J Clin Pathol 1997;50:22–26

Topoisomerase IIα mRNA and tumour cell proliferation in non-Hodgkin's lymphoma

A Lohri, J Reuter, F Gudat, R Herrmann

Abstract

***Aims*—To elucidate potential mechanisms of drug resistance, levels of topoisomerase IIα mRNA, a target for cytostatic drugs, were measured in cryopreserved tumour tissue from 36 patients with non-Hodgkin's lymphoma. To evaluate the potential association between topoisomerase IIα and cell proliferation, Ki-67 immunostaining was also assessed.**

***Methods*—The study population comprised 13 patients with low grade and 20 with high grade non-Hodgkin's lymphoma. Three patients had recurrent disease. Topoisomerase IIα mRNA was quantitated by using reverse transcription polymerase chain reaction (RT-PCR) and the PCR product measured by using HPLC. The MIB-1 monoclonal antibody was used for Ki-67 immunostaining.**

***Results*—Levels of topoisomerase IIα mRNA correlated strongly with the Ki-67 labelling index and were higher in high grade than in low grade lymphomas. Patients in complete clinical remission of high grade lymphoma had a higher Ki-67 labelling index and tended to have higher topoisomerase IIα mRNA levels.**

***Conclusions*—Although topoisomerase IIα mRNA levels may be indicative of sensitivity to drugs, it is more likely that they reflect the proliferation status of the cell, which in turn involves a large number of additional molecular systems that influence response to treatment.**

(*J Clin Pathol* 1997;**50**:22–26)

Keywords: topoisomerase IIα mRNA; Ki-67 immunostaining; non-Hodgkin's lymphoma.

Department of Internal Medicine, Division of Oncology, Kantonsspital Basel, Petersgraben 4, CH-4031 Basel, Switzerland
A Lohri
R Herrmann

Department of Research, Zentrum für Lehre und Forschung, Hebelstrasse 20, CH-4031 Basel, Switzerland
R Herrmann
J Reuter
A Lohri

Institute of Pathology, Hebelstrasse 24, CH-4003 Basel, Switzerland
F Gudat

Correspondence to:
Dr Andreas Lohri.

Accepted for publication
24 September 1996

Assessment of the proliferative status of cells in non-Hodgkin's lymphomas has become a routine diagnostic procedure for many pathologists, who, in addition to traditional morphological parameters, use various techniques, such as the numbers of cells in S phase, measured by flow cytometry, or immunocytochemical assessment of proliferation associated proteins with antibodies such as the proliferating cell nuclear antigen or Ki-67. The latter detects a nuclear proliferation associated antigen corresponding to a non-histone protein with molecular weights of 395 and 345 kilodaltons, first described by Gerdes *et al.*[1] Despite the frequent use of these techniques, the traditional lymphoma classifications currently in use[2,3] and even the Revised European-American Classification (REAL)[4] do not account specifically for these proliferation markers.

The topoisomerases are a family of proteins associated with cell proliferation, cytostatic drug pharmacology and drug resistance.[5] These proteins cleave and religate strands of DNA, thereby contributing to DNA replication, structure and repair. Topoisomerase II has two isoforms (α and β), with subunit molecular weights of 170 and 180 kilodaltons, respectively. The cleavable topoisomerase/DNA complex is the target of several cytostatic drugs, such as the podophyllotoxins and anthracyclines.[5] The α isoform of topoisomerase II is the main target of topoisomerase II inhibition.[6] In vitro observations link decreasing expression, especially of the α isoform, with drug resistance,[7] thus associating the topoisomerases with the complex system of non-MDR1 or atypical drug resistance.

We have developed a modified reverse transcription polymerase chain reaction (RT-PCR) assay which permits the quantitation of specific mRNA in fresh tumour tissue.[8] However, RT-PCR is a bulk method which may have drawbacks if used on heterogeneous tumours or on tumour tissue with a considerable admixture of reactive cells.

The aim of the present study was to test the feasibility of measuring topoisomerase IIα mRNA levels in material from our lymphoma tumour bank and to compare the results with tumour morphology, and clinical and other biological parameters, such as Ki-67 immunostaining, in patients with non-Hodgkin's lymphoma.

