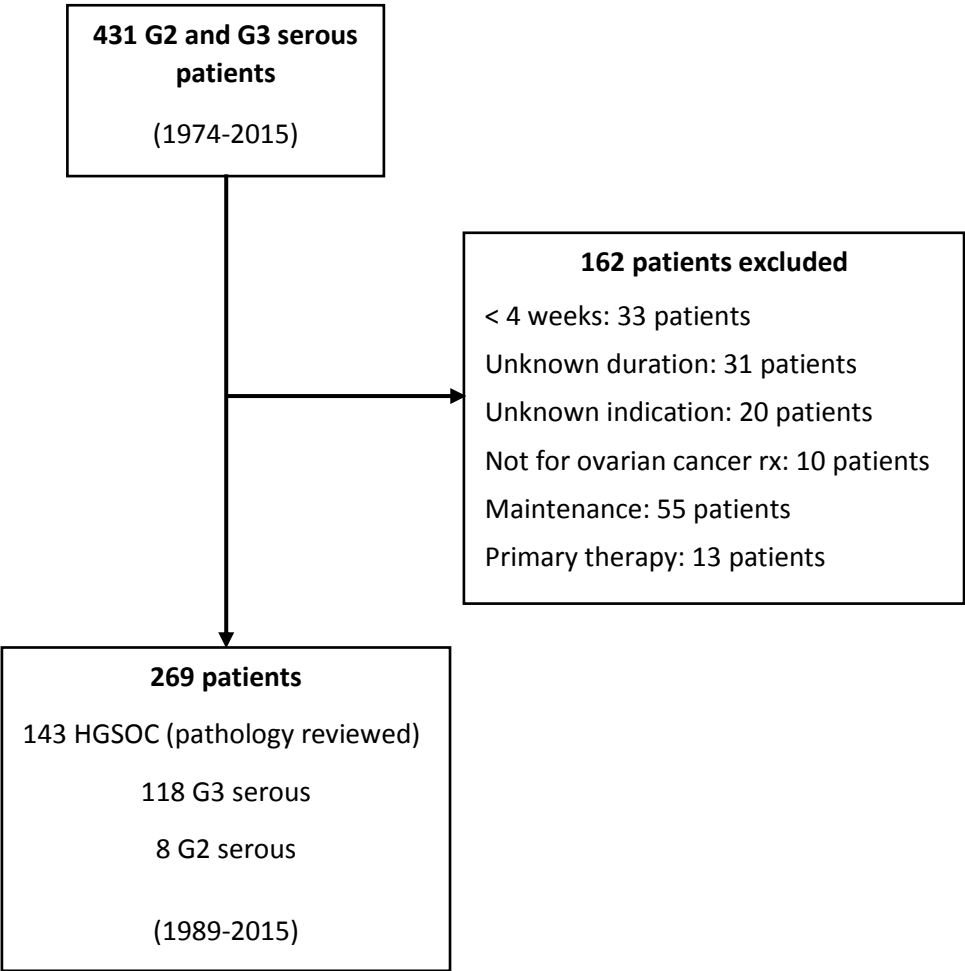ER=oestrogen receptor; TFI=treatment free interval;Megace=megesterol acetate.

Figure 1: Characteristics of patients treated with endocrine therapy.

Rx=treatment; HGSOC= high grade serous ovarian carcinoma; G=grade.
