Topoisomerase IIα and IIβ expression in childhood acute lymphoblastic leukaemia: relation to prognostic factors and clinical outcome

A J Lodge, A G Hall, M M Reid, G G McIntosh, M Steward, J J Anderson, C H W Horne, B Angus

Abstract

Background/Aims—**Many regimens used in the treatment of childhood acute lymphoblastic leukaemia (ALL) include Daunorubicin or Etoposide, which act as topoisomerase poisons. It has been suggested that there may be a relation between topoisomerase expression and response to topoisomerase poisons, based mainly on results from in vitro studies. Therefore, the aim of this study was to investigate this relation in a clinical setting and determine whether topoisomerase IIα and IIβ might be of predictive value in ALL.**
Methods—**Cellular expression of topoisomerases IIα and IIβ was assessed in 177 cases of ALL by immunohistochemistry using monoclonal antibodies to the two enzymes. The percentages of cell nuclei showing positive staining for topoisomerase IIα and IIβ expression were assessed.**
Results—**Taking the series as a whole, a clear separation of survival curves was seen with the established prognostic markers white blood cell (WBC) count, CD10 status, and sex. However, topoisomerase IIα and IIβ expression showed no relation to survival. No association was found between the topoisomerases and the prognostic markers CD10 and WBC count; however, topoisomerase IIα expression was found to be related to sex, with expression being lower in girls (p = 0.002).**
Conclusions—**These results suggest that the response to topoisomerase poisons cannot be predicted by the assessment of topoisomerase IIα and IIβ expression as defined by immunohistochemistry.**
(*J Clin Pathol* 2001;**54**:31–36)

Keywords: topoisomerase; leukaemia; prognosis; immunohistochemistry

Department of Pathology, University of Newcastle, Queen Victoria Road, Newcastle upon Tyne, NE2 4HH, UK
A J Lodge
J J Anderson
B Angus

Department of Paediatric Oncology, University of Newcastle
A G Hall

Department of Haematology, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, UK
M M Reid

Novocastra Laboratories Ltd, Balliol Business Park West, Benton Lane, Longbenton, Newcastle upon Tyne, NE12 8EW, UK
G G McIntosh
M Steward
C H W Horne

Correspondence to:
Dr Lodge
ajlodge@yahoo.co.uk

Childhood acute lymphoblastic leukaemia (ALL) has traditionally responded favourably to chemotherapy and, with the introduction of combination treatments including irradiation of the central nervous system, complete remission rates of 95%, and long term survival of around 70% can now be expected. The remainder of cases relapse and henceforth have a much reduced chance of cure, probably because of the emergence of cellular drug resistance mechanisms.

However, there is increasing evidence that intensive treatment, especially over extended periods, might have long term detrimental effects upon health, possibly increasing the risks of cardiac complications and intellectual impairment. Therefore, there is a need to determine factors that can be used to predict response to treatment so that patients who will benefit from intensive treatment receive optimum amounts, and those who will not are not overexposed needlessly.

There are already several recognised prognostic factors taken into account upon diagnosis of ALL. White blood cell (WBC) count and age are perhaps the most important, with a low count indicating a favourable prognosis, and optimum survival at 3–4 years of age at presentation. Other useful indicators include DNA ploidy and karyotype of the leukaemic blasts.[1] A further observation from the Medical Research Council (MRC) chemotherapy trial UKALL X indicated that girls respond better than boys.[2] In addition, the common ALL antigen CD10 (CALLA), when present, is associated with a strongly favourable prognosis.[3 4]

Topoisomerases IIα and IIβ have recently been suggested as possible prognostic markers in some human tumours. These enzymes modulate the topological structure of DNA during replication and transcription by introducing a transient double strand break, facilitating the passing through of a double stranded DNA helix, and then resealing the gap. Topoisomerase IIα expression and activity is linked to the cell cycle,[5] and is associated with the proliferation status of cells.[6] Topoisomerase IIβ expression is more ubiquitous across tissue types, and is characteristic of a housekeeping gene.