Methods

Table 1 summarises the clinical characteristics of 36 consecutive patients included in the study. The lymphomas were classified using the Kiel classification and the International Working Formulation (IWF). Accordingly, 13 patients had low grade (table 2) and 20 high grade (table 3) non-Hodgkin's lymphoma. Twenty six patients could also be classified according to the International Prognostic Index (IPI),[9] which emcompasses the five main risk factors for non-Hodgkin's lymphoma (age, Ann Arbor stage, serum lactate dehydrogenase (LDH) activity, extranodal disease, and ECOG performance status).

QUANTITATIVE PCR

Isolation of mRNA and preparation of cDNA

Total RNA was isolated from 10 μm frozen sections using RNAzol (a guanidinium thiocyanate/phenol containing reagent; Paesel and Lorei, Frankfurt, Germany), as described by the manufacturer, and then extracted in chloroform and precipitated with isopropanol.

Table 1 Patient characteristics (n = 36)

Characteristic	*Number of patients*
Sex	
Male	20
Female	16
Median age in years (range)	67(8–92)
Biopsy at	
Initial diagnosis (adult)	30
Initial diagnosis (child)	3
At relapse (adult)	3
Histology*	
Low grade	13
High grade	20
International Prognostic Index at diagnosis*†	
Low/low-intermediate	13
High-intermediate/high	12

*Patients with biopsy specimens available at initial diagnosis.
†Data available for 25 patients.

Single stranded cDNA was synthesised from the total RNA using the Superscript RNAse H-transcriptase kit (Gibco-BRL, Gaithesburg, Maryland, USA). RNA and cDNA were stored at −70°C pending analysis.

Primers and PCR
Sense and antisense 20–22 mer primers were designed with a GC/AT content of around 50%. For topoisomerase IIα mRNA, the following primers resulted in a 430 base pair (bp) PCR product: topoisomerase IIα sense 5'-GTAGCAATAATCTAAACCTCT-3' and antisense 5'-GGTTGTAGAATTAAGAATA GC-3'.[10] For β-actin mRNA, the following primers resulted in a 227 bp PCR product: β-actin sense 5'-GGGAAATCGTGCGTGA CATT-3' and antisense 5'-GGAGTTGA AGGTAGTTTCGTG-3'.[11] Primer pairs were specific for their respective gene sequences in the human gene bank. PCR was carried out in a final volume of 100 μl containing 10 mM Tris-HCl, pH 8.1, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% gelatine, 2.5 mM of each dNTP, 150 pmol each primer, and 2.5 units AmpliTaq polymerase (Perkin Elmer Cetus, Norwalk, Connecticut, USA). PCR conditions were as follows: 94°C for four minutes, 55°C for one minute and 72°C for one minute for the first cycle; 94°C for 50 seconds, 55°C for 40 seconds and 72°C for 40 seconds for 28 cycles, and 60°C for 10 minutes. PCR products were measured by HPLC analysis using a column packed with 2.5 mm diethylaminoethyl bonded non-porous resin particles (DEAE-NPR; Perkin Elmer Cetus), as described previously.[8] The HPLC analysis translated the amount of PCR product into an area under the curve (AUC) expressed in arbitrary units. An AUC of 1000 corresponds to about 3 ng 227 bp DNA fragments. The amount of each mRNA was normalised against the expression of a housekeeping gene, β-actin, using the formula described previously.[8] PCR products within the linear range of PCR amplification were then analysed and quantitated by HPLC. Relative gene expression was defined as a ratio of target gene expression to β-actin gene expression.

Controls
For topoisomerase IIα, H69 cell lines were used as positive controls.[12] Cells were grown in RPMI 1640, supplemented with 10% fetal calf serum, and cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2/95% air. Mononuclear cells from peripheral blood (PBMC) from healthy blood donors and mononuclear cells from the bone marrow from healthy donors of allogeneic transplants were also used as controls.