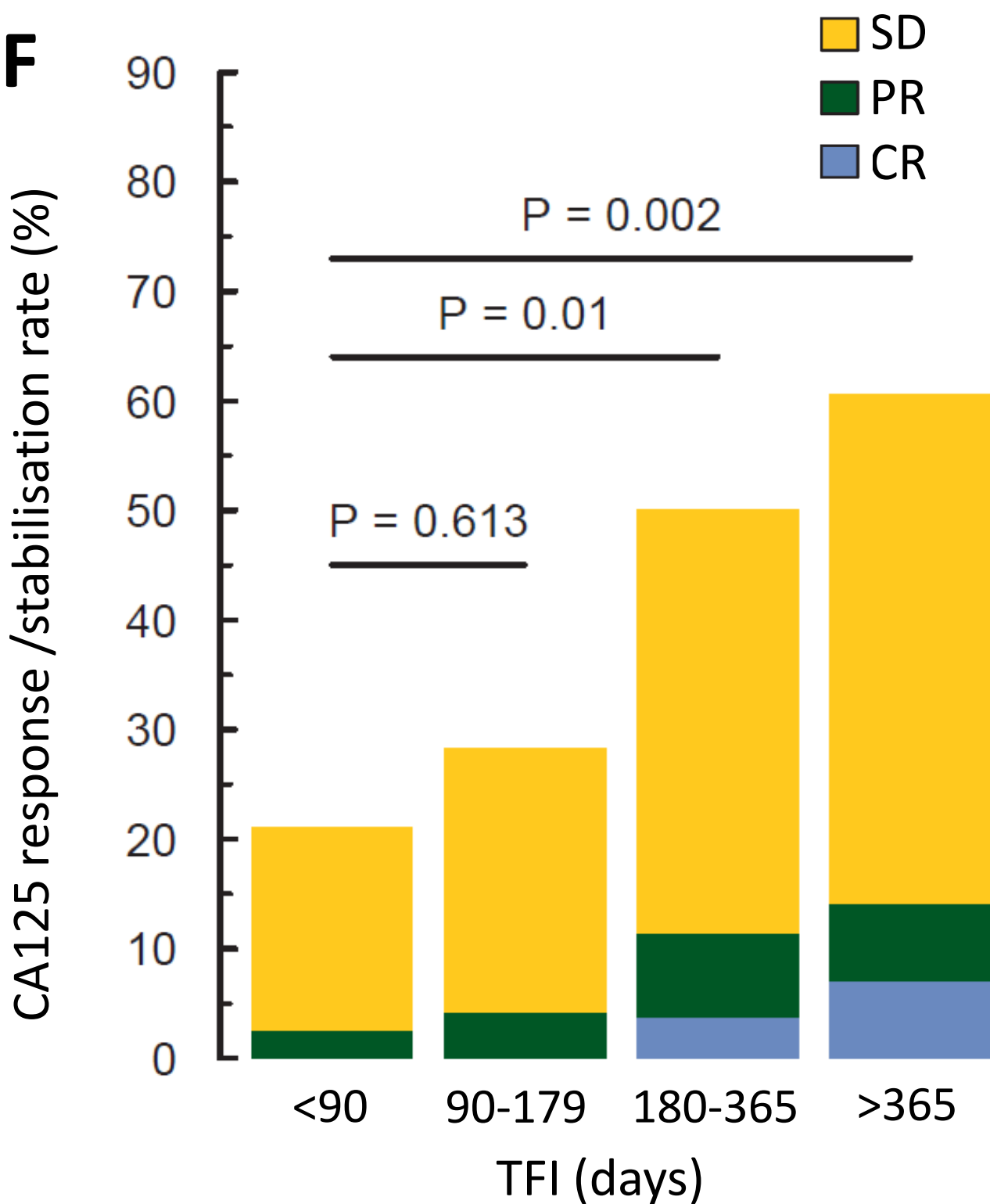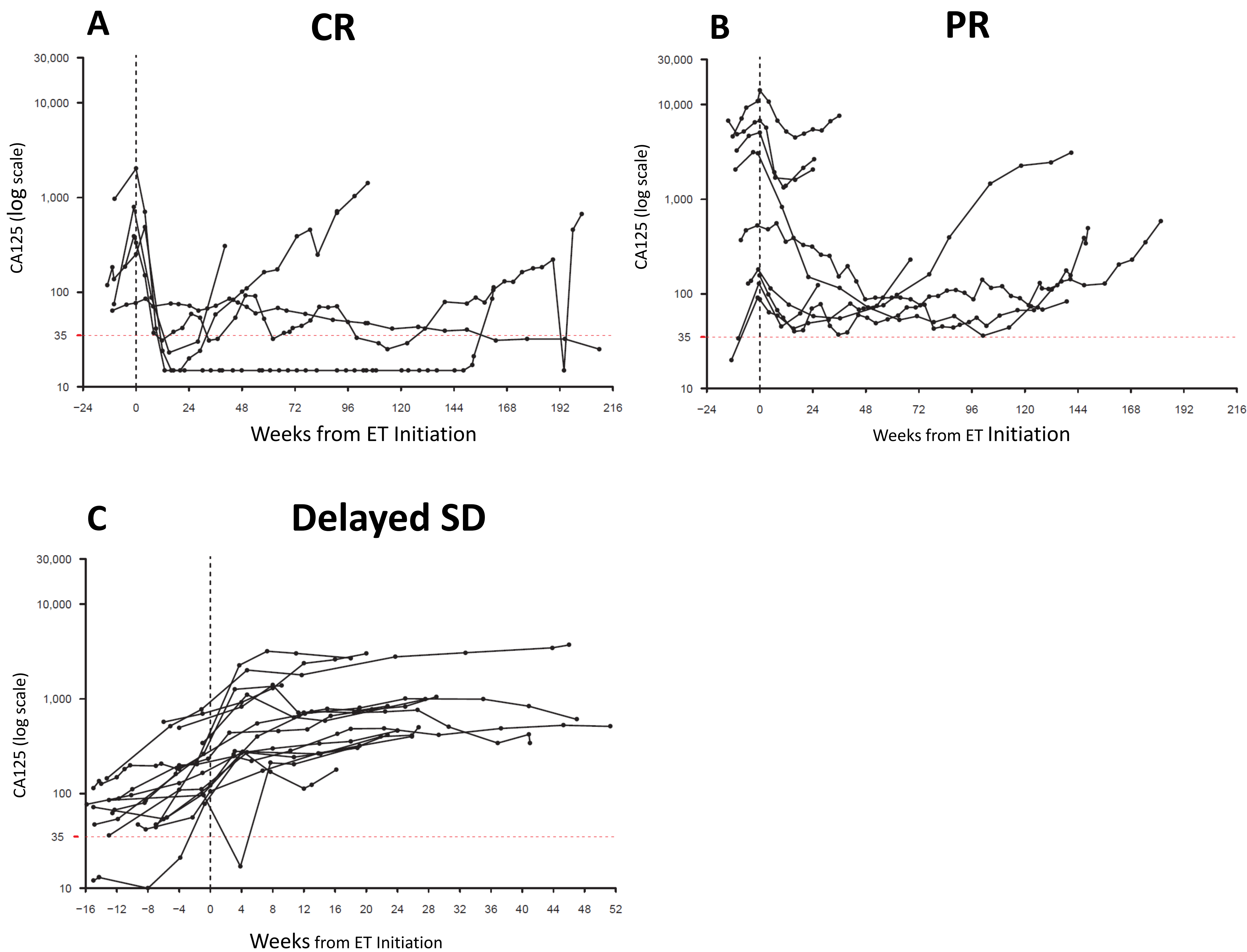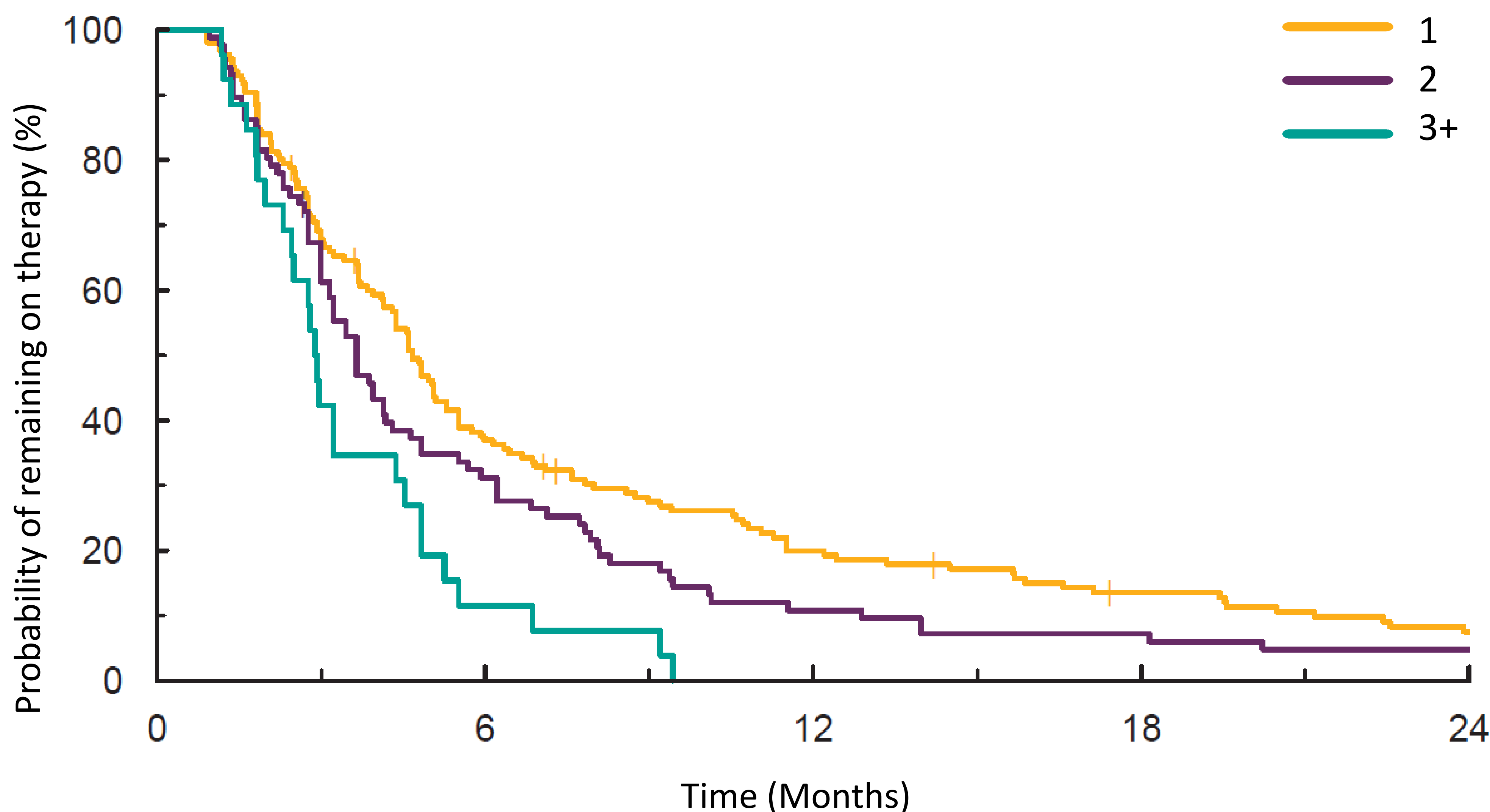
Figure 2. Duration of endocrine therapy and CA125 response rate based on ER histoscore and treatment free interval (TFI) (A) Duration of therapy versus ER histoscore, (B) CA125 response rate versus ER histoscore, (C) CA125 response versus duration of therapy, (D) CA125 response rate (including SD² patients 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; SD²=delayed SD patients; CBR=clinical benefit rate (CR+PR+SD), PD=progressive disease.
