Prognostic role of topoisomerase-IIα in advanced ovarian cancer patients

G Ferrandina1,2, M Petrillo2, A Carbone3, G Zannoni4, E Martinelli2, M Prisco2, S Pignata5, E Breda6, A Savarese7 and G Scambia2

1Department of Oncology, Catholic University, Campobasso, Italy; 2Gynecologic Oncology Unit, Catholic University, Rome, Italy; 3Institute of Human Pathology, Catholic University, Campobasso, Italy; 4Institute of Human Pathology, Catholic University, Rome, Italy; 5Medical Oncology, National Cancer Institute, Naples, Italy; 6Medical Oncology, Ospedale S. Giovanni Calibita Fatebenefratelli, Rome, Italy; 7Medical Oncology, Regina Elena Institute, Rome, Italy

To our knowledge, very few data about the role of Topoisomerase IIα (TOPO-IIα), an enzyme involved in critical steps of tumour cell proliferation and chemoresistance are currently available in ovarian cancer patients. The aim of this study was to investigate the prognostic value of TOPO-IIα expression in a large, single institution series of 96 primary untreated advanced ovarian cancer patients admitted to the Gynecologic Oncology Unit, Catholic University of Campobasso and Rome. Immunohistochemistry was carried out by using the MoAb anti-human TOPO-IIα antibody (clone Ki-S1). TOPO-IIα immunoreaction was observed in 70 out of 96 cases (72.9%), and the percentages of positively stained cells ranged between 1 and 83% (median = 10%). There was no association with clinico-pathological parameters. During the follow up period, progression and death of disease were observed in 76 (79.2%) and 45 (46.9%) cases. A statistically significant direct association between the percentages of positively immunostained tumour cells and the relative risk of death was observed (χ² = 6.6, P-value = 0.0101). In multivariate analysis, only platinum resistance, advanced stage of disease and high levels of TOPO-IIα expression retained an independent negative prognostic role for OS. The unfavourable role of high TOPO-IIα expression was maintained only in the subgroup of platinum resistant recurrent ovarian cancer patients, be TOPO-IIα expression evaluated as continuous variable (χ² = 5.1, P-value = 0.024), or by means of the defined cutoff point. Our study suggests that the assessment of TOPO-IIα could be helpful to identify poor prognosis platinum-resistant ovarian cancer patients, potentially candidates to investigational agents.

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Despite the advances in surgical efforts, and the achievement of high response rates with platinum/paclitaxel front-line treatment (McGuire et al, 1996; Eisenkop et al, 2003; Ozols et al, 2003), ovarian cancer remains the most lethal gynaecological malignancy with a 5-year survival rate of 25–30% in advanced stage disease (Jemal et al, 2007). The major determinants of clinical outcome are represented by the extent of residual tumour at primary surgery, and sensitivity to platinum-based chemotherapy (Armstrong, 2007): Indeed, in platinum-resistant ovarian cancer patients, salvage chemotherapy with non-platinum agents mostly results in short-lived response rates, and poor survival (Armstrong, 2007).

In this context, efforts aimed at identifying molecular factors eventually involved in chemotherapy resistance are actively ongoing. Several observations have recently suggested that Topoisomerase IIα (TOPO-IIα), one of the two isofoms an enzyme playing a relevant role in DNA replication, repair, and transcription (Chung et al, 1989), is involved in critical steps of tumour cell proliferation and chemoresistance (Wang, 2002). Strong TOPO-IIα expression or enzymatic activity have been documented in ovarian carcinoma compared to the hardly detectable levels in benign ovarian tumours, ovarian inclusion cysts, and normal surface epithelium (van der Zee et al, 1991; Cornarotti et al, 1996; Withoff et al, 1999; Chekerov et al, 2006). The frequency of TOPO-IIα overexpression in ovarian cancer has been reported to range between 30 and 70% (van der Zee et al, 1994; Gotlieb et al, 2001; Koshiyama et al, 2001), and a definite role of this enzyme as a marker of sensitivity not only to TOPO-IIα targeting agents, such as anthracyclines and etoposide, but also to platinum agents in vitro and in vivo has been documented (Kikuchi et al, 1997; Naniwa et al, 2007).

In particular, a significant correlation between elevated TOPO-IIα expression and tumour sensitivity to cisplatin-based chemotherapy has been shown in 37 primary untreated ovarian carcinomas (Cornarotti et al, 1996). While these observations favour the possibility that TOPO-IIα overexpression could identify ovarian cancer patients with better clinical outcome, on the other hand other authors suggested that it might serve as a marker of aggressive features and poor prognosis (Gotlieb et al, 2001; Brustmann 2004; Mano et al, 2004). The discrepancies across earlier studies might be explained by the different design, the methodologies of TOPO-IIα assessment, the small sample series, and the lack of data on salvage treatment (Cornarotti et al, 1996; Gotlieb et al, 2001; Brustmann 2004; Mano et al, 2004): indeed, it has to be taken into account that the analysis of the potential
prognostic impact of TOPO-IIz might be someway influenced by the role played by the same target as predictor of sensitivity to TOPO-IIz inhibitory drugs, often used in the salvage setting.

