PERGAMON

European Journal of Cancer 38 (2002) 393–400

European Journal of Cancer

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# DNA topoisomerase IIα expression in optic pathway gliomas of childhood

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Received 12 July 2001; received in revised form 24 September 2001; accepted 7 November 2001

**Abstract**

DNA topoisomerase IIα (Topo IIα) is linked to tumour cell growth and chemoresistance. We examined immunohistochemically Topo IIα expression levels in a series of 36 consecutive paediatric optic pathway glioma (OPG) patients. Topo IIα labelling index (LI) ranged from 0.0 to 11.6 and was significantly associated with patient age, with higher levels of Topo IIα in children $\leqslant 3$ years ($P = 0.031$). Topo IIα expression did not correlate with patient survival. Topo IIα LI was not significantly increased in specimens of repeat surgery. Topo IIα LI closely correlated with MIB-1 LI ($R = 0.781$, $P < 0.001$). We conclude that Topo IIα expression correlates with tumour cell proliferation in paediatric OPGs. Assessment of cell proliferation, however, does not assist in refining prognostic predictions. Enhanced Topo IIα expression in children $\leqslant 3.0$ years suggests that Topo IIα-interfering anticancer compounds for adjuvant treatment of OPGs may be of particular benefit to young children. 

*Keywords:* Epipodophyllotoxins; Immunohistochemistry; Multidrug resistance; MIB-1 proliferation index; Optic pathway gliomas; Topoisomerase IIα

## 1. Introduction

Optic pathway gliomas (OPGs) are uncommon neoplasms, comprising up to 5% of primary central nervous system (CNS) tumours. These lesions show a peak incidence in the first 5 years of life. Most OPGs are anatomically well circumscribed displaying features of pilocytic astrocytomas, whereas others show diffuse growth and invasion of surrounding CNS structures. OPGs are strongly associated with neurofibromatosis type 1 [1]. OPGs behave clinically in an unpredictable way. Almost half of the patients experience prolonged disease control for many years with the tumour remaining quiescent, a subset of whom appear to be 'cured' of their disease. Others (20–30%) with comparable histology and treatment show disease progression and may finally prove fatal within several months to years after diagnosis [2]. OPGs with unfavourable prognosis arise primarily in young children [3]. The basis for the widely differing behaviour of these tumours remains unknown.

Because of the unpredictable behaviour of OPGs there is a need to identify biological factors that help to improve outcome predictions and guide therapeutic decision-making. The MIB-1 antibody, which labels the Ki-67 antigen, is a convenient and widely used proliferation marker in human gliomas [4]. Previous studies have examined MIB-1 labelling in paediatric low-grade astrocytomas and have partly revealed a relationship between local tumour behaviour and MIB-1 expression [5,6]. In the case of OPG, outcome of patients seems not to be associated with MIB-1 labelling index (LI) [7]. Aside from MIB-1 LI, expression of DNA topoisomerase IIα (Topo IIα), a nuclear enzyme essential for many aspects of DNA functioning, has been highly associated with tumour cell proliferation [8].

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0959-8049/02/$ - see front matter 
PII: S0959-8049(01)00387-2

In addition to its potential role as a proliferation marker, Topo IIα has been implicated in drug resistance of tumour cells [9]. There is strong evidence that Topo IIα determines response of certain tumours to a number of anticancer compounds [9] including epipodophyllotoxins such as etoposide (VP-16) and teniposide (VM-26), doxorubicin, mitoxantrone and amsacrine [10,11]. Topo II-interfering anticancer compounds have been successfully used as an adjuvant therapeutic regimen for patients with gliomas in the adult [12–15] and paediatric [16,17] age groups. In recent years, chemotherapy has been introduced into treatment protocols of low-grade astrocytomas, including OPGs [18,19]. There is some evidence that chemotherapy targeting Topo IIα may have a prognostic impact when applied alone or as part of polydrug treatment schedules in gliomas resulting in at least short-term favourable outcomes [20,21].

To date, no information is available on Topo IIα expression in paediatric OPGs. To determine the role of Topo IIα expression as a marker of proliferation and biological prognostic factor and with regard to the potential relevance of Topo II expression for the identification of patient subpopulations for Topo II-targeting chemotherapy, we examined the intrinsic expression of Topo IIα in a well-characterised institutional cohort of children with OPGs.