IMMUNOHISTOCHEMISTRY
A monoclonal antibody directed against a formalin resistant epitope of the Ki-67 antigen was used (MIB-1, Dianova, Hamburg, Germany), following pretreatment in a microwave oven (720 W in citrate buffer), in a modification of the avidin biotin peroxidase complex technique. The percentage of Ki-67 positive cells was determined by scoring 400 tumour cells in at least three well labelled areas. Nuclei were considered positive if any nuclear staining was noted. A negative control was used in all cases.

Results
As shown in fig 1, topoisomerase IIα mRNA levels were significantly higher in high grade (lane 4) than in low grade lymphomas (lane 3; p = 0.0002, Mann-Whitney, two-tailed). Topoisomerase IIα mRNA expression was low in low grade lymphomas but was significantly higher than in normal PBMC (lane 1, negative control; p = 0.005, Mann-Whitney, two-tailed). Topoisomerase IIα mRNA was expressed moderately in bone marrow mononuclear cells (lane 2) and strongly in H69 cells (lane 5, positive controls).

Expression of topoisomerase IIα mRNA, as measured by RT-PCR, correlated strongly with

Table 2 Characteristics and results for the 13 patients with low grade lymphomas

Patient	*Sex*	*Age (years)*	*Morphology (Kiel classification)*	*IPI*	*Topoisomerase IIa mRNA ratio (× 10^{-3})*	*Ki-67 (%)*	*Follow up (months)*	*Status at follow up*
GW	F	67	Centroblastic-centrocytic, follicular, B cell	Low-intermediate	0	10.1	34	Alive, disease-free
MB	F	79	Immunocytoma (B cell)	Low	0.26	7.3	42	Alive, disease-free
GP	M	70	Immunocytoma (B cell)	High-intermediate	0.43	17	38	Alive with tumour
CS	M	64	Centroblastic-centrocytic, follicular, B cell	High-intermediate	0.65	6	22	Died of tumour
SC	M	34	Centroblastic-centrocytic, follicular, B cell	Low	0.67	15	43	Alive with tumour
LS	F	70	Lymphoepitheloid, T cell	High	0.67	6.2	26	Died of tumour
SF	M	60	Mycosis fungoides (T cell)	Low-intermediate	0.73	4.2	54	Alive with tumour
GE	F	68	Centroblastic-centrocytic, follicular, B cell	High-intermediate	0.84	11.3	28	Died of tumour
WB	M	75	B cell, MALT	Not available	0.85	4	50	Alive, disease-free
LR	F	60	AILD, T cell	High-intermediate	0.99	24	48	Alive with tumour
TR	F	62	Centroblastic-centrocytic, follicular, B cell	Not available	1.14	8	10	Alive with tumour
LH	F	59	Centroblastic-centrocytic, follicular, B cell	Low	1.71	23	60	Alive, disease-free
WW	M	76	AILD, T cell	Low-intermediate	4.05	18.3	28	Dead, disease-free

IPI = International Prognostic Index; AILD = angioimmunoblastic lymphadenopathy.

Table 3 Characteristics and results for the 20 patients with high grade lymphomas

Patient	*Sex*	*Age (years)*	*Morphology (Kiel classification)*	*IPI*	*Topoisomerase IIα mRNA ratio ($\times 10^{-3}$)*	*Ki-67 (%)*	*Follow up (months)*	*Status at follow up*
MH	M	72	Centroblastic, B cell, transformed	Low-intermediate	0.165	19.3	29	Alive with tumour
NZ	F	58	Centroblastic, B cell	High-intermediate	1.09	64	17	Alive, disease-free
KW	M	39	NOS, T cell	High-intermediate	1.28	73.2	2	Died of tumour
RV	F	70	Sezary, T cell	High-intermediate	1.31	12.3	15	Died of tumour
LH	F	92	Immunoblastic, B cell	Not available	1.72	30.2	2	Died of tumour
EB	M	71	Centroblastic, B cell, transformed?	High-intermediate	1.74	27	10	Died of tumour
FS	M	65	NOS, T cell (AILD?)	High-intermediate	1.93	41	55	Alive, disease-free
EG	F	70	Centroblastic, B cell	Low	2.25	31.2	20	Alive, disease-free
MM	M	8	Lymphoblastic, Burkitt type	Low	2.55	69.1	34	Alive, disease-free
RZ	M	14	Lymphoblastic, Burkitt type	Low-intermediate	3.31	90	26	Alive, disease-free
GO	M	42	NOS, B cell	Low	3.48	58	44	Alive, disease-free
PM	M	60	Centroblastic, B cell	High-intermediate	3.9	66.1	16	Died of tumour
BE	F	76	NOS, B cell	Not available	4.29	70.2	46	Alive with tumour
JR	M	76	Centroblastic, B cell	Low	5.4	94	43	Alive, disease-free
ST	F	13	Lymphoblastic, T cell	High	5.69	87	12	Dead, toxicity related
HB	M	40	Immunoblastic, B cell	High-intermediate	5.88	70.2	2	Died of tumour
AH	F	79	Immunoblastic, B cell	High	8.59	94	2	Died of tumour
HJ	M	68	Centroblastic, B cell	Low-intermediate	12.9	60.1	6	Died of tumour
PK	M	74	Lymphoblastic, Burkitt type	Low-intermediate	20.4	82.3	42	Alive, disease-free
LM	F	75	Centroblastic, B cell	Low-intermediate	24.09	52.2	18	Alive, disease-free

