

Response to cladribine in previously treated patients with chronic lymphocytic leukaemia identified by *ex vivo* assessment of drug sensitivity by DiSC assay

ANDREW G. BOSANQUET,¹ J. ADRIAN COPPLESTONE,² STEPHEN A. N. JOHNSON,³ ALASTAIR G. SMITH,⁴ SARA J. POVEY,⁵ JENNIFER A. ORCHARD⁶ AND DAVID G. OSCIER⁶ ¹Royal United Hospital, Bath, ²Derriford Hospital, Plymouth, ³Taunton and Somerset Hospital, ⁴Royal South Hants Hospital, Southampton, ⁵Janssen-Cilag Ltd, Buckinghamshire, and ⁶Royal Bournemouth Hospital

Received 19 October 1998; accepted for publication 14 May 1999

Summary. The ability to identify non-responders to cytotoxic chemotherapy has significant clinical and economic benefits. Differential staining cytotoxicity (DiSC) assays were performed in 34 previously treated patients with chronic lymphocytic leukaemia prior to treatment with cladribine. Of the 28 identified as *ex vivo* sensitive, 26 achieved a complete (CR) or partial response (PR) (median length of

response 1.5 years, median survival 3.37 years) and two had a >70% fall in lymphocytes: six identified as *ex vivo* resistant failed to respond. The DiSC assay can accurately identify a subgroup of patients resistant to cladribine.

Keywords: cladribine, differential staining cytotoxicity assay, phase II trial.

The purine analogue cladribine (2-chloro-deoxyadenosine) can achieve high levels of overall responses and substantial numbers of complete responses in chronic lymphocytic leukaemia (CLL). However, the ability to identify non-responders to treatment would have significant clinical and economic benefits. A well-validated *ex vivo* method, the differential staining cytotoxicity (DiSC) assay, has been previously evaluated as a tool for identifying response to treatment with purine analogues (Bosanquet, 1991; Bosanquet & Bell, 1996; Bosanquet *et al.*, 1999).

This study aimed to investigate the prognostic value of the DiSC assay in 34 patients with CLL of B-cell origin who were either unresponsive to, or had relapsed following, one or more treatments including chlorambucil, anthracycline/anthracenedione or fludarabine.

MATERIALS AND METHODS

Patients. 34 CLL patients entered the study: 23 men and 11 women (mean age 67, range 45–83). They were either unresponsive to standard therapy or required a change of therapy because of shortened remission duration or

advanced disease. Patients were excluded if they had a life expectancy of <6 months.

DiSC assay. DiSC assays were performed before cladribine treatment using published methods (Bosanquet & Bell, 1996). Mononuclear cells were isolated from blood samples and incubated with cladribine at 1.024, 0.256, 0.064, 0.016 and 0.004 µg/ml and the LC₉₀ determined (minimum concentration of cladribine required to kill 90% of lymphocytes). The results were not made known to the clinicians managing the patients.

Cladribine treatment. Cladribine (Leustat, Janssen-Cilag Ltd) was administered as an intravenous (i.v.) 2 h infusion at a dose of 0.12 mg/kg each day for 5 consecutive days. Treatment was repeated at 28 d intervals. Depending on the onset and degree of response, patients received between four and six cycles. Those who showed no response to one cycle received no further treatment. Patients received full supportive care. Cycles could be postponed in the event of infection or thrombocytopenia (defined as a drop in platelets of >50% of pre-treatment values or <60 × 10⁹/l on the day before the next cycle). Responses were assessed using the National Cancer Institute (NCI) criteria to determine study outcomes (Cheson *et al.*, 1996). Following cessation of treatment, patients were monitored for duration of response (calculated from 4 weeks after last cladribine treatment) and survival (calculated from treatment start).

Correspondence: Dr A. G. Bosanquet, Bath Cancer Research Unit, Royal United Hospital, Bath BA1 3NG. e-mail: a.g.bosanquet@bath.ac.uk.