## 2. Patients and methods

### 2.1. Clinical patient data

36 consecutive children diagnosed with OPG between 1978 and 2000 (in one instance the patient was diagnosed in 1971) at our institution were retrospectively identified. Clinical patient data are shown in Table 1. Age at diagnosis ranged from 4 months to 17.6 years. All but 1 patient (case no. 17 in Table 1) had surgery at the time of diagnosis. The mean age at surgery was 5.9 years. All children underwent preoperative and postoperative neuroimaging by computed tomography (CT) or magnetic resonance imaging (MRI). In 30 patients, detailed information on tumour location and extent of resection based on imaging and operative criteria has been provided in a previous study [7]. In 6 new patients (cases 9–14), the same criteria were used to determine tumour site and resection extent. A second operation was performed in 11/36 patients, either due to tumour recurrence, disease progression, or to achieve a more complete resection from a different surgical approach. 3 patients were operated on three times and 1 patient six times. Radiation therapy was applied before first surgery in 1 case (patient no. 17 in Table 1), following the first operation in 9 cases, and after second (implanted iodine-125 seed) and after third surgery in 1 case, respectively. Adjuvant chemotherapy was performed in 8 patients, consisting of carboplatin and vincristine (VCR) in all cases and an additional six cycles of a combination of etoposide (VP-16) and carboplatin in alternate cycles with VP-16/ifosfamide/cisplatin (CDDP) in 1 case. Patient outcome was assessed using neuroimaging, hospital and outpatient charts, and telephone interview. Follow-up ranged from 4.5 months to 22.3 years, and 29 years in the single case operated upon in 1971. 2 patients (cases 16 and 28) were lost to follow-up. Last information on the disease status of these patients was obtained in December 1997. Information obtained on the other patients was current as of July 2000.

### 2.2. Tissue specimens and tumour histopathology

Haematoxylin-eosin stained sections of all tumour specimens were reviewed by a neuropathologist. All specimens were determined to be low-grade astrocytomas. 32 cases showed features of pilocytic astrocytoma and 4 cases of diffuse fibrillary astrocytoma, according to standard criteria [1].

### 2.3. Immunohistochemistry

Immunohistochemical analysis was performed on paraffin-embedded tissues. The monoclonal antibodies JH2.7 (Neomarkers) against TopoIIα and MIB-1 (Dianova) against the Ki-67 antigen were used as the primary antibodies. Sections were cut at 4 μm and antigen retrieval was performed by boiling sections in citrate buffer (pH 6.0, 20 min for JH2.7; pH 6.6, 10 min for MIB-1). Antibody binding was visualised with the ABC technique and DAB as the chromogen.

JH2.7 and MIB-1 binding was apparent as nuclear staining. Evaluation of JH2.7 and MIB-1 immunostained sections was performed by counting all cell nuclei, regardless of staining intensity, excluding vascular endothelial cells and haematogenous cells. For each specimen, 500 tumour cells were counted in fields showing the highest density of immunopositive tumour cells and labelled nuclei were expressed as a percentage.

### 2.4. Statistical analysis

For statistical analysis, the study cohort was subdivided into two groups with one demonstrating a low Topo IIα LI of $\leqslant 4.0$ and one with an increased Topo IIα LI $>4.0$. Univariate analysis of overall patient survival was performed as outlined by Kaplan–Meier [22]. Overall survival was defined from the day of surgery until the death of the patient. Death from a cause other than OPG or survival until the end of the observation period were considered censoring events. In a single patient (case no. 17 in Table 1), who had radiotherapy before surgery, survival was calculated from the day of radiotherapy. The correlation between Topo IIα LI and