IPI = International Prognostic Index; AILD = angioimmunoblastic lymphadenopathy.

the Ki-67 labelling index, measured by immunostaining (Spearman r = 0.773, p = 0.0001, n = 36 (including the three patients with biopsy specimens at relapse), two-tailed). Owing to the lower numbers of paired samples tested, the correlation coefficient was lower if the groups with high grade (r = 0.53, p = 0.016, two-tailed, n = 20) and particularly with low grade (r = 0.35, p = 0.24, two-tailed, n = 13) disease were analysed separately.

TOPOISOMERASE IIα mRNA AND Ki-67 EXPRESSION IN PATIENTS WITH LOW GRADE LYMPHOMA

The median topoisomerase IIα ratio in low grade lymphomas was 0.73×10^{-3} (range $0–4.05 \times 10^{-3}$) and median Ki-67 staining was 10.1% (range 4–24%). Only three patients had topoisomerase IIα ratios above 1.0×10^{-3}. The patient with the highest ratio (4.05×10^{-3}) had angioimmunoblastic lymphadenopathy (T cell) with mixed small and large lymphoid elements and a Ki-67 labelling index of 18.3%. Therefore, the allocation of this case to the low grade group was very arbitrary. The patient died 29 months after diagnosis of a paraneoplastic vasculitis. Interestingly, the other two patients both had extranodal lymphoma. Patient LH had a centroblastic-centrocytic lymphoma of the thyroid with some admixture of large cells (table 2), a topoisomerase IIα mRNA ratio of 1.7×10^{-3} and a Ki-67 labelling index of 23%. It may well be that the specimen examined was chosen from an area containing more blastic cells. Patient TR had a low grade B cell lymphoma of the left breast with a low content of larger cells, a topoisomerase IIα mRNA ratio of 1.14×10^{-3} and a Ki-67 labelling index of less than 10%. The topoisomerase IIα mRNA ratio in this patient probably reflects the upper limit of what may be expected in typical material from low grade lymphomas.

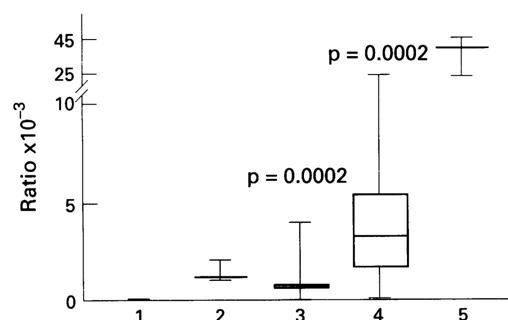

Figure 1 Topoisomerase (Topo) IIa mRNA expression. 1, normal peripheral blood mononuclear cells from 14 healthy blood donors (control 1); 2, normal bone marrow mononuclear cells from four healthy donors (residual marrow from donors of allogeneic transplants); 3, samples from 13 patients with low grade non-Hodgkin's lymphoma; 4, samples from 20 patients with high grade non-Hodgkin's lymphoma; 5, H69 cells (positive control).

