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

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

Barbara Stanley[1, 2*], Robert L. Hollis[1*], Hugo Nunes[3], Jonathan D. Towler[2], Xiangfei Yan[2], Tzyvia Rye[1], Carol Dawson[1], Melanie J. Mackean[2], Fiona Nussey[2], Michael Churchman[1], C. Simon Herrington[1], Charlie Gourley[1, 2]

[1]Nicola Murray Centre for Ovarian Cancer Research, Cancer Research UK Edinburgh Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK

[2]Edinburgh Cancer Centre, Western General Hospital, Edinburgh, UK

[3]Instituto Português de Oncologia de Lisboa Francisco Gentil

*These authors contributed equally to this work.

Corresponding author:

Professor Charlie Gourley, Cancer Research UK Edinburgh Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Crewe Road South, Edinburgh, UK; telephone: 00441316518608; fax: 00441316518711; email: charlie.gourley@ed.ac.uk

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.

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

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

2. METHODS

2.1 Patient Identification

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

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

2.2 Inclusion and Exclusion Criteria

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

2.3 Recorded data

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

2.4 Treatment efficacy

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

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

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

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

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

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

3.3 Delayed SD patients

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

3.4 ER Histoscore

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

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

3.5 Treatment free interval

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

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

3.6 Prior lines of chemotherapy

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

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

3.7 Type of ET

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

3.8 Characteristics of patients deriving greatest benefit from ET

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

The median duration of therapy in the 14 patients who achieved CR or PR was significantly longer at 878 days (range 180-3981 days) compared to 241 days in the 49 patients who 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].

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

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

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

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

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

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

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

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

Although line of therapy was not an independent predictor in our study, the close association described between line of therapy and TFI in the literature[22] may suggest that patients with HGSOC are most likely to benefit from early introduction of ET for relapsed disease (i.e. when patients are more likely to have the longest TFI). This is supported by several studies of ET in patients with mixed histology [4, 23, 24]. Notably, an analysis of several tamoxifen 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

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

CONFLICT OF INTEREST

CG reports grants and personal fees from Astrazeneca, personal fees from Roche, personal fees from Clovis, grants and personal fees from Tesaro, personal fees from Roche, grants and personal fees from Nucana, grants from Aprea, grants from Novartis, personal fees from Foundation One, outside the submitted work. In addition, CG has a patent Molecular Diagnostic Test for Cancer issued. CD reports personal fees and non-financial support from the University of Edinburgh, grants from Cancer Research UK, grants from the Chief Scientist Office, grants and non-financial support from ECMC and non-financial support from NHS Lothian, during the conduct of the study.

FUNDING

- BS was supported by the Oncology Endowment Fund (University of Edinburgh) and Edinburgh Lothian Health Fund.
- RLH was supported by an MRC PhD Studentship.

AUTHORS' CONTRIBUTIONS

- BS contributed to the design of the study, data collection, data interpretation, and drafting the manuscript.
- RLH contributed to the design of the study, data analysis and interpretation, and critical revision of the manuscript.
- HN contributed to the design of the study and data collection.
- JDT and XY contributed to the data collection.

- TR prospectively collected the data as part of the Edinburgh Ovarian Cancer Database.
- CD contributed to the data collection.
- MJM and FN contributed to the data collection and critical review of the manuscript.
- MC contributed to the data collection and the critical review of the manuscript.
- CSH contributed to the design of the study, data collection, data interpretation, and critical review of the manuscript.
- CG contributed to the design of the study, data interpretation, critical revision of the manuscript and overall supervision of this study.

ACKNOWLEDGEMENTS

We would like to extend our thanks to the Edinburgh Ovarian Cancer Database, to all of the patients who contributed to this study, and to the Nicola Murray Foundation for their generous support of the Nicola Murray Centre for Ovarian Cancer Research.

SUPPLEMENTARY MATERIAL

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

REFERENCES

[1] Langdon SP, Hawkes MM, Lawrie SS, Hawkins RA, Tesdale AL, Crew AJ, et al. Oestrogen receptor expression and the effects of oestrogen and tamoxifen on the growth of human ovarian carcinoma cell lines. British journal of cancer. 1990;62:213-6.

[2] Nash JD, Ozols RF, Smyth JF, Hamilton TC. Estrogen and anti-estrogen effects on the growth of human epithelial ovarian cancer in vitro. Obstetrics and gynecology. 1989;73:1009-16.

[3] Langdon SP, Gourley C, Gabra H, Stanley B. Endocrine therapy in epithelial ovarian cancer. Expert review of anticancer therapy. 2017;17:109-17.

[4] Paleari L, Gandini S, Provinciali N, Puntoni M, Colombo N, DeCensi A. Clinical benefit and risk of death with endocrine therapy in ovarian cancer: A comprehensive review and meta-analysis. Gynecologic oncology. 2017;146:504-13.

[5] Lindemann K, Gibbs E, Avall-Lundqvist E, dePont Christensen R, Woie K, Kalling M, et al. Chemotherapy vs tamoxifen in platinum-resistant ovarian cancer: a phase III, randomised, multicentre trial (Ovaresist). British journal of cancer. 2017;116:455-63.

[6] Vaughan S, Coward JI, Bast RC, Jr., Berchuck A, Berek JS, Brenton JD, et al. Rethinking ovarian cancer: recommendations for improving outcomes. Nature reviews Cancer. 2011;11:719-25.

[7] Sieh W, Kobel M, Longacre TA, Bowtell DD, deFazio A, Goodman MT, et al. Hormone-receptor expression and ovarian cancer survival: an Ovarian Tumor Tissue Analysis consortium study. The Lancet Oncology. 2013;14:853-62.

[8] Gershenson DM, Sun CC, Iyer RB, Malpica AL, Kavanagh JJ, Bodurka DC, et al. Hormonal therapy for recurrent low-grade serous carcinoma of the ovary or peritoneum. Gynecologic oncology. 2012;125:661-6.

[9] Gershenson DM, Bodurka DC, Coleman RL, Lu KH, Malpica A, Sun CC. Hormonal Maintenance Therapy for Women With Low-Grade Serous Cancer of the Ovary or Peritoneum. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2017;35:1103-11.

[10] Heinzelmann-Schwarz V, Knipprath Meszaros A, Stadlmann S, Jacob F, Schoetzau A, Russell K, et al. Letrozole may be a valuable maintenance treatment in high-grade serous ovarian cancer patients. Gynecologic oncology. 2018;148:79-85.

[11] Bowman A, Gabra H, Langdon SP, Lessells A, Stewart M, Young A, et al. CA125 response is associated with estrogen receptor expression in a phase II trial of letrozole in ovarian cancer: identification of an endocrine-sensitive subgroup. Clinical cancer research : an official journal of the American Association for Cancer Research. 2002;8:2233-9.

[12] Smyth JF, Gourley C, Walker G, MacKean MJ, Stevenson A, Williams AR, et al. Antiestrogen therapy is active in selected ovarian cancer cases: the use of letrozole in estrogen receptor-positive patients. Clinical cancer research : an official journal of the American Association for Cancer Research. 2007;13:3617-22.