RESULTS

DiSC assay

Data were available from all 34 patients. Patients could be separated into two distinct groups: 6/34 patients were *ex vivo* resistant ($LC_{90} > 1 \mu\text{g/ml}$) and 28/34 patients were *ex vivo* sensitive ($LC_{90} < 0.3 \mu\text{g/ml}$). Assay results correlated with clinical response (Fig 1): 26 of the 'sensitive' group achieved a complete response (CR) or partial response (PR), and two had $>70\%$ reduction in lymphocytes but withdrew after one cycle because of adverse events (fatal myocardial infarction and haemolytic anaemia). All six patients in the resistant group failed to respond: two showed a minor and short-lived reduction in their lymphocyte count. The proportion of patients for whom the DiSC assay correctly identified clinical outcome was therefore 32/34 (94%; 95% CI 86–100%).

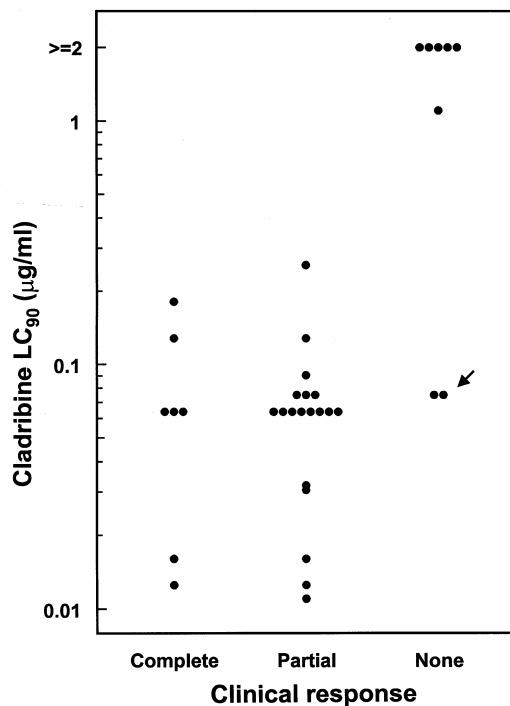


Fig 1. Comparison of cladribine sensitivity by DiSC assay (LC_{90} , $\mu\text{g/ml}$) with subsequent patient response to cladribine. The two *ex vivo* sensitive patients who achieved a $>70\%$ reduction in lymphocyte count are indicated by the arrow. ≥ 2 , LC_{90} was not observed at the maximum concentration of cladribine tested ($1.024 \mu\text{g/ml}$).

Response and survival

7/34 patients (21%; CI 7–34%) achieved a CR, 19/34 (56%; CI 39–73%) achieved a PR. Two patients showed a 71% and 88% reduction in lymphocytes but did not meet the full response criteria before withdrawing from the study. The remainder (6/34, 18%; CI 5–30%) had no response. Response rates stratified by Binet stage and W.H.O. performance status at baseline are shown in Table I.

Median remission duration of the responders was 1.50 years (range 0–3.23) for the PR patients and 2.30 years (range 0.71–3.74+) for the CR patients. Three of the CR patients and one of the PR patients remain in remission

Table I. Response rates stratified according to Binet stage and W.H.O. performance status at presentation.

	N	CR	PR	Overall response (%)
Binet stage				
A	8	4	4	100
B	13	2	8	77
C	13	1	7	62
W.H.O. performance status				
0	8	4	4	100
1	19	2	12	74
2	6	1	3	67
3	1	0	0	0

>3 years after treatment; one PR patient died of pneumonia while still in PR.

Survival of DiSC-assay-sensitive versus DiSC-assay-resistant patients is presented in Fig 2. DiSC-assay-sensitive patients survived a median of 3.37 years (CI 2.2–4.6 years). 11 patients were still alive at analysis at 3.6–4.8 years (mean 4.2 years). Three of the assay-resistant patients died very soon after cladribine treatment commenced, a similar trend to that seen following fludarabine treatment of *ex vivo* fludarabine-resistant patients (Bosanquet *et al.*, 1999). Of the other three patients, one responded to splenectomy and two survived with supportive care but poor quality of life for >3 years. Due to the long survival of these three patients, no significant difference in survival was seen between the DiSC-assay-sensitive and DiSC-assay-resistant patients ($P = 0.12$).