Table 1
Clinical features, Topo IIα and MIB-1 LIs at first operation in a series of 36 OPGs of childhood

| Case | Age (years) | Sex | NF1[a] | Site[b] | Resect.[c] | XRT[d] (Gy) | Chemo[e] | Topo (LI)[f] | MIB-1 (LI) | Progr.[g] | OS[h] (years) | F/U[i] (years) | Status[j] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.4 | m | – | P | S | 54 | – | 3.2 | 3.0 | Yes | 13.6 | | DOD[k] |
| 2 | 10.7 | m | + | P | B | 63.3 | – | 2.8 | 1.8 | Yes | 13.5 | | DOD |
| 3 | 3.7 | f | + | P | P | 66 | – | 0 | 0 | Yes | 6.8 | | DOD |
| 4 | 10 | f | – | P | S | 54[l] | – | 3.6 | 1.9 | Yes | 5.2 | | DOD |
| 5 | 10.5 | m | – | P | B | 58.5 | – | 4.4 | 2.3 | Yes | 3.8 | | DOD |
| 6 | 7.4 | m | – | P | B | – | – | 4.8 | 5.5 | Yes | 2.8 | | DOD |
| 7 | 3.5 | f | – | P | B | – | – | 1.4 | 2.5 | Yes | 1.9 | | DOD |
| 8 | 6.8 | f | – | P | P | 60 | – | 2.4 | 3 | Yes | 1.4 | | DOD |
| 9 | 17.6 | m | – | P | B | – | – | 0 | 0.4 | Yes | | 29 | Stable[u] |
| 10 | 3.5 | f | – | P | S | 55 | – | 3 | 4.0 | Yes | | 22.3 | Stable |
| 11 | 3.2 | f | – | ON | T | – | – | 4.2 | 5.1 | No | | 20.4 | CR[t] |
| 12 | 18 | m | – | P | S | – | – | 0.8 | 1 | No | | 17.8 | Stable |
| 13 | 3.9 | f | – | P | P | 30 | – | 1 | 3.2 | Yes | | 17.3 | Stable |
| 14 | 8 | m | – | P | P | – | – | 5.8 | 5.7 | No | | 15.8 | Improved[v] |
| 15 | 16.3 | m | – | ON | T | – | – | 2 | 1 | No | | 15.2 | CR |
| 16 | 1.8 | m | + | P | P | 49 | – | 9.2 | 17.2 | Yes | | 14.5 | Stable[p] |
| 17 | 3.5 | f | – | P | P | +[s] | – | 2.8 | 1.9 | No | | 13.5 | Improved |
| 18 | 2.8 | f | – | P | P | +[r] | + | 5.4 | 4.2 | Yes | | 12 | Improved |
| 19 | 4.7 | m | – | P | P | 50 | – | 1.8 | 1.4 | No | | 11.3 | Stable |
| 20 | 4.6 | m | – | P | B | 56[q] | – | 4.2 | 3.8 | Yes | | 10.2 | Stable |
| 21 | 5.6 | f | – | P | S | – | – | 8 | 5 | Yes | | 10.3 | Stable |
| 22 | 3 | m | – | P | S | – | – | 4.4 | 2.5 | No | | 9.9 | Improved |
| 23 | 8 mo[m] | f | – | P | B | – | – | 7.4 | 7 | Yes | | 8.8 | Improved |
| 24 | 2.8 | f | + | C | B | – | + | 4.2 | 4.5 | Yes | | 7 | Stable |
| 25 | 5.7 | m | – | P | S | – | + | 6.6 | 6.4 | No | | 5.8 | CR |
| 26 | 8.7 | f | – | P | B | – | – | 2.6 | 4.5 | No | | 5.5 | Stable |
| 27 | 4.5 | m | – | P | S | – | + | 5.4 | 6.1 | Yes | | 4.4 | Stable |
| 28 | 1.7 | m | + | C | B | – | – | 11.6 | 10 | Yes | | 4.3 | Stable[p] |
| 29 | 10 mo | m | – | P | B | – | + | 0 | 0 | No | | 2.8 | Stable |
| 30 | 4 mo | m | – | P | B | – | +[n] | 7.6 | 6.3 | Yes | | 2.8 | PD[o] |
| 31 | 11 mo | m | – | P | B | – | + | 11.2 | 23.9 | No | | 1 | Stable |
| 32 | 7.6 | m | – | P | T | – | – | 0.6 | 2.4 | No | | 11 mo | CR |
| 33 | 3.6 | f | – | P | S | – | – | 4.4 | 6 | Yes | | 9.5 mo | Stable |
| 34 | 2.2 | f | + | P | B | – | + | 7.4 | 10.2 | No | | 9 mo | Improved |
| 35 | 6.4 | m | – | P | P | – | – | 4.2 | 4.7 | No | | 5 mo | Stable |
| 36 | 15.2 | f | + | P | S | – | – | 2.8 | 5.2 | No | | 4.5 mo | Stable |