[13] Kirkegaard T, Edwards J, Tovey S, McGlynn LM, Krishna SN, Mukherjee R, et al. Observer variation in immunohistochemical analysis of protein expression, time for a change? Histopathology. 2006;48:787-94.

[14] Malpica A, Deavers MT, Lu K, Bodurka DC, Atkinson EN, Gershenson DM, et al. Grading ovarian serous carcinoma using a two-tier system. The American journal of surgical pathology. 2004;28:496-504.

[15] Rustin GJ, Vergote I, Eisenhauer E, Pujade-Lauraine E, Quinn M, Thigpen T, et al. Definitions for response and progression in ovarian cancer clinical trials incorporating RECIST 1.1 and CA 125 agreed

by the Gynecological Cancer Intergroup (GCIG). International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2011;21:419-23.

[16] Hall MR, Petruckevitch A, Pascoe J, Persic M, Tahir S, Morgan JS, et al. Using serum CA125 to assess the activity of potential cytostatic agents in ovarian cancer. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2014;24:676-81.

[17] George A, McLachlan J, Tunariu N, Della Pepa C, Migali C, Gore M, et al. The role of hormonal therapy in patients with relapsed high-grade ovarian carcinoma: a retrospective series of tamoxifen and letrozole. BMC cancer. 2017;17:456.

[18] Bonaventura A, O'Connell RL, Mapagu C, Beale PJ, McNally OM, Mileshkin LR, et al. Paragon (ANZGOG-0903): Phase 2 Study of Anastrozole in Women With Estrogen or Progesterone Receptor-Positive Platinum-Resistant or -Refractory Recurrent Ovarian Cancer. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2017;27:900-6.

[19] Kobel M, Kalloger SE, Boyd N, McKinney S, Mehl E, Palmer C, et al. Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. PLoS medicine. 2008;5:e232.

[20] Papadimitriou CA, Markaki S, Siapkaras J, Vlachos G, Efstathiou E, Grimani I, et al. Hormonal therapy with letrozole for relapsed epithelial ovarian cancer. Long-term results of a phase II study. Oncology. 2004;66:112-7.

[21] Stasenko M, Plegue M, Sciallis AP, McLean K. Clinical response to antiestrogen therapy in platinum-resistant ovarian cancer patients and the role of tumor estrogen receptor expression status. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2015;25:222-8.

[22] Eng KH, Hanlon BM, Bradley WH, Szender JB. Prognostic factors modifying the treatment-free interval in recurrent ovarian cancer. Gynecologic oncology. 2015;139:228-35.

[23] Markman M, Webster K, Zanotti K, Rohl J, Belinson J. Use of tamoxifen in asymptomatic patients with recurrent small-volume ovarian cancer. Gynecologic oncology. 2004;93:390-3.

[24] Perez-Gracia JL, Carrasco EM. Tamoxifen therapy for ovarian cancer in the adjuvant and advanced settings: systematic review of the literature and implications for future research. Gynecologic oncology. 2002;84:201-9.

[25] Thurlimann B, Keshaviah A, Coates AS, Mouridsen H, Mauriac L, Forbes JF, et al. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. The New England journal of medicine. 2005;353:2747-57.

[26] Mouridsen H, Gershanovich M, Sun Y, Perez-Carrion R, Boni C, Monnier A, et al. Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: analysis of survival and update of efficacy from the International Letrozole Breast Cancer Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21:2101-9.

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;

PR=partial response; SD=stable disease; SD^2=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.

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

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

Barbara Stanley[1, 2*], Robert L. Hollis[1*], Hugo Nunes[3], Jonathan D. Towler[2], Xiangfei Yan[2], Tzyvia Rye[1], Carol Dawson[1], Melanie J. Mackean[2], Fiona Nussey[2], Michael Churchman[1], C. Simon Herrington[1], Charlie Gourley[1, 2]

[1]Nicola Murray Centre for Ovarian Cancer Research, Cancer Research UK Edinburgh Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK

[2]Edinburgh Cancer Centre, Western General Hospital, Edinburgh, UK

[3]Instituto Português de Oncologia de Lisboa Francisco Gentil

*These authors contributed equally to this work.

Corresponding author:

Professor Charlie Gourley, Cancer Research UK Edinburgh Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Crewe Road South, Edinburgh, UK; telephone: 00441316518608; fax: 00441316518711; email: charlie.gourley@ed.ac.uk

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.

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

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

2. METHODS

2.1 Patient Identification

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

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

2.2 Inclusion and Exclusion Criteria

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

2.3 Recorded data

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

2.4 Treatment efficacy

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

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

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

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

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 (fFigure 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

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

3.3 Delayed SD patients

16 patients demonstrated delayed stabilisation of their CA125 (supplemental figure S1~~Figure 2~~C). The median time to first CA125 progression was 42 days (21-114 days). The delayed SD group (patients whose CA125 rose then stabilised according to the criteria outlined above) had a significantly longer median duration of therapy than those whose disease progressed on CA125 criteria without subsequent stabilisation (196 days versus 84 days, *P*<0.0001). The median duration of therapy between the GCIG-defined SD group and delayed SD group were comparable (P=0.288) (f~~F~~igure 2~~3~~C). In view of this, a second CBR (CBR2) which included the delayed SD patients as part of the GCIG-defined SD cohort was calculated and compared for each variable as an exploratory analysis.

3.4 ER Histoscore

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

The median duration of therapy was significantly longer at 140 days and 161 days in the ER201-250 (multivariable: HR 0.62, 95% CI 0.42-0.91, *P*=0.016) and ER251-300 groups (multivariable: HR 0.63, 95% CI 0.41-0.96, *P*=0.032) when compared to 88.5 days in those with ER ≤150 (table 2, figure 2~~3~~A). There were no significant differences in median duration of therapy between the ER151-200 and ER ≤150 groups.

3.5 Treatment free interval

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

The median duration of therapy was significantly longer at 161 days and 209 days in those with TFI 180-365 days (multivariable: HR 0.32, 95% CI 0.21-0.48, *P* <0.0001) and TFI>365 days (multivariable: HR 0.28, 95% CI 0.17-0.45, *P* <0.0001) compared to 84 days in those with TFI<90days (table 2, figure 2~~3~~E). There were no significant differences in duration of therapy between those with TFI<90days and 90-179 days.

3.6 Prior lines of chemotherapy

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

2, supplemental figure S2~~1~~). However, these differences were not significant upon multivariable analyses (P = 0.67 and P = 0.406, respectively).

3.7 Type of ET

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

3.8 Characteristics of patients deriving greatest benefit from ET

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

The median duration of therapy in the 14 patients who achieved CR or PR was significantly longer at 878 days (range 180-3981 days) compared to 241 days in the 49 patients who achieved GCIG defined SD (HR = 0.30 [0.15-0.60] P < 0.001) (figure 2~~3~~C).