Furthermore, both enzymes are known to be the targets of a range of antineoplastic drugs including anthracyclines and epipodophylotoxins. These topoisomerase poisons trap the enzyme on the chromosome, forming a complex, whereby re-ligation of the double strand break is prevented.[7] This can induce chromosome fragmentation and ultimately cell death. The topoisomerase poisons Daunorubicin and Etoposide are commonly used as part of the treatment regimen of childhood ALL.

Previous studies have reported variable results regarding topoisomerases as prognostic markers. It has been suggested that high concentrations of topoisomerase IIα may be associated with features of high grade, poor differentiation, or high proliferation, and studies using cell lines have suggested that cells with high amounts of the enzyme might be more sensitive to topoisomerase poisons.[8–11] These

results implied that it might be possible to predict response to treatment with topoisomerase poisons based on enzyme expression. Some previous studies involving leukaemia have found no relation between topoisomerase expression and sensitivity to topoisomerase poisons.[12] [13] However, no large in vivo studies of topoisomerase related chemosensitivity in ALL have been published to our knowledge.

We aimed to investigate the relation between topoisomerase expression in ALL and response to treatment with topoisomerase poisons in a clinical setting. We have therefore investigated topoisomerase IIα and IIβ expression by immunohistochemistry in paraffin wax embedded sections of bone marrow trephines taken at diagnosis from children with ALL. Full follow up data for the patients were obtained and topoisomerase IIα and IIβ expression was compared statistically with the known prognostic factors: WBC count, CD10 (CALLA) status, and sex, and also analysed with respect to survival.

Methods

PATIENTS STUDIED

The patients studied comprised a series of 160 cases presenting to the Royal Victoria Infirmary, Newcastle upon Tyne, UK, between 1985 and 1997, from whom bone marrow trephine biopsies were taken at diagnosis. Follow up data including age, sex, presenting WBC count, and survival times were available for all patients.

All patients were entered into either UKALL X (n = 73), XI (n = 24), or XI'92 (n = 63), clinical trials established by the MRC to assess the relative merits of different treatment regimens for childhood ALL. All patients received standard induction treatment containing Daunorubicin, a type II topoisomerase inhibitor, followed by intensification treatment containing Daunorubicin and Etoposide, another such inhibitor. The timing and number of intensification bursts varied in each trial; details can be found in Richards *et al*.[14]

ANTIBODIES

The two monoclonal antibodies used in our study were NCL-TOPOIIA (Novocastra, Newcastle, UK) (GG McIntosh *et al*, 2000, unpublished data), and TOPO2B-8.71, which was generated in our laboratories. Both antibodies were generated by utilising recombinant proteins as immunogens in the production of specific hybridoma cell lines. Recombinant protein corresponding to the C-terminal of both topoisomerase IIα and IIβ was generated using the pET recombinant protein expression system (Novogen, Madison, USA), as described previously by Lodge *et al*.[15] Primer sequences used to generate cDNA were as follows: topoisomerase IIα primers, 5'-GCTCGA GATGGCTGAAGTTTTGCCTTCTCCG-3' and 5'-GCTCGAGTTAAAACA GATCATC TTCATCTGACTC-3'; and topoisomerase IIβ primers, 5'-GGGATCCGAAAC AGACA GATAAAGTTCCAAGT-3' and 5'-GCTCGA GTCATCTGTTCTGAGGACAAC AAAAG GG-3'. The generated recombinant proteins
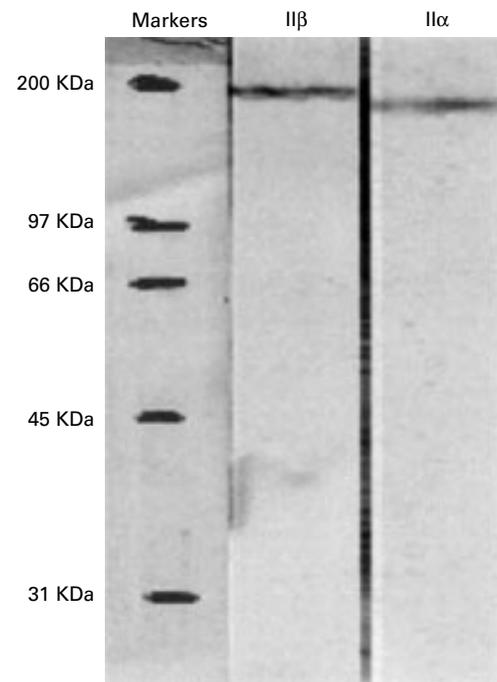