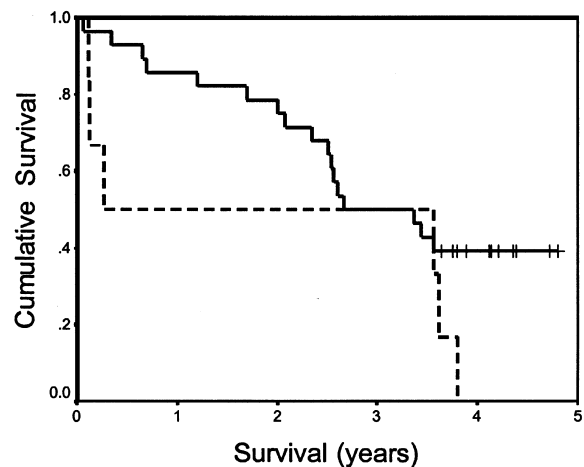


Fig 2. Survival of DiSC assay-sensitive (—) and assay-resistant (---) patients from beginning of cladribine treatment. Difference in survival was not significant ($P = 0.12$).

Previous treatment

Fourteen patients had received fludarabine; 12 responded but later relapsed. The two other patients who had not responded to previous fludarabine treatment were resistant to cladribine by DiSC assay and withdrew from the study

because of persistent thrombocytopenia after one or two cycles of cladribine. Another patient developed autoimmune haemolytic anaemia during cladribine treatment and therefore withdrew, having previously stopped fludarabine treatment for the same condition.

Adverse events

Five patients developed severe haemolysis shortly after treatment; 4/5 had positive direct antiglobulin tests (DAGT), two subsequently died, all four had previously received chlorambucil and fludarabine. Haemolysis occurred during the first (one), second (three) or third (one) cycle of cladribine, from 5–24 (median 18) days after cladribine administration. All four patients who received more than one cycle of cladribine achieved a partial response. These cases have been reported by Chasty *et al* (1998). Six patients experienced persistent thrombocytopenia. Two other patients died during the course of treatment (from a myocardial infarct and cerebrovascular accident). Three patients developed further malignancies or disease progression.

DISCUSSION

In this study the DiSC assay identified resistance to cladribine, having previously been shown to detect *ex vivo* sensitivity and resistance accurately in both solid and haematological tumours (Bosanquet, 1991; Bosanquet & Bell, 1996; Bosanquet *et al*, 1999). Other groups have published preliminary results using *ex vivo* drug sensitivity tests in CLL with mixed success (Bromidge *et al*, 1998; Liu *et al*, 1997; Lambert *et al*, 1992; Hansen *et al*, 1991). However, the results of this work and the powerful prognostic factor of the fludarabine DiSC assay sensitivity for response and survival after fludarabine (Bosanquet *et al*, 1999) suggest that the DiSC assay should be used routinely before purine analogues are administered to patients.

Knowledge of *ex vivo* resistance enables patients who are unlikely to respond to avoid ineffective and potentially debilitating treatment. Patients with good performance status and in an early stage of their disease (i.e. W.H.O. grade 1 or Binet stage A) are more likely to achieve a CR or PR with cladribine (Table I).

The overall response rate observed in this study (76%) is similar to that reported for untreated patients (73–87.5%) (Mulligan *et al*, 1996; Saven *et al*, 1995; Delannoy *et al*, 1995; Juliusson *et al*, 1996). This group is therefore more sensitive than most previously treated patients – indeed many patients had responded to their previous treatments. The median response duration compares favourably with previous reports.

In conclusion, the DiSC assay can delineate CLL patients who could, or who will not, benefit from cladribine treatment.