To our knowledge, very few data about the role of TOPO-IIz expression in predicting clinical outcome of ovarian cancer patients is currently available (Gotlieb et al, 2001; Brustmann 2004).

The aim of this study was to investigate the prognostic value of immunohistochemically assessed TOPO-IIz expression in a large, single institution series of primary untreated advanced ovarian cancer patients.

**PATIENTS AND METHODS**

**Patients**

The study included 96 ovarian cancer patients admitted to the Gynecologic Oncology Unit, Catholic University of Campobasso and Rome. In our Institution a written informed consent is routinely requested to patients for collection of their clinical data, as well as paraffin embedded sections for research use. Clinico-pathological characteristics of the overall series are summarised in Table 1.

Median age was 60 years (range, 27–80). Seventy-seven cases (80.2%) were stage III and 19 (19.8%) cases were stage IV disease.

**Table 1 Clinico-pathological characteristics of the overall series, and TOPO-IIz expression**

| Characteristics       | No. of patients (%) | Median (range) | P-value* |
|-----------------------|---------------------|----------------|----------|
| All cases             | 96                  | 5 (0–83)       |          |
| Age (years)           |                     |                |          |
| ≤ 65                  | 31 (32.3)           | 4 (0–50)       | 0.4      |
| > 65                  | 65 (67.7)           | 5 (0–83)       |          |
| FIGO Stage            |                     |                |          |
| III                   | 77 (80.2)           | 5 (0–83)       | 0.5      |
| IV                    | 19 (19.8)           | 3 (0–50)       |          |
| Grade                 |                     |                |          |
| G1-2                  | 18 (18.7)           | 5 (0–60)       | 0.8      |
| G3                    | 67 (69.8)           | 5 (0–83)       |          |
| n.a.                  | 11                  |                |          |
| Histotype             |                     |                |          |
| Serous                | 84 (87.5)           | 5 (0–83)       |          |
| Other                 | 12 (12.5)           | 4 (0–50)       | 0.6      |
| Residual tumour       |                     |                |          |
| < 1 cm                | 41 (42.7)           | 4 (0–60)       |          |
| > 1 cm                | 15 (15.6)           | 4 (0–40)       |          |
| Exploratory laparotomy| 40 (41.7)           | 7 (0–83)       | 0.7b     |
| Primary chemotherapy  |                     |                |          |
| Platinum/paclitaxel   | 91 (94.8)           | 5 (0–83)       |          |
| Platinum-based        | 5 (5.2)             | 5 (0–18)       | 0.8      |
| Response to CT        |                     |                |          |
| Yes                   | 48 (50.0)           | 4.5 (0–60)     | 0.8      |
| No                    | 48 (50.0)           | 5 (0–83)       |          |

n.a. = not available. *Calculated by Mann–Whitney nonparametric test. bCalculated by Kruskall–Wallis sum test.

According to the standard guidelines, maximal surgical effort has been attempted in all patients resulting in optimal debulking (residual tumour <1 cm) in 41 (42.7%) cases, which underwent surgical removal of tumour masses, along with total abdominal hysterectomy, adnexectomy, radical omentectomy appendectomy, multiple biopsies, and additional surgery (intestinal resections, diaphragm stripping) when required. Radical pelvic and paraaortic lymphadenectomy was performed in all patients undergoing primary cytoreduction who had residual tumour <1 cm. Suboptimal cytoreduction (residual tumour >1 cm) was achieved in 15 (15.6%) cases. Forty cases (41.7%) were judged to be unresectable at first surgery because of extensive peritoneal bulky carcinomatosis, agglutinated bowel/mesentery and infiltration of the upper gastrointestinal tract and /or the major vessels, and were submitted only to multiple biopsies. All patients received platinum-based chemotherapy (75–100 mg m⁻² for cisplatin, AUC = 5 for carboplatin, per cycle), including also paclitaxel (135–175 mg m⁻² for each cycle) in 91 (94.8%) cases. As far as patients undergoing only exploratory laparotomy are concerned, they received 3–4 cycles of chemotherapy before attempting a second cytoreductive surgery, unless they showed clinical progression during treatment. Response to chemotherapy was assessed according to WHO criteria (World Health Organization, 1979). In the subgroup of patients who were not susceptible to be cytoreduced at first surgery, a direct assessment of the extent of response to chemotherapy was carried out at time of second laparotomy. At recurrence/progression of disease platinum sensitive patients were triaged to platinum/taxane-containing regimen, while platinum-resistant patients were administered pegylated liposomal doxorubicin (PLD) according to clinical trials ongoing in our Institution (Ferrandina et al, 2007, 2008).