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

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

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

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

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

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

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

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

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

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

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

5. CONCLUSION

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

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

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

CONFLICT OF INTEREST

CG reports grants and personal fees from Astrazeneca, personal fees from Roche, personal fees from Clovis, grants and personal fees from Tesaro, personal fees from Roche, grants and personal fees from Nucana, grants from Aprea, grants from Novartis, personal fees from Foundation One, outside the submitted work. In addition, CG has a patent Molecular Diagnostic Test for Cancer issued. CD reports personal fees and non-financial support from the University of Edinburgh, grants from Cancer Research UK, grants from the Chief Scientist Office, grants and non-financial support from ECMC and non-financial support from NHS Lothian, during the conduct of the study.

FUNDING

- BS was supported by the Oncology Endowment Fund (University of Edinburgh) and Edinburgh Lothian Health Fund.
- RLH was supported by an MRC PhD Studentship.

AUTHORS' CONTRIBUTIONS

- BS contributed to the design of the study, data collection, data interpretation, and drafting the manuscript.
- RLH contributed to the design of the study, data analysis and interpretation, and critical revision of the manuscript.
- HN contributed to the design of the study and data collection.
- JDT and XY contributed to the data collection.

- TR prospectively collected the data as part of the Edinburgh Ovarian Cancer Database.
- CD contributed to the data collection.
- MJM and FN contributed to the data collection and critical review of the manuscript.
- MC contributed to the data collection and the critical review of the manuscript.
- CSH contributed to the design of the study, data collection, data interpretation, and critical review of the manuscript.
- CG contributed to the design of the study, data interpretation, critical revision of the manuscript and overall supervision of this study.

ACKNOWLEDGEMENTS

We would like to extend our thanks to the Edinburgh Ovarian Cancer Database, to all of the patients who contributed to this study, and to the Nicola Murray Foundation for their generous support of the Nicola Murray Centre for Ovarian Cancer Research.

SUPPLEMENTARY MATERIAL

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

REFERENCES

[1] Langdon SP, Hawkes MM, Lawrie SS, Hawkins RA, Tesdale AL, Crew AJ, et al. Oestrogen receptor expression and the effects of oestrogen and tamoxifen on the growth of human ovarian carcinoma cell lines. British journal of cancer. 1990;62:213-6.

[2] Nash JD, Ozols RF, Smyth JF, Hamilton TC. Estrogen and anti-estrogen effects on the growth of human epithelial ovarian cancer in vitro. Obstetrics and gynecology. 1989;73:1009-16.

[3] Langdon SP, Gourley C, Gabra H, Stanley B. Endocrine therapy in epithelial ovarian cancer. Expert review of anticancer therapy. 2017;17:109-17.

[4] Paleari L, Gandini S, Provinciali N, Puntoni M, Colombo N, DeCensi A. Clinical benefit and risk of death with endocrine therapy in ovarian cancer: A comprehensive review and meta-analysis. Gynecologic oncology. 2017;146:504-13.

[5] Lindemann K, Gibbs E, Avall-Lundqvist E, dePont Christensen R, Woie K, Kalling M, et al. Chemotherapy vs tamoxifen in platinum-resistant ovarian cancer: a phase III, randomised, multicentre trial (Ovaresist). British journal of cancer. 2017;116:455-63.

[6] Vaughan S, Coward JI, Bast RC, Jr., Berchuck A, Berek JS, Brenton JD, et al. Rethinking ovarian cancer: recommendations for improving outcomes. Nature reviews Cancer. 2011;11:719-25.

[7] Sieh W, Kobel M, Longacre TA, Bowtell DD, deFazio A, Goodman MT, et al. Hormone-receptor expression and ovarian cancer survival: an Ovarian Tumor Tissue Analysis consortium study. The Lancet Oncology. 2013;14:853-62.

[8] Gershenson DM, Sun CC, Iyer RB, Malpica AL, Kavanagh JJ, Bodurka DC, et al. Hormonal therapy for recurrent low-grade serous carcinoma of the ovary or peritoneum. Gynecologic oncology. 2012;125:661-6.

[9] Gershenson DM, Bodurka DC, Coleman RL, Lu KH, Malpica A, Sun CC. Hormonal Maintenance Therapy for Women With Low-Grade Serous Cancer of the Ovary or Peritoneum. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2017;35:1103-11.

[10] Heinzelmann-Schwarz V, Knipprath Meszaros A, Stadlmann S, Jacob F, Schoetzau A, Russell K, et al. Letrozole may be a valuable maintenance treatment in high-grade serous ovarian cancer patients. Gynecologic oncology. 2018;148:79-85.

[11] Bowman A, Gabra H, Langdon SP, Lessells A, Stewart M, Young A, et al. CA125 response is associated with estrogen receptor expression in a phase II trial of letrozole in ovarian cancer: identification of an endocrine-sensitive subgroup. Clinical cancer research : an official journal of the American Association for Cancer Research. 2002;8:2233-9.

[12] Smyth JF, Gourley C, Walker G, MacKean MJ, Stevenson A, Williams AR, et al. Antiestrogen therapy is active in selected ovarian cancer cases: the use of letrozole in estrogen receptor-positive patients. Clinical cancer research : an official journal of the American Association for Cancer Research. 2007;13:3617-22.

[13] Kirkegaard T, Edwards J, Tovey S, McGlynn LM, Krishna SN, Mukherjee R, et al. Observer variation in immunohistochemical analysis of protein expression, time for a change? Histopathology. 2006;48:787-94.

[14] Malpica A, Deavers MT, Lu K, Bodurka DC, Atkinson EN, Gershenson DM, et al. Grading ovarian serous carcinoma using a two-tier system. The American journal of surgical pathology. 2004;28:496-504.

[15] Rustin GJ, Vergote I, Eisenhauer E, Pujade-Lauraine E, Quinn M, Thigpen T, et al. Definitions for response and progression in ovarian cancer clinical trials incorporating RECIST 1.1 and CA 125 agreed

by the Gynecological Cancer Intergroup (GCIG). International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2011;21:419-23.

[16] Hall MR, Petruckevitch A, Pascoe J, Persic M, Tahir S, Morgan JS, et al. Using serum CA125 to assess the activity of potential cytostatic agents in ovarian cancer. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2014;24:676-81.

[17] George A, McLachlan J, Tunariu N, Della Pepa C, Migali C, Gore M, et al. The role of hormonal therapy in patients with relapsed high-grade ovarian carcinoma: a retrospective series of tamoxifen and letrozole. BMC cancer. 2017;17:456.

[18] Bonaventura A, O'Connell RL, Mapagu C, Beale PJ, McNally OM, Mileshkin LR, et al. Paragon (ANZGOG-0903): Phase 2 Study of Anastrozole in Women With Estrogen or Progesterone Receptor-Positive Platinum-Resistant or -Refractory Recurrent Ovarian Cancer. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2017;27:900-6.

[19] Kobel M, Kalloger SE, Boyd N, McKinney S, Mehl E, Palmer C, et al. Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. PLoS medicine. 2008;5:e232.

[20] Papadimitriou CA, Markaki S, Siapkaras J, Vlachos G, Efstathiou E, Grimani I, et al. Hormonal therapy with letrozole for relapsed epithelial ovarian cancer. Long-term results of a phase II study. Oncology. 2004;66:112-7.