Patients 1–8 died due to disease progression. Patients 9–36 are arranged in order of follow-up time. Age, overall survival and follow-up are listed in years. In 26 patients, follow-up is longer than 5 years, in 21 patients follow-up is longer than 10 years. OPG, optic pathway gliomas.

[a] Neurofibromatosis type 1.
[b] Tumour site: P, posterior; ON, optic nerve; C, chiasm.
[c] Resection extent: B, biopsy; P, partial; S, subtotal; T, total.
[d] XRT, radiotherapy.
[e] Chemo, chemotherapy, consisting of carboplatin and vincristine (VCR).
[f] Topo (LI), DNA topoisomerase IIα labelling index.
[g] Progression after first operation.
[h] OS, overall survival.
[i] F/U, follow up (years to last contact (patient alive)).
[j] Status at last follow-up.
[k] DOD, dead of disease.
[l] After second surgery.
[m] mo, months.
[n] In addition, six cycles of chemotherapy consisting of etoposide (VP-16) + carboplatin alternately with VP-16 + ifosfamide + cisplatin (CDDP).
[o] PD, progressive disease.
[p] Lost to follow-up, information to outcome as of December 1997.
[q] After third surgery.
[r] Iodine-125 seed after second surgery.
[s] Before first surgery, dose unknown.
[t] CR, complete remission.
[u] No tumour progression on imaging.
[v] Tumour reduction on imaging.

MIB-1 LI was investigated using non-parametric Spearman's coefficient of correlation ($R$). Kruskal–Wallis test, Mann–Whitney test, Chi-square test and Fisher's Exact test were used as appropriate. For all tests, a two-tailed $P$ value of $\leqslant 0.05$ was considered as significant.

## 3. Results

### 3.1. Patient characteristics

Relevant clinical features of the study cohort are summarised in Table 1. 7 patients (19%) had a concomitant diagnosis of neurofibromatosis type 1 (NF1). 13/36 (36%) patients were irradiated, and 8/36 (22%) had received chemotherapy. Overall survival (OS) was 85 and 71% at 5 and 10 years follow-up, respectively. 28/36 (78%) patients were alive at the last follow-up. 13 of these patients remained progression-free after the first operation. At last follow-up, 4 patients enjoyed a complete remission, 6 patients demonstrated an improved status, 17 patients had stable disease, and 1 showed disease progression. Log-rank test revealed no significant difference in OS between patients with or without chemotherapy ($P=0.2729$) and between patients with or without irradiation ($P=0.1087$).

### 3.2. Topo IIα labelling indices

Topo IIα LIs of specimens obtained from first surgery are listed together with MIB LIs in Table 1, LIs of specimens from repeat surgery in Table 2. Topo IIα LIs varied widely in our patient population, ranging from no labelling in 3 patients to a LI of $>10\%$ in 2 patients (mean ± standard deviation (S.D.), $3.86 \pm 2.53$). Based on this mean value, a cut-off level of Topo IIα LI of $\leqslant 4.0\%$ was chosen. A larger portion of specimens ($n=32$; 59%) exhibited low Topo IIα LIs of $\leqslant 4.0$, and a smaller portion ($n=22$; 41%) exhibited increased Topo IIα LIs of $>4.0$. Fig. 1 shows representative immunohistochemistry results in a patient with low Topo IIα LI (patient 10) and in a patient with increased Topo IIα LI (case 28).