**Immunohistochemistry**

Pretreatment tumour tissues biopsies were obtained at first surgery in all cases. Tissue specimens were fixed in 10% formalin and paraffin-embedded according to standard procedures. Immunostaining was performed on 3 μm tissue sections mounted on poly-l-lysine-coated slides and dried at 37°C overnight. After the slides were deparaffinised in xylene, and rehydrated conventionally, the endogenous peroxidase activity was blocked with 3% H₂O₂ in TBS for 5 min. Antigen retrieval procedure was performed by microwave oven heating in citrate buffer (pH = 6). Sections were incubated with 20% normal goat serum for 30 min at room temperature to reduce nonspecific binding, then with the monoclonal mouse anti-human TOPO-IIz antibody (clone Ki-S1) (diluted 1:50) (Dako Cytomation, Denmark) in 20% goat serum. TOPO-IIz detection was evaluated by a labelled polymer The En Vision-mouse + System-HRP System (DAKO, Carpinteria, CA, USA) was used. Diaminobenzidine was used as a chromogen (DAB substrate System, DAKO). Positive controls for TOPO-IIz was represented by sections taken from the breast. Results were expressed as the proportion of immunostained tumour cells. The analysis of all tissue sections was done without any prior knowledge of the clinical parameters by two authors (AC, GFZ) by inter-observer variability (mean ± s.d. = 8% ± 2, and 12% ± 3, respectively).

**Statistical analysis**

Wilcoxon signed rank sum test was used to analyse the expression levels of TOPO-IIz according to clinico-pathological parameters. Time to progression and overall survival (OS) were calculated from
Figure 1 shows representative examples of high vs low TOPO-II expression in primary ovarian cancer. (A) Positive control (human breast cancer tissue specimen), (B) negative control (ovarian carcinoma) for TOPO-II staining. Representative examples of high (C) and low (D) TOPO-II expression. (A, B, C, D) Magnification × 200.

The percentages of positively stained cells ranged between 1 and 83% (median = 10%). The large inter-tumour variability, absence of a defined scoring system, and need to minimise any source of bias related to the use of a specific cutoff value (TOPO-II expression as a continuous variable.)

The percentages of TOPO-II immunoreactive tumour cells were found not to be associated with any of the clinico-pathological parameters examined. Moreover, no association with response to first-line treatment was documented (Table 1).

Follow-up data were available for all patients. As of December 2007, the median follow up was 37 months (range, 6 – 120). During the follow up period, progression and death of disease were observed in 76 (79.2%) and 45 (46.9%) cases.

Figure 2 shows the plot of the estimates of the relative risk of progression or death as a prediction of TOPO-II values, calculated by COX’s proportional hazard regression model: there was no association between the percentage values of positively expressed TOPO-II immunostained tumour cells and the relative risk of progression of disease ($\chi^2 = 2.3, P-value = 0.12$).

On the other hand, a statistically significant direct association between the percentages of TOPO-II expressed positively immunostained tumour cells and the relative risk of death was observed ($\chi^2 = 6.6, P-value = 0.0101$) (see also Table 2).

We were then prompted at defining the cutoff value of TOPO-II expression that more closely correlated with the risk of death: the most significant association was observed at the cutoff value of 25% TOPO-II immunoreactive cells: cases with high TOPO-II expression has a shorter OS (median OS = 35 months) than cases with low TOPO-II levels (median = 34 months) ($P-value = 0.048$) (Figure 3).

In univariate analysis of OS, platinum resistance, more advanced stage of disease, and suboptimal residual tumour at primary surgery were also found to be associated with a high risk of death of disease (Table 2). In multivariate analysis, only platinum resistance, more advanced stage of disease and high levels of TOPO-II expression retained an independent negative prognostic role for OS (Table 2).

We were then prompted at analyzing the prognostic relevance of TOPO-II expression in platinum-sensitive vs platinum-resistant ovarian cancer patients: interestingly enough, the unfavourable role of high TOPO-II expression was maintained only in the subgroup of platinum-resistant recurrent ovarian cancer patients, be it evaluated as continuous variable ($\chi^2 = 5.1, P-value = 0.024$), or by means of the defined cutoff point: cases with high TOPO-II expression has a shorter OS (median OS = 18 months) than cases with low TOPO-II levels (median = 35 months) ($P-value = 0.041$) (data not shown).