[21] Stasenko M, Plegue M, Sciallis AP, McLean K. Clinical response to antiestrogen therapy in platinum-resistant ovarian cancer patients and the role of tumor estrogen receptor expression status. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2015;25:222-8.

[22] Eng KH, Hanlon BM, Bradley WH, Szender JB. Prognostic factors modifying the treatment-free interval in recurrent ovarian cancer. Gynecologic oncology. 2015;139:228-35.

[23] Markman M, Webster K, Zanotti K, Rohl J, Belinson J. Use of tamoxifen in asymptomatic patients with recurrent small-volume ovarian cancer. Gynecologic oncology. 2004;93:390-3.

[24] Perez-Gracia JL, Carrasco EM. Tamoxifen therapy for ovarian cancer in the adjuvant and advanced settings: systematic review of the literature and implications for future research. Gynecologic oncology. 2002;84:201-9.

[25] Thurlimann B, Keshaviah A, Coates AS, Mouridsen H, Mauriac L, Forbes JF, et al. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. The New England journal of medicine. 2005;353:2747-57.

[26] Mouridsen H, Gershanovich M, Sun Y, Perez-Carrion R, Boni C, Monnier A, et al. Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: analysis of survival and update of efficacy from the International Letrozole Breast Cancer Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21:2101-9.

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

Figure 2~~3~~. 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; PR=partial response; SD=stable disease; SD^2=delayed SD patients; CBR=clinical benefit rate (CR+PR+SD), PD=progressive disease.

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

Formatted: Left

S2~~1~~. Prior lines of chemotherapy versus duration of endocrine therapy.

S3~~2~~. Type of endocrine therapy versus duration of endocrine therapy.

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 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

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)

			median DOT	univariate			multivariate		
	N	%	days	HR	95% CI	P	HR	95% CI	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.

5. Figure 1 revised

Click here to download 5. Figure: GYN-18-1005R1_Figure 1_revised.docx

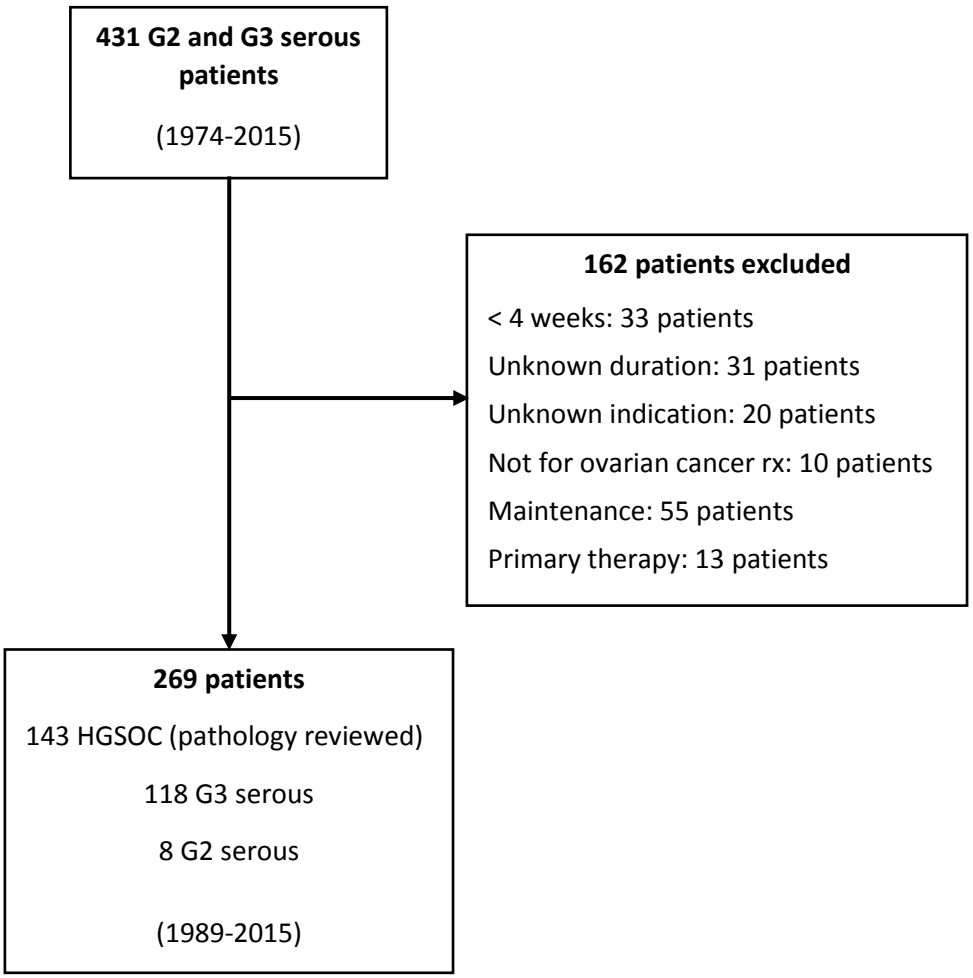
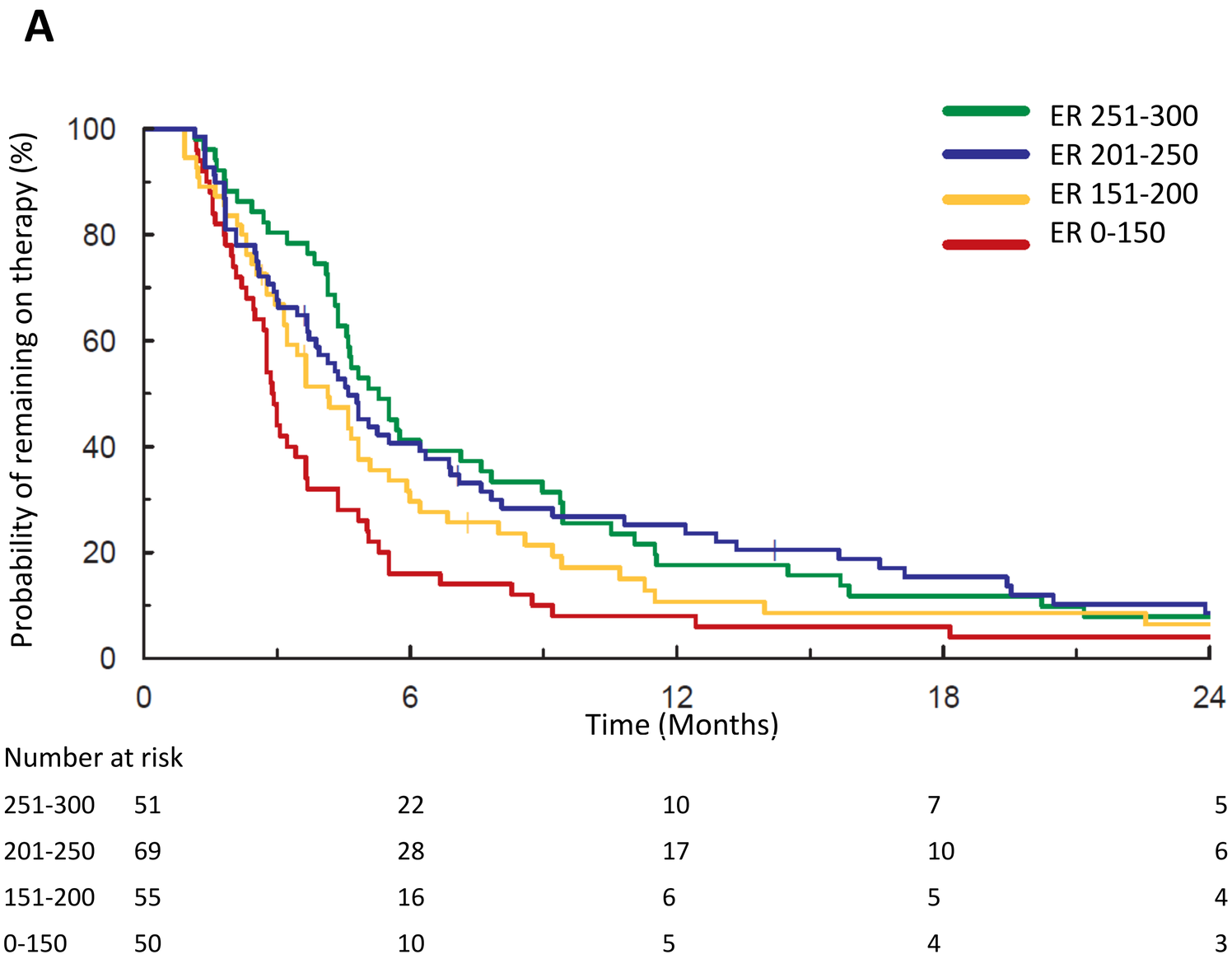
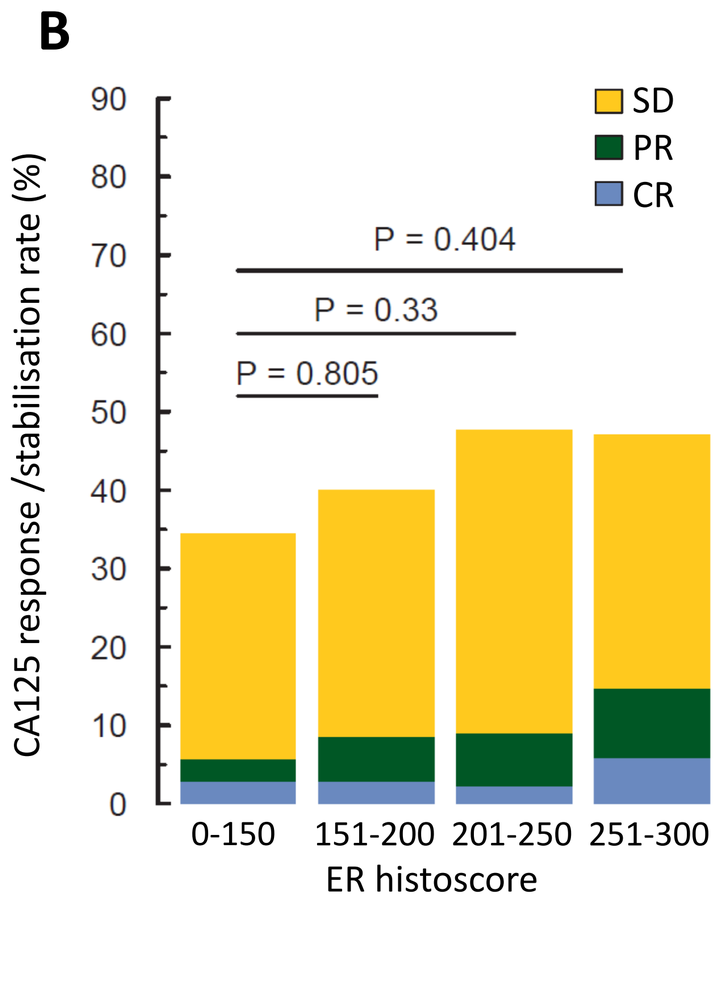