Figure 1 Western blot showing immunoreactivity of TOPO2β-8.71 with a 180 kDa protein (left) and NCL-TOPOIIA with a 170 kDa protein (right) using a lysate of a RAJI cell line.

were then used as immunogens and as screening tools in the production and selection of hybridoma cell lines secreting monoclonal antibodies, as described previously.[15]

Both NCL-TOPOIIA and TOPO2β-8.71 have been characterised extensively by enzyme linked immunosorbent assay (ELISA), western blotting, and immunohistochemistry. On western blots, the two antibodies react with single, clear bands at 170 kDa and 180 kDa, respectively (fig 1). When used in immunohistochemistry, NCL-TOPOIIA displays the established proliferation related nuclear staining pattern and TOPO2B-8.71 shows a typical ubiquitous, diffuse nuclear staining pattern with some nucleolar enhancement (fig 2).

IMMUNOHISTOCHEMISTRY

Immunohistochemistry was carried out using an indirect streptavidin ABC method as described previously.[15] Briefly, sections were dewaxed and hydrated before blocking of endogenous peroxide using methanol/ hydrogen peroxide. Antigen retrieval was carried out at high temperature and pressure using citrate buffer (200 mM citric acid, 500 mM NaOH, pH 6.0). Sections were then washed in 1× TBS (140 mM NaCl, 50 mM Tris/HCl, pH 7.6) and blocked using 20% normal rabbit serum before incubation with primary antibody (NCL-TOPOIIA at a dilution of 1/30 and TOPO2β-8.71 at 1/20). Sections were incubated with biotinylated rabbit antimouse secondary antibody (DAKO, Cambridgeshire, UK) at a dilution of 1/500, followed by streptavidin–biotin (DAKO). The reaction was developed using 3,3'-diaminobenzidine (Sigma, Poole, Dorset, UK) in a TBS/hydrogen peroxide solution for three
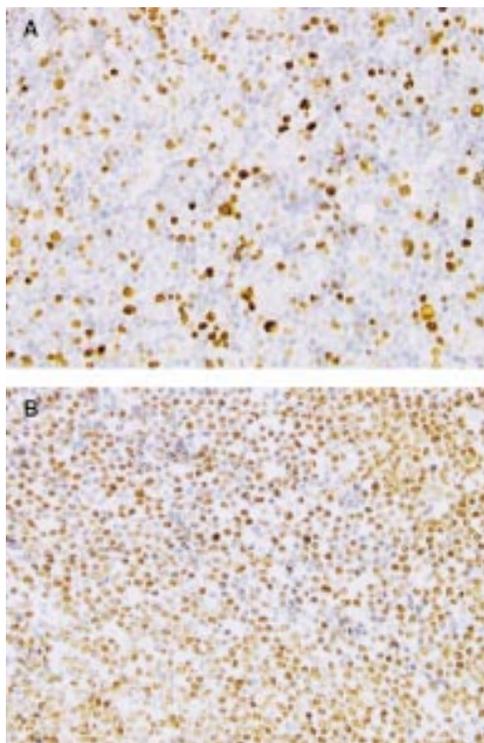
Figure 2   Immunohistochemical staining of (A) topoisomerase IIα and (B) topoisomerase IIβ in an acute lymphoblastic leukaemia bone marrow trephine showing extensive infiltration. Note intensive staining of tumour cell nuclei in both instances.

minutes. Finally, sections were counterstained using Harris's haematoxylin before being blued, and then dehydrated and mounted in DPX.