TOPOISOMERASE IIα mRNA AND Ki-67 EXPRESSION IN PATIENTS WITH HIGH GRADE LYMPHOMA

The median topoisomerase IIα mRNA ratio was 3.4×10^{-3} (range $0.16–24.09 \times 10^{-3}$) and median Ki-67 staining was 65% (range 12.3–94%). Only one patient had a topoisomerase IIα mRNA ratio below 1.0×10^{-3}. This patient (MH; table 3) had a resection of a tonsillar lymphoma, which was diagnosed initially as a centroblastic, high grade lymphoma, although Ki-67 staining was relatively low (19.3%). Areas of centroblastic-centrocytic morphology were present, however, so the patient probably had a secondary centroblastic lymphoma with a low grade component. The course of this patient's disease was indolent. Five years after initial diagnosis, the patient is currently on interferon, is in partial remission and retains the retroperitoneal nodes.

Three patients had topoisomerase IIα mRNA ratios above 10×10^{-3}. The highest value was seen in patient LM with an Ann Arbor Stage II B lymphoma involving the spleen. On histology, this was a large cell (centroblastic) lymphoma with a Ki-67 labelling index of 52.2%. The patient is in complete remission three years after splenectomy and anthracycline chemotherapy. Patient KP had Ann Arbor Stage II disease with involvement of the left tonsil and the cervical nodes. Morphologically, this patient had a small, non-cleaved Burkitt type lymphoma (IWF J) with a Ki-67

labelling index of 82.3%. He also is in complete remission five years after resection, brief anthracycline chemotherapy and radiation treatment. Patient HJ had Ann Arbor Stage IV lymphoma with initial central nervous system involvement. He also had a large cell (centroblastic) lymphoma with a Ki-67 labelling index of 60.1%. This patient died without attaining complete remission.

We also investigated whether any major clinical parameter or risk factor for outcome correlated with the level of expression of topoisomerase IIα mRNA or the Ki-67 labelling index. The percentage of Ki-67 positive nuclei was significantly higher in tumours from the 12 patients with high grade lymphomas achieving a complete remission on initial treatment compared with the eight patients who never achieved complete remission ($p = 0.034$, Mann Whitney, two-tailed). Levels of topoisomerase IIα mRNA also tended to be higher in patients in complete remission, but the differences were not significant. No obvious correlations with the International Prognostic Index were found and neither topoisomerase IIα mRNA nor Ki-67 labelling index values seemed to have a significant impact on clinical outcome (disease-free and overall survival). However, these results need to be regarded with caution owing to the small number of patients investigated.

Discussion

In our series of non-Hodgkin's lymphomas, we found a close correlation between topoisomerase IIα mRNA levels, measured by quantitative RT-PCR, and the percentage of Ki-67 positive tumour cells, measured by immunohistochemistry. As this correlation is most likely because the association of both genes with cell proliferation, it was not surprising to find a significant difference between the levels of topoisomerase IIα mRNA in low and high grade tumours. We also found that patients with high grade lymphomas who achieved complete remission on initial treatment had significantly higher Ki-67 labelling indexes. Levels of topoisomerase IIα mRNA also tended to be higher in patients with complete clinical remission, but this did not reach statistical significance.

Quantitative RT-PCR measures bulk mRNA, whereas Ki-67 immunostaining is evaluated on individual tumour cells. Non-Hodgkin's lymphomas are generally heterogeneous and contain an admixture of reactive cells, both features being more pronounced in low grade tumours. Therefore, we would not have expected such a good correlation between a bulk method and a method based on the analysis of sections of relatively heterogeneous tumour tissue. This may be explained by the fact that normal lymphoid or reactive cells express topoisomerase IIα mRNA at a much a lower level than tumour cells. In our experience, mononuclear cells from normal peripheral blood often express topoisomerase IIα mRNA at the limit of detection, whereas mononuclear cells from normal bone marrow, which represent a cell fraction with lymphoid and immature myeloid cells with raised cell turnover, have a higher topoisomerase IIα mRNA content. Similar findings were reported by Kaufmann who used Western blots to detect topoisomerase IIα mRNA.[13] In comparison with the more aggressive lymphomas, we found that the topoisomerase IIα mRNA ratio measured in bone marrow mononuclear cells was relatively low, with a ratio around 1.0×10^{-3}. Dilution of topoisomerase IIα mRNA from non-malignant resting or reactive lymphoid cells does not seem to play a major role. We therefore think that RT-PCR may be a useful tool to quantitate topoisomerase IIα mRNA in lymphoma tissue.