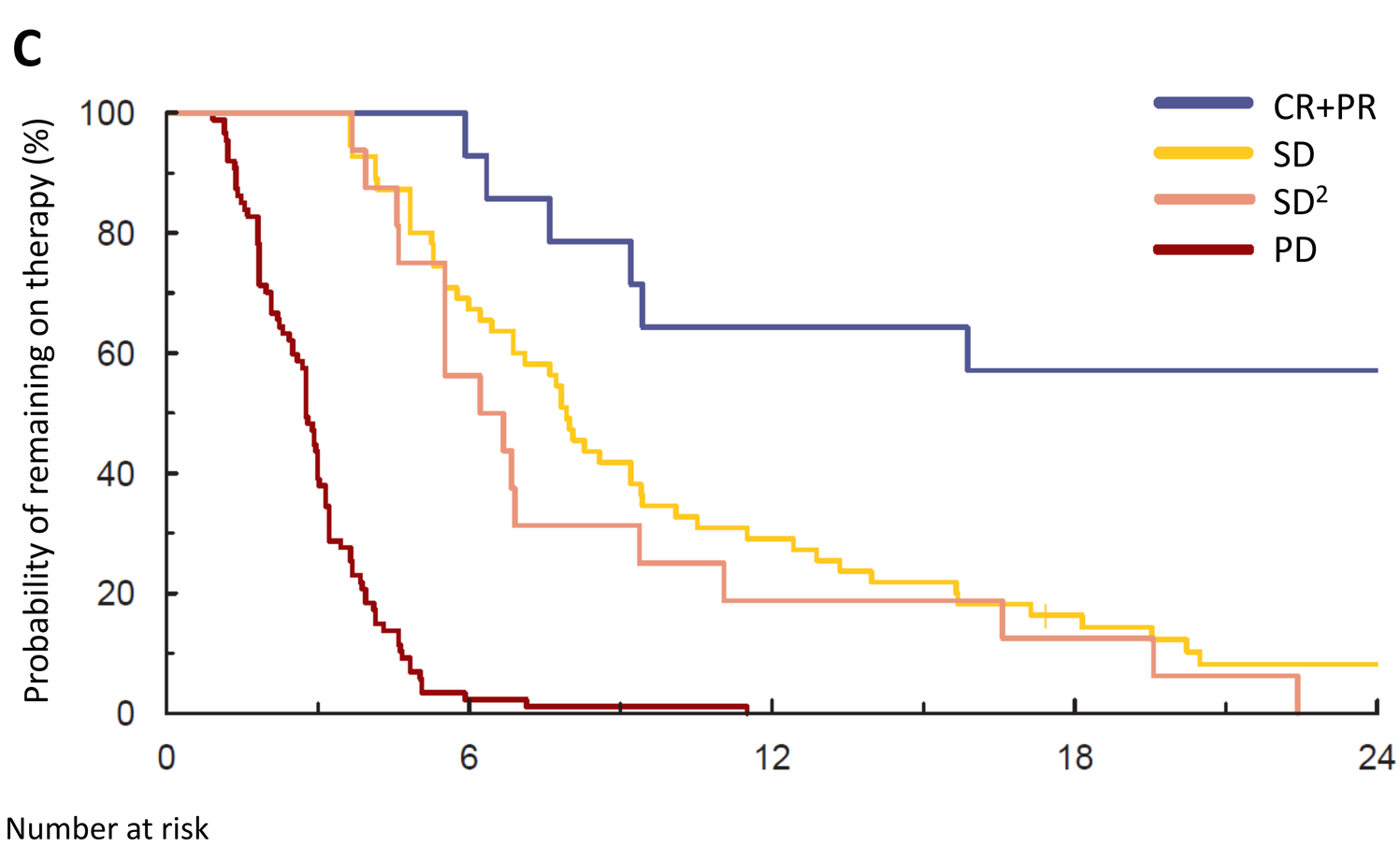
Figure 1: Characteristics of patients treated with endocrine therapy.