ASSESSMENT OF MYELOID CELL CONTENT

To assess the proportion of myeloid cells within the samples, a random selection of 20 cases were analysed by immunohistochemical staining using a monoclonal antibody to myeloperoxidase (NCL-MYELOp; Novocastra). The proportion of myeloid cell nuclei never exceeded 1%, and we therefore concluded that it was not necessary to adjust our results for this possible confounding factor.

SCORING METHODS

Scoring was carried out using Adobe Photoshop according to the method described by Lodge *et al.*[16] Briefly, using a light microscope, three fields of view (×400 magnification) were captured as digitised images to a conventional PC using Image Grabber (Neotech, London, UK); each image was then opened in Photoshop. A small brown dot of a previously defined threshold intensity ($R = 71$, $G = 38$, $B = 51$) was then spotted on to the image and selected using the "magic wand" tool with the tolerance set at 40. Using the "similar" command in the "select" menu, all other brown pixels in the image were selected, and counted using the "histogram" tool in the "image" menu. Selected brown pixels were then deleted and the process repeated with the counterstained blue pixels. Cell positivity was calculated as a percentage with the number of brown pixels expressed as a proportion of the total (brown + blue pixels). This analysis provides an accurate estimate of the proportion of positively stained nuclei above a defined threshold.

STATISTICAL ANALYSIS

To assess the relations between topoisomerases IIα and IIβ and WBC count (continuous), sex (categorical), and CD10 status (categorical) several statistical tests were applied. Relations between the continuous variables were assessed by Spearman's test for rank correlations (bivariate). In addition, the continuous variables were categorised (split into two groups (high and low) around the median), and then compared with the categorical variables using Fisher's exact test. In addition, to evaluate sex related trends in the distribution of the data for topoisomerases IIα and IIβ, the Mann-Whitney U test was also used.

For survival analysis, Kaplain Meier step graphs were constructed and survival differences compared using the log rank test. The data were analysed with respect to topoisomerases IIα and IIβ, sex, WBC count, and CD10 status. All analysis was carried out using SPSS statistical software.

Results

GENERAL

The series comprised 96 boys and 64 girls between the ages of 0 and 14. One hundred and sixteen patients were classed as CD10 positive at diagnosis, whereas 44 were negative. The presenting WBC count for the whole series ranged from 1.4 to 1000×10^9/litre, with a mean value of 48.94×10^9/litre. Twenty five patients were diagnosed with T cell ALL (T-ALL), 133 with B cell ALL (B-ALL), and two patients were classed as biphenotypic.

IMMUNOSTAINING FOR TOPOISOMERASES IIα AND IIβ

Topoisomerase IIα and IIβ staining was mostly confined to the nuclei of the neoplastic cells (fig 2A and B). For topoisomerase IIα, the range for the whole series was 0.2–66.12% cells positive, with a mean value of 11.27% and 25th and 75th centiles of 2.70% and 15.20%, respectively. For topoisomerase IIβ, the range for the whole series was 1.66–90.9% cells positive, with a mean value of 43.53% and 25th and 75th centiles of 17.79% and 68.08%, respectively.