ACKNOWLEDGMENTS

We thank the following for their help and collaboration with this study: Mrs A. Burlton, Mr P. Bell (Royal United Hospital,

Bath); Dr A. G. Prentice, Dr M. D. Hamon, Sister E. Leppard (Derriford Hospital, Plymouth); Dr M. J. Phillips, Sister C. Ibberson (Taunton and Somerset Hospital); Dr A. S. Duncombe, Mrs S. M. Course (Royal Southants Hospital, Southampton). The study was funded by Janssen-Cilag Ltd and we acknowledge the contributions of Elizabeth Hope (study monitoring), Lorna Legg (statistical analysis) and Elizabeth Wager (manuscript preparation).

REFERENCES

- Bosanquet, A.G. (1991) Correlations between therapeutic response of leukaemias and in-vitro drug sensitivity assay. *Lancet*, **337**, 711–714.
- Bosanquet, A.G. & Bell, P.B. (1996) Enhanced *ex vivo* drug sensitivity testing of chronic lymphocytic leukemia using refined DiSC assay methodology. *Leukemia Research*, **20**, 143–153.
- Bosanquet, A.G., Johnson, S.A. & Richards, S.M. (1999) Prognosis for fludarabine therapy of chronic lymphocytic leukaemia based on *ex vivo* drug response by DiSC assay. *British Journal of Haematology*, **106**, 71–77.
- Bromidge, T.J., Turner, D.L., Howe, D.J., Johnson, S.A. & Rule, S.A.J. (1998) *In vitro* chemosensitivity of chronic lymphocytic leukaemia to purine analogues: correlation with clinical course. *Leukemia*, **12**, 1230–1235.
- Chasty, R.C., Myint, H., Oscier, D.G., Orchard, J.A., Bussutil, D.P., Hamon, M.D., Prentice, A.G. & Copplestone, J.A. (1998) Autoimmune haemolysis in patients with B-CLL treated with chlorodeoxyadenosine (CDA). *Leukaemia and Lymphoma*, **29**, 391–398.
- Cheson, B.D., Bennett, J.M., Grever, M., Kay, N., Keating, M.J., O'Brien, S. & Rai, K.R. (1996) National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood*, **87**, 4990–4997.
- Delannoy, A., Martiat, P., Gala, J.L., Deneys, V., Ferrant, A., Bosly, A., Schieff, J.M. & Michaux, J.L. (1995) 2-Chlorodeoxyadenosine for patients with previously untreated chronic lymphocytic leukemia. *Leukemia*, **9**, 1130–1135.
- Hanson, J.A., Bentley, D.P., Bean, E.A., Nute, S.R. & Moore, J.L. (1991) *In vitro* chemosensitivity testing in chronic lymphocytic leukemia patients. *Leukemia Research*, **15**, 565–569.
- Juliusson, G., Christiansen, I., Hansen, M.M., Johnson, S., Kimby, E., Elmhorn-Rosenborg, A. & Liliemark, J. (1996) Oral cladribine as primary therapy for patients with B-cell chronic lymphocytic leukemia. *Journal of Clinical Oncology*, **14**, 2160–2166.
- Lambert, E., Rees, J.K. & Twentyman, P.R. (1992) Resistance circumvention strategies tested in clinical leukemia specimens using the MTT colorimetric assay. *Leukemia*, **6**, 1063–1071.
- Liu, K.Z., Schultz, C.P., Johnston, J.B., Lee, K. & Mantsch, H.H. (1997) Comparison of infrared spectra of CLL cells with their *ex vivo* sensitivity (MTT assay) to chlorambucil and cladribine. *Leukemia Research*, **21**, 1125–1133.
- Mulligan, S.P., Gill, D., Eliadis, P., Dale, B., Bunce, I., Bashford, J., Page, F., Matthews, J. & Bradstock, K. (1996) 2-Chlorodeoxyadenosine by shortened infusion results in high response rate in previously untreated chronic lymphocytic leukemia: an Australian Leukemia Study Group Trial. *British Journal of Haematology*, **93**, (Suppl. 2), 116.
- Saven, A., Lemon, R.H., Kosty, M., Beutler, E. & Piro, L.D. (1995) 2-Chlorodeoxyadenosine activity in patients with untreated chronic lymphocytic leukemia (CLL). *Journal of Clinical Oncology*, **13**, 570–574.