The correlation of the topoisomerases with cell proliferation has been described in non-malignant cells.[14] However, it is not clear whether these proteins are regulated differentially in malignant cells,[15] especially in the lymphomas. This is particularly interesting in view of the fact that the topoisomerases are targets for the major cytostatic drugs, such as the anthracyclines and the epipodophyllotoxins. We know from in vitro experiments on human tumour cell lines that, at least in some systems, response to cytostatic agents depends on the amount of topoisomerase II mRNA present.[16] Therefore, it could be concluded that topoisomerase IIα mRNA levels in tumour cells are a predictive factor in the response to cytostatic drugs directed against topoisomerase. In chronic lymphocytic leukaemia—for example, very low levels of topoisomerase IIα mRNA[17] were found, which led to the conclusion that such low levels may be the reason why chronic lymphocytic leukaemia responds poorly to the anthracyclines and the epipodophyllotoxins. However, there are no data suggesting that the opposite is true. Although our data suggest a higher response rate in lymphomas with a high Ki-67 labelling index or high topoisomerase IIα mRNA levels, the number of patients studied was too small for reliable evaluation.

The traditional risk factors implemented in the International Prognostic Index[9] seem to have a much stronger impact on clinical outcome. Topoisomerase IIα mRNA levels have not been studied extensively in non-Hodgkin's lymphoma, but in other diseases, such as acute myeloid leukaemia, two groups[15 18] did not find correlations with clinical outcome, and, although a similar correlation between levels of topoisomerase IIα mRNA and Ki-67 immunostaining was seen in paediatric acute lymphoblastic leukaemia by Stammler *et al*,[19] no impact on outcome was observed. Ki-67 immunostaining has been investigated extensively in lymphomas and again, the data are contradictory. Gerdes *et al*[20] and Miller *et al*[21] reported that high Ki-67 labelling indexes were inversely related to prognosis, whereas Hall *et al*[22] identified a group of patients with very high indexes which fared better.

The wider availability of monoclonal antibodies directed against topoisomerase IIα[23] and the more frequent use of molecular methods, such as quantitative RT-PCR, will facilitate the study of topoisomerase IIα mRNA expression in larger numbers of lymphomas at initial diag-

nosis. Future studies should also focus on tissue obtained at relapse, when patients have already been exposed to cytostatic drugs. This will help to solve the question whether topoisomerase IIα is a marker of proliferation and therefore is correlated with Ki-67 in this disease, or whether the expression of these two markers is regulated independently.