RELATIONS BETWEEN VARIABLES

Considering the relations between the variables, significant correlations were found between topoisomerases IIα and IIβ ($p < 0.0001$) when assessed as both continuous and categorical variables. CD10 positivity was associated with low WBC count ($p < 0.0001$, Fisher's exact test). Low topoisomerase IIα expression was associated with female sex ($p = 0.01$, Fisher's exact test; $p = 0.002$, Mann-Whitney U test). No other associations were found.
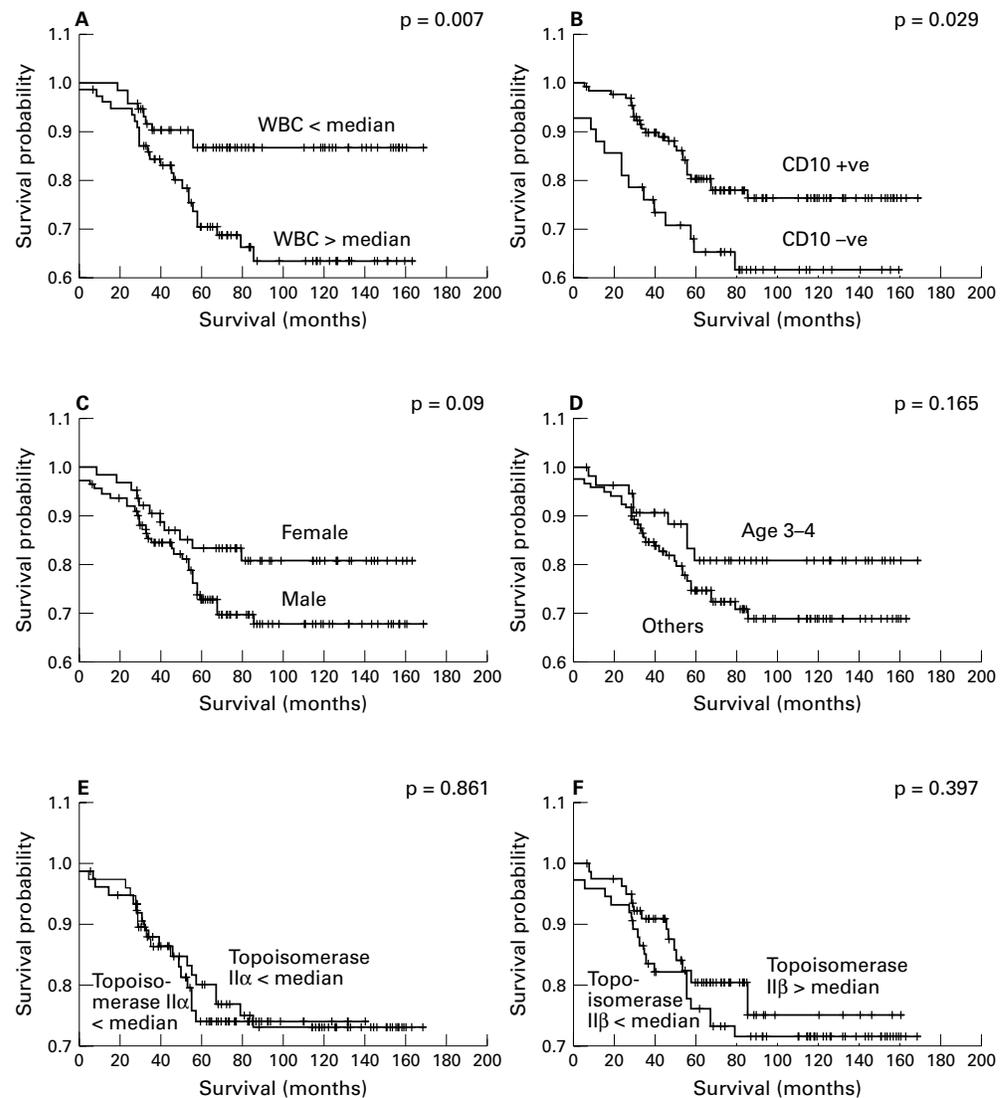
Figure 3   Kaplain Meier survival analysis for all patients (n = 160) according to (A) presenting white blood cell (WBC) count, (B) CD10, (C) sex, (D) age, (E) topoisomerase IIα, and (F) topoisomerase IIβ.

SURVIVAL ANALYSIS

Considering the series as a whole, low WBC count (p = 0.007), CD10 positivity (p = 0.029), female sex (p = 0.09), and age 3–4 at presentation (p = 0.165) were favourable prognostic markers, with the former two reaching significance (fig 3A–D). However, there was no separation of the survival curves for topoisomerases IIα and IIβ (fig 3E and F).

It should be noted that one of the treatment groups in UKALL X received no intensification treatment with topoisomerase poisons; full analysis was therefore repeated with the patients in this group omitted. However, it was found that there was little or no change in the results obtained, and thus future references (in this paper) to these results refer to those obtained using the whole series.