DISCUSSION

This is the first study analysing the association between the expression of TOPO-II protein and clinical outcome in a large series of primary untreated ovarian cancer patients.
We showed that patients whose tumours express high levels of TOPO-IIα experience a shorter OS compared to cases with low TOPO-IIα content, as also suggested by preliminary studies (Gotlieb et al., 2001; Mano et al., 2004).

The analysis of the percentages of positively TOPO-IIα immunostained cells as a continuous value allowed to avoid the potential bias inherent in the use of an arbitrary cutoff point, and further supported the association of high TOPO-IIα expression with a high risk of death of disease.

The independent role of high TOPO-IIα expression as marker of poor prognosis is sustained by the lack of association with any of the clinico-pathological parameters examined, and also by the results of multivariate analysis documenting the persistence of the unfavourable significance of high TOPO-IIα expression after adjusting for stage of disease, residual tumour, and platinum responsiveness.

The association between TOPO-IIα overexpression and poor prognosis has been reported in other human tumours, including breast and bladder carcinomas (Kruger et al., 2005; O’Connor et al., 2006) as well as glioblastoma and lymphoma (Ho et al., 2003; Schrader et al., 2004); however, the assessment of whether the prognostic impact of this parameter is due to its value as predictor of response to treatment-based regimen or as a marker of intrinsic tumour aggressiveness (pure prognostic factor), is difficult to be established. Indeed, the data about the role of TOPO-IIα in determining susceptibility to platinum agents are very controversial since increased levels of TOPO-IIα have been found both in cell lines or small subsets of patients resistant to alkylating agents or platinum drugs (de Jong et al., 1990; Chu, 1994; Kikuchi et al., 1997), as well as in in vitro models and ovarian cancer patients exhibiting sensitivity to cisplatin (Giaccone et al., 1992; Cornarotti et al., 1996; Koshiyama et al., 2001). In our series, we could not detect any difference in the distribution of TOPO-IIα values according to response to first line chemotherapy, or time to progression, the latter one being strictly associated with treatment susceptibility, thus suggesting that overexpression of TOPO-IIα might more likely indicate tumour-intrinsic biological aggressiveness, rather than represent only a marker of platinum sensitivity. In this context, it is noteworthy that TOPO-IIα is involved in several biological pathways of tumour aggressiveness: for instance, TOPO-IIα is correlated with the proliferation associated marker ki67 (Costa et al., 2000), the Vascular Endothelial Growth Factor

### Table 2: Univariate and multivariate analysis of clinico-pathological parameters and TOPO-IIα as prognostic factors for overall survival in advanced ovarian cancer patients

| Variable                          | Univariate | Multivariate* |
|-----------------------------------|------------|---------------|
|                                   | RR1 | χ² | P-value | RR2 | χ² | P-value |
| Age (years)                       |     |    |        |     |    |        |
| <65                               | 1.0 | 0  | —      | —   | —  | —      |
| >65                               | 1.2 | 0.3| 0.6    | —   | —  | —      |
| Stage                             |     |    |        |     |    |        |
| III                               | 1.0 | 0  | —      | —   | —  | —      |
| IV                                | 3.0 | 9.4| 0.0021 | 1.96| 7.1| 0.008  |
| Extent of residual tumour         |     |    |        |     |    |        |
| <1 cm                             | 1.0 | 0  | —      | —   | —  | —      |
| >1 cm                             | 3.1 | 9.5| 0.0020 | 1.63| 1.5| 0.22   |
| Response to treatment             |     |    |        |     |    |        |
| No                                | 1.0 | 0  | —      | —   | —  | —      |
| Yes                               | 9.7 | 35.0| 0.0001 | 8.2 | 30.2| 0.0001 |
| Topo-IIα percentages              |     |    |        |     |    |        |
| Continuous data                   | 1.02| 6.6| 0.0101 | 1.02| 3.9| 0.0477 |

*Reference category. χ² of the model = 45.7; P-value = 0.0001. *Only variables with P-value < 0.20 in the univariate analysis were included in the multivariate model. RR1 = unadjusted relative risk, RR2 = relative risk after adjusting for all the factors listed. *Relative risk per percentage unit of TOPO-IIα increase.

Figure 2  Plot of the estimates of the relative risk of progression (A) and death (B) of disease as a prediction of TOPO-IIα values, calculated by COX’s proportional hazard regression model.

Figure 3  Overall survival curves in ovarian cancer patients according to the status of TOPO-IIα.
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In conclusion, although these findings need to be confirmed in a larger series, our study suggests that the assessment of TOPO-IIa could be helpful to identify poor prognosis platinum resistant ovarian cancer patients, potentially candidates to investigational agents.

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