### 3.3. Relationship between Topo IIα LI and patient outcome or other clinical variables

For correlation of Topo IIα LI and outcome, we divided the 36 specimens obtained from first surgery into two populations with low or increased Topo IIα LIs (cut-off point: 4.0 LI) and performed an univariate analysis. Overall survival was not significantly different between both populations showing a weak tendency of patients with Topo IIα LIs of $>4.0$ to exhibit a more favourable outcome ($P=0.2442$, log-rank test). Similarly, there was no significant association between Topo IIα LI and disease progression (yes *or* no) after first surgery ($P=0.736$, Chi-square test). Topo IIα LIs were not significantly increased in specimens of repeat surgery compared with specimens of the first operation.

There was no correlation between Topo IIα expression and either gender, tumour location, extent of resection, or concomitant diagnosis of NF1 ($P>0.05$). However, Topo IIα LI at first surgery correlated with patient age at diagnosis: Topo IIα LI was significantly higher in young infants using cut-off points of 4.0 for Topo IIα LI and 3.0 years for patient age ($P=0.031$, Chi-square test). Only 2/11 patients $\leqslant 3$ years of age had a Topo IIα LI of $\leqslant 4.0$, whereas 15/25 patients $>3$ years of age demonstrated Topo IIα LIs of $\leqslant 4.0$ (Table 3). Mean Topo IIα LI ± S.D. was $6.51 \pm 3.50$ in the age group of $\leqslant 3$ years versus $3.18 \pm 2.05$ in the age group of $>3$ years. In a previous study, a link between the amount of immunohistochemically detectable Topo IIα expression and the age of the archived tissue specimens was noted [23]. To test for such a possible association, we performed in our series a regression analysis.

Table 2
Topo IIα and MIB-1 labelling indices of surgical OPG specimens obtained from first operation versus indices of recurrent surgery

| Case | First op. (year): Topo IIα/MIB-1 LI | Second op. (year): Topo IIα/MIB-1 LI | Third op. (year): Topo IIα/MIB-1 LI | Fourth op. (year): Topo IIα/MIB-1 LI | Fifth op. (year): Topo II α/MIB-1 LI | Sixth op. (year): Topo IIα/MIB-1 LI |
|---|---|---|---|---|---|---|
| 1 | 1984: 3.2/3.0 | 1984: 3.3/4.5 | 1988: 2.6/3.5 | 1988: 3.4/2.8 | 1993: 4.0/3.1 | 1994: 4.8/3.6 |
| 3 | 1980: 0.0/0.0 | 1987: 4.0/6.0 | | | | |
| 4 | 1993: 3.6/1.9 | 1995: 3.0/2.9 | 1998: 2.0/3.2 | | | |
| 8 | 1980: 2.4/3.0 | 1981: 4.0/3.9 | | | | |
| 9 | 1971: 0.0/0.4 | 1985: 1.6/2.9 | 1997: 1.6/1.5 | | | |
| 11 | 1980: 4.2/5.1 | 1980: 3.8/3.2 | | | | |
| 18 | 1988: 5.4/4.2 | 1989: 2.4/4.8 | | | | |
| 20 | 1990: 4.2/3.8 | 1994: 2.0/3.0 | | | | |
| 22 | 1990: 4.4/2.5 | 1990: 5.2/8.8 | | | | |
| 27 | 1996: 5.4/6.1 | 1997: 2.8/3.8 | | | | |
| 30 | 1997: 7.6/6.3 | 1998: 4.3/6.9 | 2000: 2.6/3.6 | | | |

op, operation; Topo IIα, DNA topoisomerase IIα.

Linear regression revealed a discrete trend towards lower LIs in older specimens for both Topo IIα ($P=0.035$, $R^2=0.124$) and MIB-1 ($P=0.047$, $R^2=0.111$). However, a comparison of the mean age of tissue specimens from children less than 3 years of age with those of older children did not disclose a statistically significant difference ($P>0.05$, Mann–Whitney test), indicating that the age of the specimens was not a confounding factor with respect to the observed enhanced expression of Topo IIα in young patients. Due to the low number of patients in the various subgroups, it was not possible to evaluate the influence of Topo IIα and MIB-1 LI on the response to chemotherapy.