Discussion

Several investigators have carried out studies of the topoisomerases with respect to prognosis and predictive potential in human tumours. Most studies involve topoisomerase IIα. Expression of this enzyme has been found to be associated with other prognostic markers—for example, Ki67 in tumours such as non-Hodgkin's lymphoma,[17] breast cancer,[18] and gastric cancer,[19] and other proliferation markers such as S phase fraction in breast cancer[18] and cyclin A in leukaemia.[20]

Regarding the relation between topoisomerase expression and sensitivity to topoisomerase poisons, most previous studies have been carried out using cell lines. As stated previously, several studies have suggested that cells expressing high amounts of the enzyme are more sensitive to topoisomerase poisons,[8–10] and also that a common method of drug resistance is for cells to express decreased amounts of topoisomerase.[21 22] This would fit the hypothesis that higher concentrations of the target enzyme confer greater sensitivity to topoisomerase poisons. However, previous studies involving leukaemia have not found this relation. A study by Klumper *et al* involving ALL samples found that although topoisomerase IIα expression correlated with the percentage of ALL cells in S and G_2M phase, it did not correlate with in vitro sensitivity to Duanoru-

bicin and Teniposide.[12] In another study by Kaufmann *et al* of patients with acute myeloid leukaemia (AML), no relation was seen between topoisomerase IIα or IIβ expression and clinical outcome.[13] However, a study by McKenna *et al* of topoisomerase IIα mRNA expression in patients with AML (n = 23) suggested that further studies were warranted.[23] No large in vivo studies of topoisomerase related chemosensitivity in ALL have been published to our knowledge.

There is little published data on the potential prognostic and predictive value of topoisomerase IIβ in human tumours. A study by Houlbrook *et al* in breast cancer cell lines suggested that whereas topoisomerase IIα showed sensitivity to Amsacrine, topoisomerase IIb was more susceptible to Etoposide.[24] Another study indicated that prognostic associations seen for topoisomerase IIα might also be mediated by the IIb isoform.[11] However, a study of topoisomerase IIβ mRNA expression in AML samples by McKenna *et al* found no link with therapeutic response[25]; however, patient numbers were small (n = 26).

The most important prognostic factors in childhood ALL are presenting WBC count, CD10 status, sex, and age, and we assessed these as part of our study. We did not assess immunophenotype, because this has been shown to be of little prognostic relevance in UKALL X,[26] or initial response to treatment.

Our results are consistent with other larger studies of prognostic factors in ALL. We found that favourable outcome was associated with low WBC count at diagnosis, CD10 positivity, female sex, and age between 3–4 years. In our study, the first two factors were significant and the latter two approached significance. Confirmation of these trends provides some degree of confidence that our results are genuine. However, upon analysis of the data for topoisomerase IIα and IIβ, the survival curves showed little or no separation, and thus no relation with clinical outcome was seen.

Our analysis also showed some interesting correlations between variables. There was a significant association between the expression of topoisomerase IIα and that of topoisomerase IIβ, suggesting that expression and/or activity of the two may be coordinated. Topoisomerase IIα expression was found to be related to sex, being significantly lower in girls than in boys. This might be a characteristic of the superior survival rate seen in girls and might be related to the intrinsic biology of ALL, which clearly shows differences between girls and boys and their response to treatment. In addition, we also found a significant association between CD10 and presenting WBC count, which to our knowledge has not been documented before.

It should be noted that there are some limitations to the methods that we used in our study. First, we used immunohistochemistry to assess topoisomerase values in preserved tissue. This assay only takes into account protein concentrations and does not give an indication of enzyme activity, which would probably be more relevant. Ideally, a functional assay

should be used,[27] but such methods are technically difficult and can be performed only in cases where fresh frozen tumour cells are available. Immunohistochemistry has the great advantage of simple application using routinely processed paraffin wax blocks of bone marrow trephines, and thus large numbers of cases can be simply assessed in a short space of time.