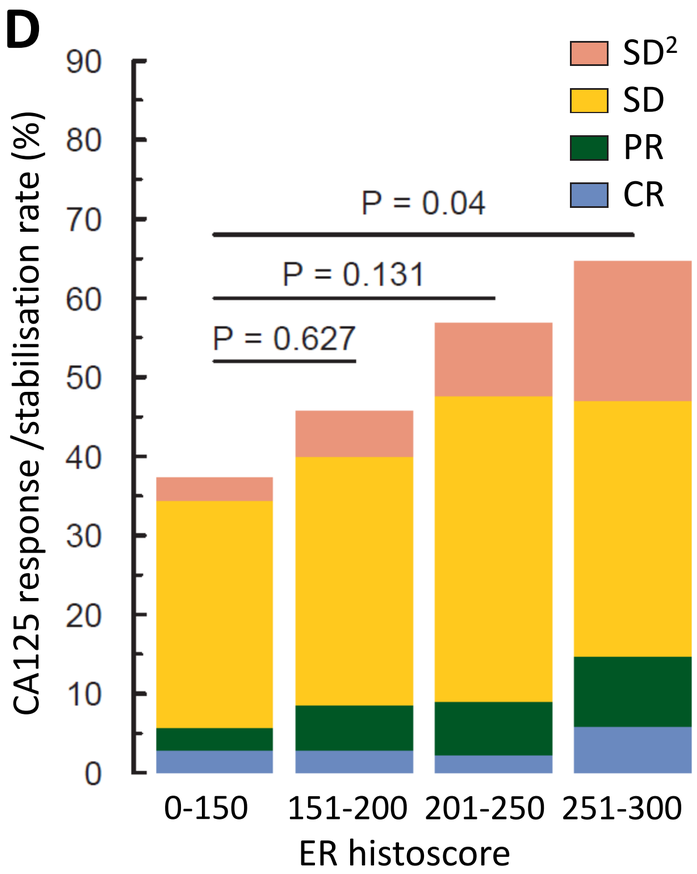
Rx=treatment; HGSOC= high grade serous ovarian carcinoma; G=grade.

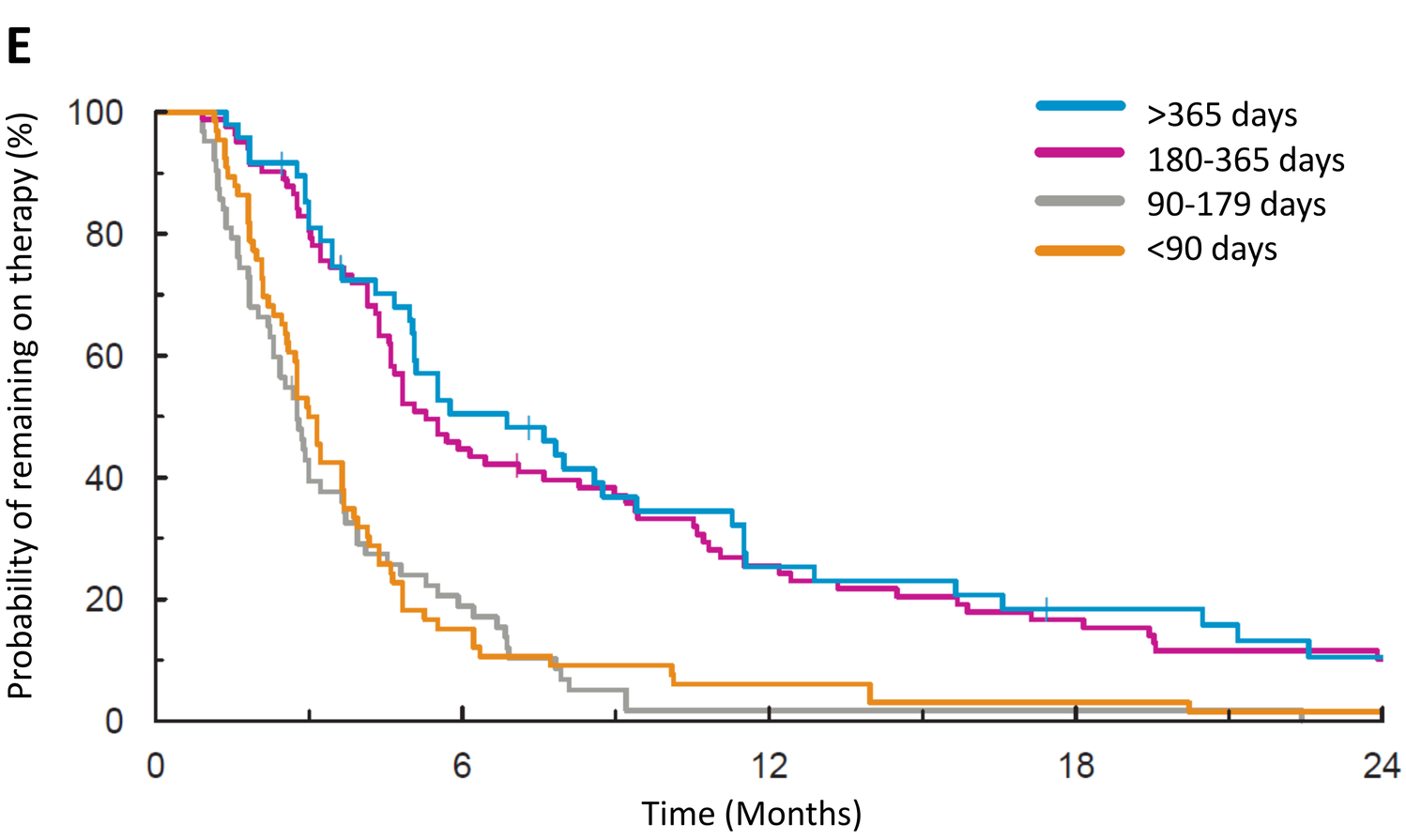
5. Figure
Click here to download 5. Figure: GYN-18-1005R1_Figure 2_revised2.pptx