### 3.4. Relationship between Topo IIα and MIB-1 labelling indices

In view of its potential role as a marker of tumour cell growth, Topo IIα expression was correlated with the proliferation index as assessed by MIB-1 labelling of the Ki-67 nuclear antigen on specimens of first operation (range of MIB-1 LI: 0.0 to 23.9; mean ± S.D., 4.6 ± 3.9). This analysis revealed a strong association between Topo IIα and MIB-1 LIs, which approached high statistical significance ($R=0.781$, $P<0.001$, Spearman's coefficient of correlation) (Fig. 2). In 27/54 surgical specimens, there was a low expression (⩽4.0 LI) of both Topo IIα and Ki-67, and 18/54 surgical specimens had an increased expression of both Topo IIα and Ki-67 (LI > 4.0). Subdividing our patients into these two groups, however, did not disclose an association between combined Topo IIα/MIB-1 LI and patient outcome. Furthermore, combined Topo IIα/MIB-1 LI did not correlate with any of the other clinical variables ($P>0.05$).

## 4. Discussion

Topo IIα, which has been identified as a major scaffold protein of mitotic chromosomes being present in the interphase nuclear matrix [24], is a homodimeric nuclear enzyme essential for many aspects of DNA functioning, in particular negative supercoiling of DNA organised as a superhelix, a process necessary for replication, recombination and transcription of nuclear DNA [25]. This enzyme is synthesised in late $G_1$ or the early S-phase of the cell cycle, is present throughout the $G_2$ and M phases, and finally degrades as the cell enters $G_1$ [8,23,26]. In malignant cells, the half-life of Topo II is several magnitudes greater when compared with non-neoplastic cells, with undegraded Topo IIα enzyme persisting during the $G_1$ phase [8,26].

Assessment of Topo IIα expression via immunohistochemical analysis in formalin-fixed, paraffin-embedded tissue has been proven to be a reliable method to identify the percentage of cycling cells in human malignancies, including high-grade gliomas [23,27],