Second, a problem arose when deciding which patients should be used for our study. Ideally, patients would have been taken from the same trial, but because of problems in obtaining tissue blocks outside the local region it was decided to carry out our study using local blocks from different trials. We felt that the treatment regimens used for UKALL X, XI, and XI'92 were sufficiently similar for the patients to be pooled. In addition, patients should ideally have received treatment with topoisomerase poisons in isolation, to minimise variation resulting from other effects. However, modern treatment of leukaemia is such that patients always receive multitreatment regimens comprising other drugs in addition to topoisomerase poisons, and the UKALL trials used here are no exception (GG McIntosh *et al*, 2000, unpublished data). We therefore feel that this is a problem that is largely beyond our control.

Therefore, it could be that the effects of differing amounts of topoisomerase IIα and IIβ expression might have been obscured by other overriding factors. However, we feel that we have minimised variation to the best of our ability, and that the validity of our study is not greatly threatened. All patients received similar amounts of topoisomerase poisons and, if a correlation had been present, a strong trend at least should have shown up in our analysis. We therefore suggest that if such a correlation does exist, it is not powerful enough to be of clinical use, and is unlikely to outweigh existing prognostic factors.

In conclusion, our results have not provided support for the hypothesis that high topoisomerase IIα and/or IIβ expression might serve as a predictor of increased survival by indicating likelihood of response to topoisomerase poisons, at least as assessed by immunohistochemistry. Although several in vitro studies have suggested that there might be a link between topoisomerase expression and response to topoisomerase poisons, our results and other studies involving leukaemic samples and cell lines have suggested that this relation is unlikely to apply in a clinical setting. However, further studies will be required to assess the true prognostic potential of topoisomerases in ALL.

1 Cassano WF, Eskenazi AE, Frantz CN. Therapy for childhood acute lymphoblastic leukemia. *Curr Opin Oncol* 1993;**5**:42–52.
2 Chessells JM, Richards SM, Bailey CC, *et al*. Gender and treatment outcome in childhood lymphoblastic leukemia—report from the MRC UKALL trials. *Br J Haematol* 1995;**89**:364–72.
3 Bene MC, Faure GC. CD10 in acute leukemias. *Haematologica* 1997;**82**:205–10.
4 Greaves MF, Brown G, Rapson NT, *et al*. Antisera to acute lymphoblastic leukemia cells. *Clin Immunol Immunopathol* 1975;**4**:67–84.
5 Woessner RD, Mattern MR, Mirabelli CK, *et al*. Proliferation-dependent and cell cycle-dependent differ-

ences in expression of the 170-kilodalton and 180-kilodalton forms of topoisomerase II in NIH-3T3 cells. *Cell Growth Differ* 1991;**2**:209–14.

6 Holden JA, Rolfson DH, Wittwer CT. Human DNA topoisomerase II: evaluation of enzyme activity in normal and neoplastic tissues. *Biochemistry* 1990;**29**:2127–34.

7 Tewey KM, Rowe TC, Yang L, *et al*. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science* 1984;**226**:466–8.

8 Davies SM, Robson CN, Davies SL, *et al*. Nuclear topoisomerase II levels correlate with the sensitivity of mammalian cells to intercalating agents and epipodophyllotoxins. *J Biol Chem* 1988;**263**:17724–9.

9 Fry AM, Chresta CM, Davies SM, *et al*. Relationship between topoisomerase II level and chemosensitivity in human tumor cell lines. *Cancer Res* 1991;**51**:6592–5.

10 Sullivan DM, Latham MD, Ross WE. Proliferation-dependent topoisomerase II content as a determinant of antineoplastic drug action in human, mouse, and Chinese hamster ovary cells. *Cancer Res* 1987;**47**:3973–9.

11 Brown GA, McPherson JP, Gu L, *et al*. Relationship of DNA topoisomerase II alpha and topoisomerase II beta expression to cytotoxicity of antineoplastic agents in human acute lymphoblastic leukemia cell lines. *Cancer Res* 1995;**55**:78–82.

12 Klumper E, Giaccone G, Pieters R, *et al*. Topoisomerase IIα gene expression in childhood acute lymphoblastic leukemia. *Leukaemia* 1995;**9**:1653–60.

13 Kaufmann SH, Karp JE, Jones RJ, *et al*. Topoisomerase II levels and drug sensitivity in adult acute myelogenous leukaemia. *Blood* 1994;**83**:517–30.