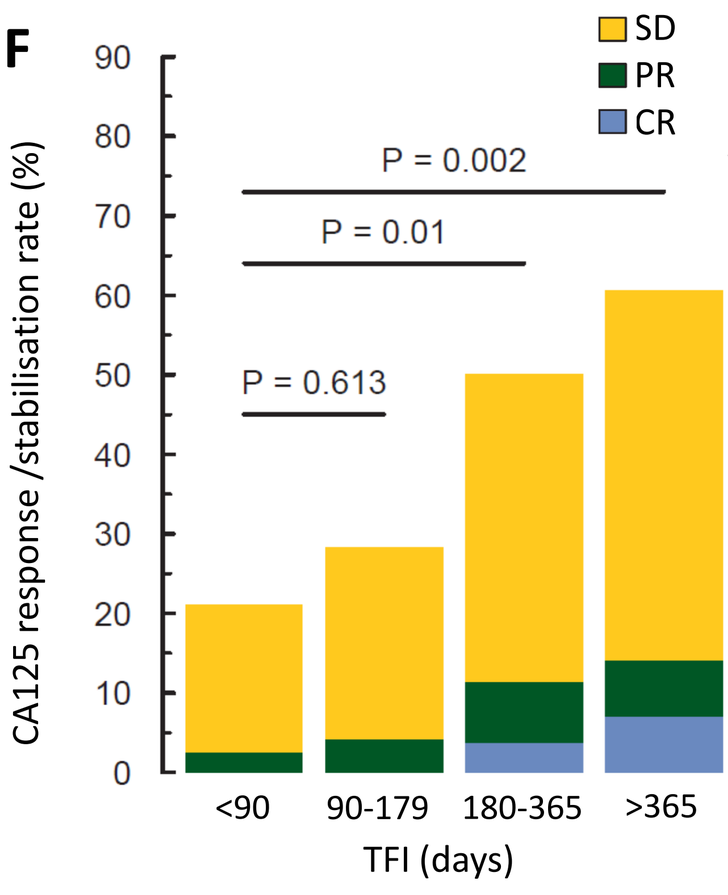
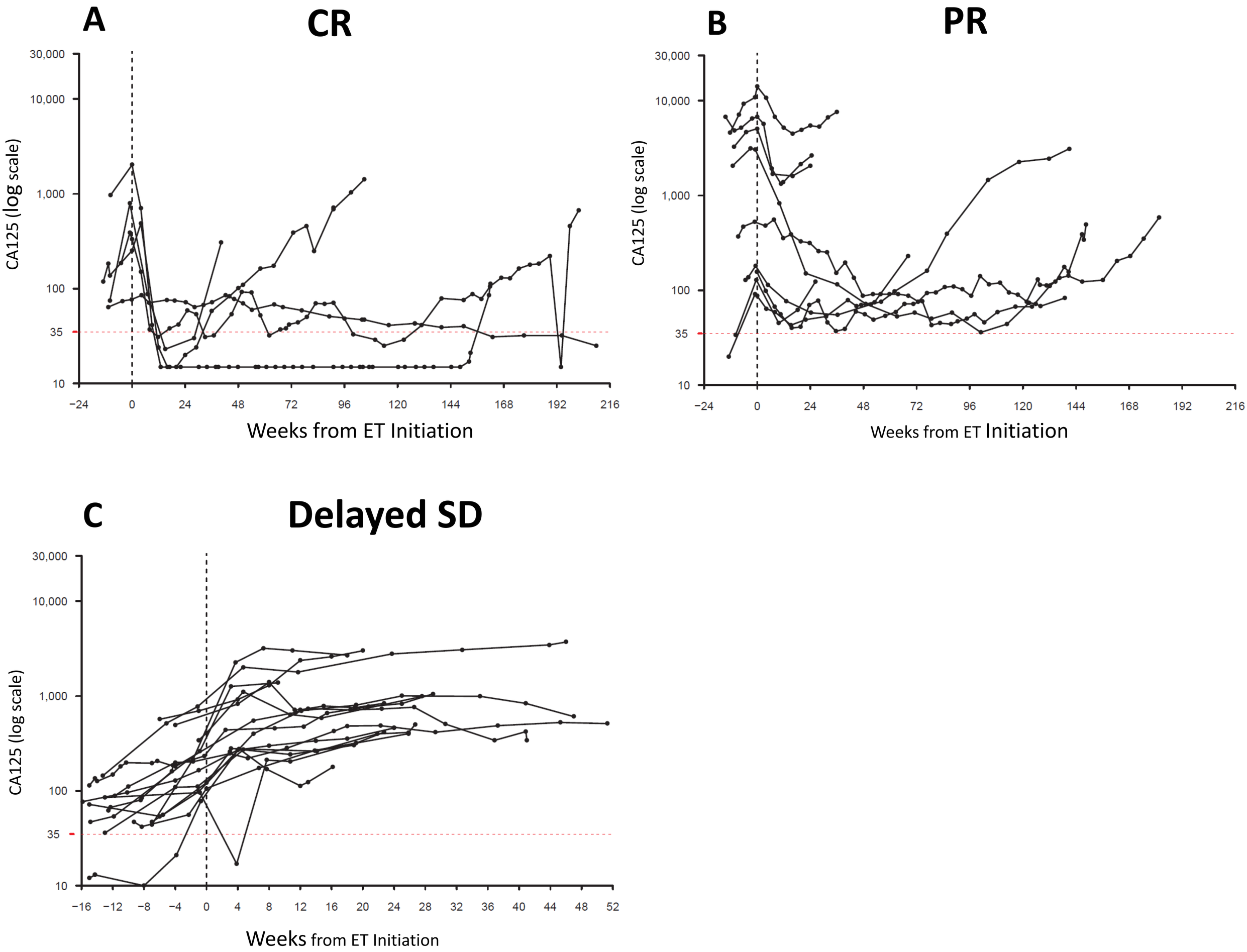