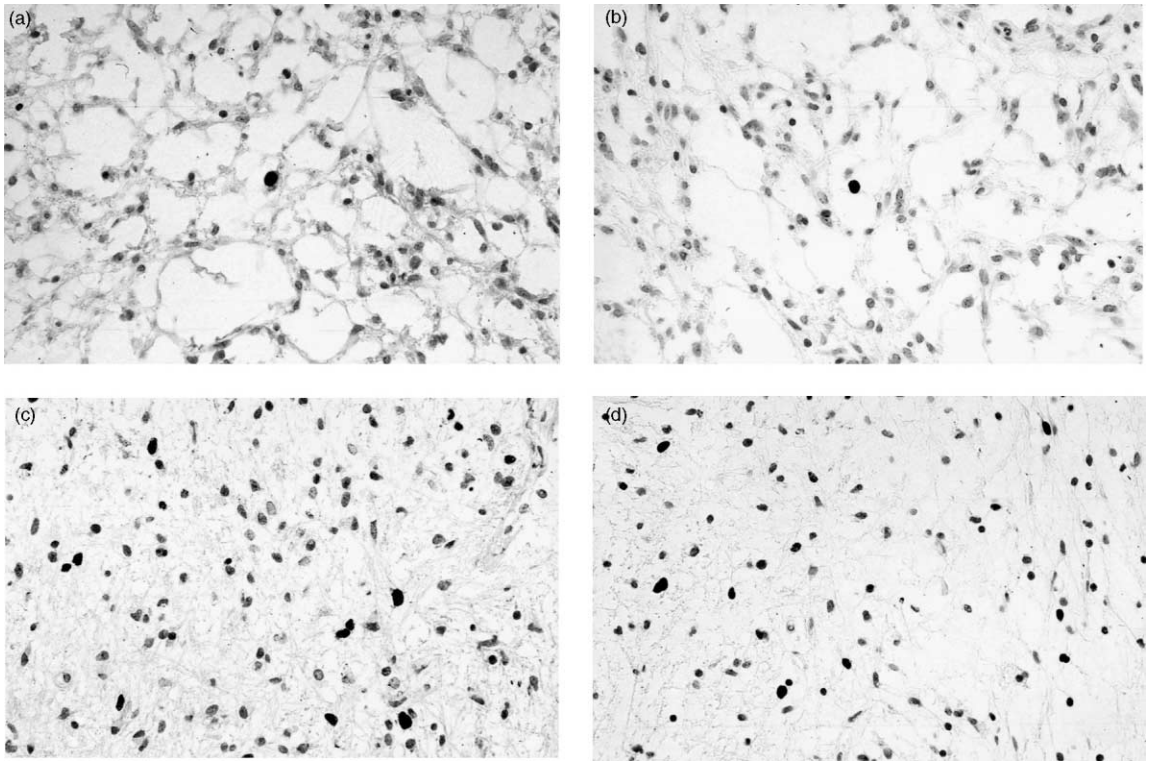


Fig. 1. Nuclear DNA topoisomerase IIα (Topo IIα) and MIB-1 immunolabelling in two tumour specimens, one with low Topo IIα (a) and MIB-1 (b) labelling indices (LIs) and another with increased Topo IIα (c) and MIB-1 (d) LIs. Original magnification: ×400.

suggesting that Topo IIα expression may be directly related to the proliferation rate in these lesions. In line with this observation, Topo IIα expression has been shown to correlate with other cell growth markers in human gliomas, in particular with the proliferation index as assessed by MIB-1 labelling of the Ki-67 nuclear antigen [23,27]. In our cohort of OPGs, we showed variable Topo IIα LIs ranging from 0.0 to 11.6. Analysing our data, we disclosed a striking correlation between Topo IIα and MIB-1 LIs, which approached high statistical significance. Hence, Topo IIα expression may serve as a biological correlate of tumour cell proliferation in OPGs of childhood. However, neither Topo IIα LIs *per se* or combined Topo IIα/MIB-1 LIs correlated with patient outcome nor with any clinical variable analysed. Therefore, assessment of cell proliferation in paediatric OPGs does not appear to assist in refining prognostic predictions and thus does not support clinical decision-making. However, our results of the survival analysis in relation to Topo IIα expression have to be judged carefully. In fact, it has to be taken into account that the follow-up period of some of our patients—particularly those in the subgroup of patients alive—is rather short with 5 patients followed-up for less than a year. It may well be that a correlation between any laboratory parameter and length of patient survival may only be possible in this class of slowly growing tumours when there is a follow-up period in the order of 20 years. Furthermore, adjuvant treatment was applied in a non-standardised fashion in this retrospective patient cohort.

Besides its role as a marker of proliferative activity, there is compelling evidence that Topo IIα is a pivotal factor in determining the response of certain tumours to a number of anticancer compounds [9]. In particular, two groups of Topo II-inhibiting drugs exist [10]. First, Topo II poisons, such as etoposide (VP-16) and teniposide (VM-26), which act to stabilise the enzyme in a so-called 'cleavable complex' formed between Topo II and DNA, thereby inhibiting its proper function [10]. A second group of catalytic inhibitors of Topo II, including doxorubicin, mitoxantrone and amsacrine, prevents Topo II from carrying out its essential physiological functions [10]. Alteration of Topo II, namely reduced expression or activity of the enzyme, has been associated with a special form of multidrug resistance (MDR), which has become known as *atypical* MDR (*at*-MDR) [28,29]. Recently, it has been shown that the incorporation of Topo II-targeted anticancer compounds into polydrug treatment protocols provided an active therapeutic regimen for patients with gliomas [12–15]. In addition, it has been demonstrated that salvage therapy with etoposide was well tolerated and showed efficacy in children with juvenile pilocytic cerebellar astrocytomas [17]. Similarly, Castello and colleagues [18] reported that in the management of non-resectable symptomatic low-grade astrocytoma, chemotherapeutic protocols including etoposide can be used to postpone radiotherapy in young children and even, in some cases, to avoid radiotherapy. In paediatric OPGs, Topo II-interfering agents have been applied in combination with other chemotherapeutic drugs [20]. In addition, it has been shown that single agent chemotherapy with oral etoposide is well tolerated and shows efficacy in children with recurrent chiasmatic-hypothalamic gliomas [21].