1 Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer* 1983;**31**:13–20.
2 Brittinger G, Bartels H, Common H, Duhmke E, Fulle HH, Gunzer U, *et al.* Clinical and prognostic relevance of the Kiel classification of non-Hodgkin lymphomas. Results of a prospective multicenter study by the Kiel lymphoma study group. *Hematol Oncol* 1984;**2**:269–306.
3 NCI non-Hodgkin's Classification Project Writing Committee. Classification of non-Hodgkin's lymphomas. Reproducibility of major classification systems. *Cancer* 1985;**55**:91–5.
4 Harris NL, Jaffe ES, Stein H, Banks BM, Chan JKC, Cleary ML, *et al.* A revised European-American classification of lymphoid neoplasms: A proposal from the International Lymphoma Study Group. *Blood* 1994;**84**:1361–92
5 Hofmann GA, Mattern MR. Topoisomerase II in multiple drug resistance. *Cytotechnology* 1993;**12**:137–54.
6 Drake FH, Hofmann GA, Bartus HF, Mattern MR, Crooke ST, Mirabelli CK. Biochemical and pharmacological properties of p170 and p180 forms of topoisomerase II. *Biochemistry* 1989;**28**:8154–60.
7 Evans CD, Mirski SE, Danks MK, Cole SPC. Reduced levels of topoisomerase II alpha and II beta in a multidrug-resistant lung-cancer cell line. *Cancer Chemother Pharmacol* 1994;**34**:242–8.
8 Van Hille B, Lohri A, Reuter J, Herrmann R. Nonradioactive quantification of mdr1-mRNA by polymerase chain reaction amplification coupled with HPLC. *Clin Chem* 1995;**41**:1087–93.
9 Shipp M. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med* 1993;**329**:987–94.
10 Chung TD, Drake FH, Tan KB, Per SR, Crooke ST, Mirabelli CK. Characterization and immunological identification of cDNA clones encoding two human DNA topoisomerase II isozymes. *Proc Natl Acad Sci USA* 1989;**86**: 9431–5.
11 Nakajima Iijima S, Hamada H, Reddy P, Kakunaga T. Molecular structure of the human cytoplasmic beta-actin gene: interspecies homology of sequences in the introns. *Proc Natl Acad Sci USA* 1985;**82**:6133–7.
12 Cole SPC, Chanda ER, Dicke FP, Gerlach JH, Mirski SEL. Non-p-glycoprotein mediated multidrug resistance in a small cell lung cancer cell line: evidence for decreased susceptibility to drug-induced DNA damage and reduced levels of topoisomerase II. *Cancer Res* 1991;**51**:3345–52.
13 Kaufmann SH, McLaughlin SJ, Kasten MB, Liu LF, Karp JE, Burke PJ. Topoisomerase II levels during granulocytic maturation in vitro and in vivo. *Cancer Res* 1991;**51**:3534–43.
14 Sullivan DM, Glisson BS, Hodges PK, Smallwood-Kentro S, Ross WE. Proliferation dependence of topoisomerase II mediated drug action. *Biochemistry* 1986;**25**:2248–56.
15 Kaufmann SH, Karp JE, Jones RJ, Miller CB, Schneider E, Zwelling LA, *et al.* Topoisomerase II levels and drug sensitivity in adult acute myelogenous leukemia. *Blood* 1994;**83**: 517–30.
16 Fry AM, Chresta CM, Davies SM, Walker MC, Harris AL, Hartley JA, *et al.* Relationship between topoisomerase II level and chemosensitivity in human tumor cell lines. *Cancer Res* 1991;**51**:6592–5.
17 Beck J, Niethammer D, Gekeler V. High mdr1- and mrp-, but low topoisomerase II alpha-gene expression in B-cell chronic lymphocytic leukaemias. *Cancer Lett* 1994;**86**:135–42.
18 McKenna SL, West RR, Whittaker JA, Padua RA, Holmes JA. Topoisomerase II alpha expression in acute myeloid leukaemia and its relationship to clinical outcome. *Leukemia* 1994;**8**:1498–502.
19 Stammler G, Sauerbrey A, Volm M. Messenger RNA expression of resistance factors and their correlation to the proliferative activity in childhood acute lymphoblastic leukemia. *Cancer Lett* 1995;**89**:129–35.
20 Gerdes J, Stein H, Pileri S, Rivano MT, Gobbi M, Ralfkiaer E, *et al.* Prognostic relevance of tumour-cell growth fraction in malignant non-Hodgkin's lymphomas [letter]. *Lancet* 1987;**ii**:448–9.
21 Miller TP, Grogan TM, Dahlberg S, Spier CM, Braziel RM, Banks PM, *et al.* Prognostic significance of the Ki-67-associated proliferative antigen in aggressive non-Hodgkin's lymphomas: A prospective Southwest Oncology Group trial. *Blood* 1994;**83**:1460–6.
22 Hall PA, Richards MA, Gregory WM, d'Ardenne AJ, Lister TA, Stansfeld AG. The prognostic value of Ki67 immunostaining in non-Hodgkin's lymphoma. *J Pathol* 1988;**154**: 223–35.
23 Boege F, Andersen A, Jensen S, Zeidler R, Kreipe H. Proliferation-associated nuclear antigen Ki-S1 is identical with topoisomerase II alpha. Delineation of a carboxy-terminal epitope with peptide antibodies. *Am J Pathol* 1995;**146**:1302–8.