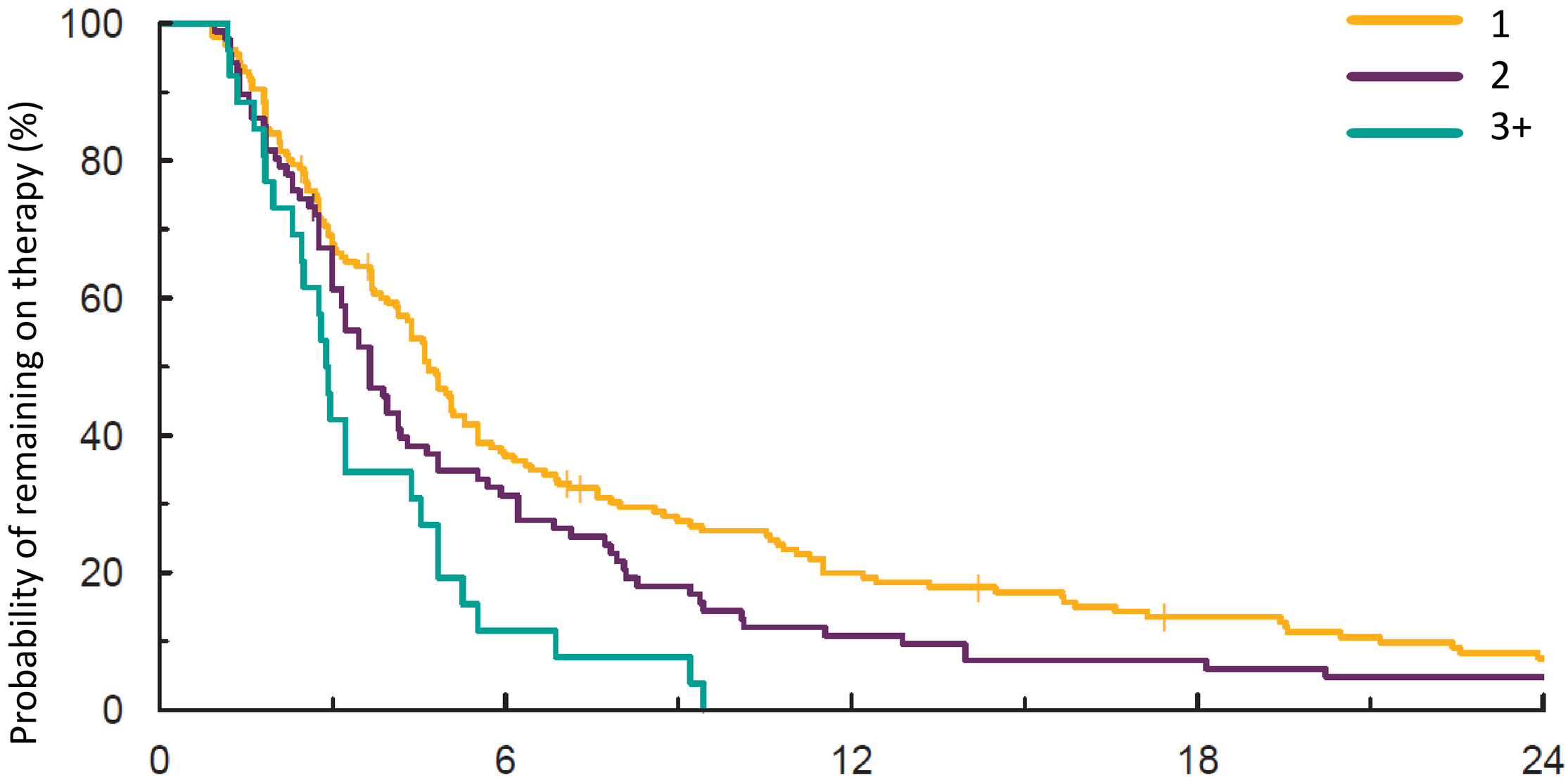
Figure 2. Duration of endocrine therapy and CA125 response rate based on ER histoscore and treatment free interval (TFI) (A) Duration of therapy versus ER histoscore, (B) CA125 response rate versus ER histoscore, (C) CA125 response versus duration of therapy, (D) CA125 response rate (including SD^2 patients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

6. Supplementary Material

Click here to download 6. Supplementary Material: GYN-18-1005R1_Supp_revised2.pptx



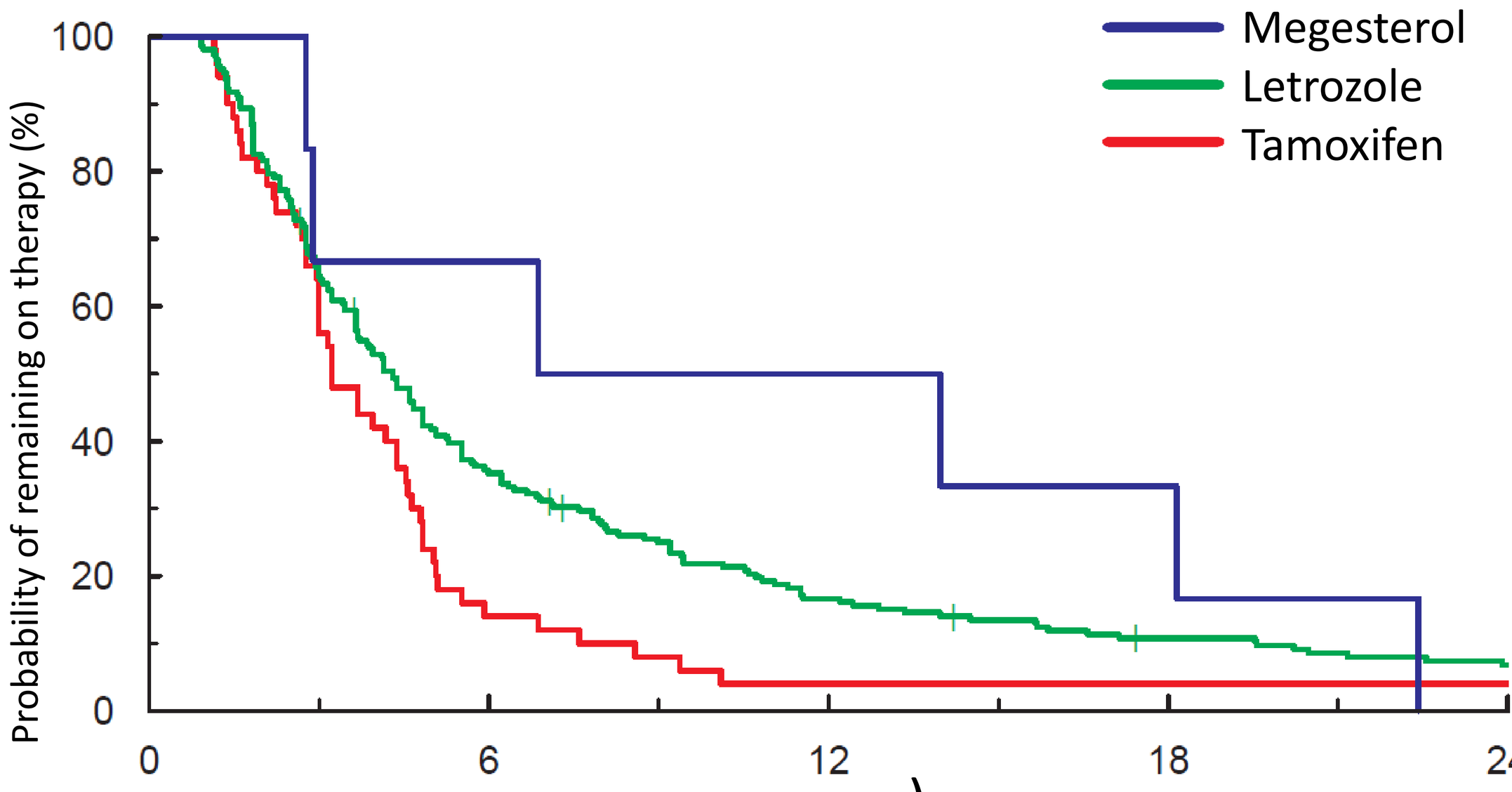
S1. Pattern of CA125 response in patients. (A) complete response (CR), (B) partial response (PR), (C) delayed stable disease (SD).



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.