In view of the potential use of anticancer agents targeting Topo II for the therapy of paediatric OPGs, we

Table 3
Age-dependency of Topo IIα expression in 36 paediatric OPG patients[a]

| | Topo IIα labelling index | | |
|---|---|---|---|
| | ⩽4.0 | >4.0 | No. of cases |
| Age (years) | | | |
| ⩽3.0 | 2 | 9 | 11 |
| >3.0 | 15 | 10 | 25 |
| No. of cases | 17 | 19 | 36 |

OPG, optic pathway gliomas; LI, labelling index; S.D., standard deviation.

[a] Topo IIα expression was found to be significantly related to patient age ($P=0.031$). A subdivision of our study cohort into two groups with a cut-off age of 3.0 years demonstrated a higher LI with patients ⩽3.0 years of age compared with patients older than 3 years of age. Mean LI±S.D. was 6.51±3.50 in the former age group versus 3.18±2.05 in the latter.

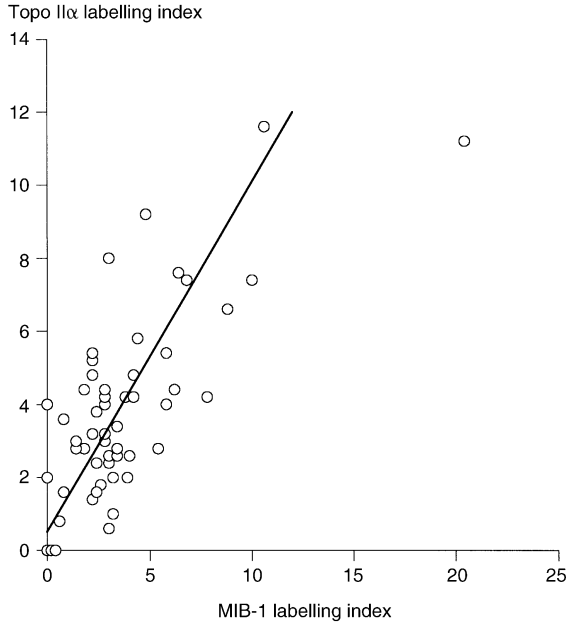


Fig. 2. Scatterplot showing the significant correlation between Topo IIα and MIB-1 labelling indices (LIs) in our series of optic pathway gliomas (OPGs). The correlation coefficient determined was $R=0.781$ ($P<0.001$).

correlated in our cohort of OPGs the intrinsic expression of Topo IIα with clinical variables. For assessment of a relationship between Topo IIα expression and age, we subdivided the study cohort into distinct groups of young and older children and tumours showing low or increased Topo IIα expression. We defined as cut-off points a Topo IIα LI of 4.0 and a patient age of 3.0 years. We found a significantly enhanced Topo IIα expression in young children less than 3 years of age. This association, however, was not absolute; 2/11 patients with a Topo IIα LI of $\leqslant$4.0 were less than 3 years of age, and 10/25 patients older than 3 years showed Topo IIα expression in more than 4.0% of the tumour cells.

Our observation of enhanced Topo IIα expression in OPGs of children less than 3 years of age could indicate that this age group may particularly benefit from a Topo II-directed chemotherapy. Such considerations are of importance in view of the deleterious toxic side-effects of radiotherapy on the developing brain in the first years of life. Irradiation is known to be particularly harmful to the diencephalon that is located directly in the radiation field in the case of OPG, and also to the brain vasculature, particularly in patients with NF1 [30]. However, it has to be mentioned that in young infants the application of Topo IIα inhibitors such as etoposide can be complicating as well. This treatment regimen particularly bears a risk for secondary leukaemia in this age group.

In clinical practice, it will be difficult to correlate a biological marker like Topo IIα with response to chemotherapy, since most often Topo IIα-interfering compounds are applied as part of a polydrug protocol to OPG patients. However, a small group of young children with recurrent OPG has been treated with oral etoposide monotherapy and a beneficial effect was noted [21]. Nevertheless, verification of an independent cytotoxic effect of Topo IIα-targeted (mono)chemotherapy in context with Topo IIα expression levels will require evaluation in large clinicopathological trials.

## Acknowledgements

This study was supported by the Forschungsgesellschaft für cerebrale Tumore, Austria. The authors are grateful to Helga Flicker and Birgit Schmid for their excellent technical assistance.

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