14 Richards S, Burrett J, Hann I, *et al*. Improved survival with early intensification: combined results from the Medical Research Council childhood ALL randomised trials, UKALL X and UKALL XI. *Leukemia* 1998;**12**:1031–6.

15 Lodge AJ, Anderson JJ, Ng SW, *et al*. Expression of topoisomerase IIIα in normal and neoplastic tissues determined by immunohistochemistry using a novel monoclonal antibody. *Br J Cancer* 2000;**83**:498–505.

16 Lodge AJ, Anderson JJ, Angus B. Pixel-based image cytometry for quantification of nuclear antigen expression using Adobe Photoshop. *J Cell Pathol* 2000;**4**:245–9.

17 Lohri A, Reuter J, Gudat F, *et al*. Topoisomerase II alpha mRNA and tumour cell proliferation in non-Hodgkin's lymphoma. *J Clin Pathol* 1997;**50**:22–6.

18 Jarvinen TA, Kononen J, Pelto HM, *et al*. Expression of topoisomerase II alpha is associated with rapid cell proliferation, aneuploidy, and c-erbB2 overexpression in breast cancer. *Am J Pathol* 1996;**148**:2073–82.

19 Yabuki N, Sasano H, Kato K, *et al*. Immunohistochemical study of DNA topoisomerase II in human gastric disorders. *Am J Pathol* 1996;**149**:997–1007.

20 Beck J, Handgretinger R, Dopfer R, *et al*. Expression of mdr1, mrp, topoisomerase II alpha/beta, and cyclin A in primary or relapsed states of acute lymphoblastic leukemias. *Br J Haematol* 1995;**89**:356–63.

21 Fernandes DJ, Danks MK, Beck WT. Decreased nuclear matrix DNA topoisomerase II in human leukemia cells resistant to VM-26 and M-Amsa. *Biochemistry* 1990;**29**:4235–41.

22 Towatari M, Adachi K, Marunouchi T, *et al*. Evidence for a critical role of DNA topoisomerase II alpha in drug sensitivity revealed by inducible antisense RNA in a human leukaemia cell line. *Br J Haematol* 1998;**101**:548–51.

23 McKenna SL, West RR, Whittaker JA, *et al*. Topoisomerase IIα expression in acute myeloid leukaemia and its relationship to clinical outcome. *Leukaemia* 1994;**8**:1498–502.

24 Houlbrook S, Addison CM, Davies SL, *et al*. Relationship between expression of topoisomerase II isoforms and intrinsic sensitivity to topoisomerase II inhibitors in breast cancer cell lines. *Br J Cancer* 1996;**74**:1154–4.

25 McKenna SL, Jackson H, Padua RA, *et al*. Topoisomerase II beta mRNA expression in acute myeloid leukaemia relationship to clinical outcome. *Blood* 1995;**86**(suppl 10):3067.

26 Chessells JM, Bailey C, Richards SM, *et al*. Intensification of treatment and survival in all children with lymphoblastic leukemia—results of UK Medical Research Council trial UKALL X. *Lancet* 1995;**345**:143–8.

27 Cattan AR, Levett D, Douglas EA, *et al*. Method for quantifying expression of functionally active topoisomerase II in patients with leukemia. *J Clin Pathol* 1996;**49**:848–52.

WHAT'S YOUR VIEW?

Pathology Interactive: useful or useless?

To make *Pathology Interactive* a more useful tool to help you in your CPD

. . . we need your views

Please take time to complete a self completion questionnaire and fax it back to us

Questionnaire available for printing out on:
    Pathology Interactive CD Vol 1 issue 3 (Dec 1999)
    Pathology Interactive CD Vol 2 2000
    Journal of Clinical Pathology website - www.jclinpath.com

Fax completed forms to Moira Sarsfield, *Journal of Clinical Pathology* (+44 (0)20 7383 6668).

Your time and help are appreciated.

Pathology Interactive 2000 offers up to three CPD credits per article; later issues feature case study and picture quiz formats