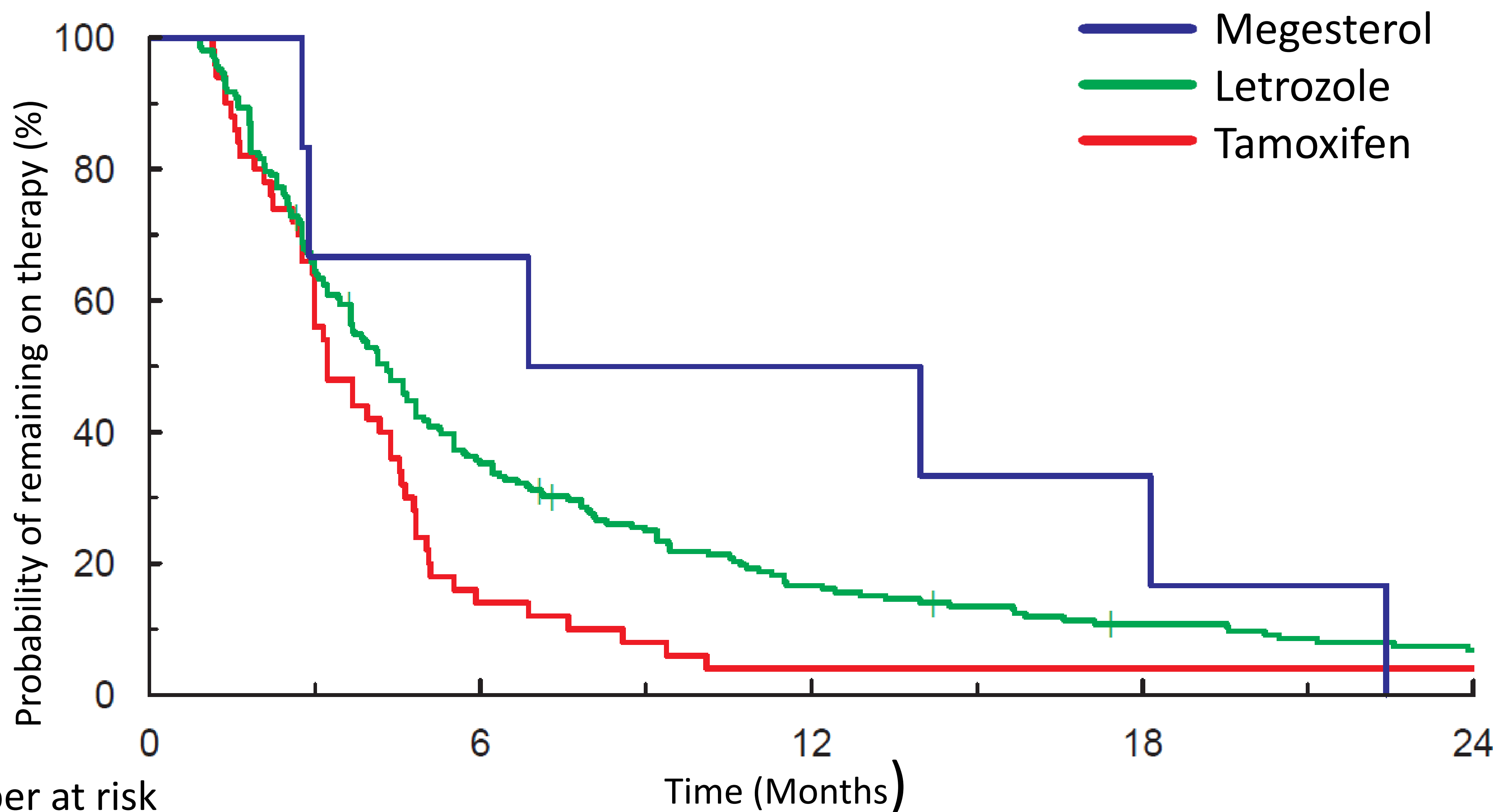
S1. Pattern of CA125 response in patients. (A) complete response (CR), (B) partial response (PR), (C) delayed stable disease (SD).

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

Number at risk

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

S3. Type of endocrine therapy 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

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.