measures to prevent disease transmission and progression. Therefore, counselling and testing for HIV infection should be offered routinely to all pregnant women.

REFERENCES

1. Centers for Disease Control. AIDS and the human immunodeficiency virus infection in the United States: 1988 update. *MMWR* 1989; **38:** S1–S38.
2. Novick LF, Berns D, Stricof R, Stevens R, Pass K, Wethers J. HIV seroprevalence in newborns in New York State. *JAMA* 1989; **261:** 1745–50.
3. Hoff R, Berardi VP, Werblen BJ, Mahoney-Trout L, Mitchell ML, Grady GF. Seroprevalence of human immunodeficiency virus among childbearing women. *N Engl J Med* 1988; **318:** 525–30.
4. Maryland AIDS update. State of Maryland Communicable Disease Bulletin. Maryland Department of Health and Mental Hygiene, August, 1990.
5. Peckham CS, Tedder RS, Briggs M, et al. Prevalence of maternal HIV infection based on unlinked anonymous testing of newborn babies. *Lancet* 1990; **335:** 516–19.
6. Ippolito G, Stegagno M, Costa Angeloni P, Angeloni U, Guzanti E. Detection of anti-HIV antibodies in newborns: a blind serosurvey in 92 Italian hospitals. Les implications du SIDA pour la mere et l'infant. Paris, Nov 27–30, 1989: 141 (abstr).
7. Centers for Disease Control. Recommendations for assisting in the prevention of perinatal transmission of human T-lymphotropic virus type III/lymphadenopathy-associated virus and acquired immunodeficiency syndrome. *MMWR* 1985; **34:** 721–31.
8. Prevention of human immune deficiency virus infection and acquired immune deficiency syndrome. Washington: American College of Obstetricians and Gynecologists, 1987: 1–8.
9. Minkoff HL, Landesman SH. The case for routinely offering prenatal testing for human immunodeficiency virus. *Am J Obstet Gynecol* 1988; **159:** 793–96.
10. Barbacci MB, Dalabetta GA, Repke JT, Talbot BL, Polk BF, Chaisson RE. Human immunodeficiency virus infection in women attending an inner-city obstetrics clinic: ineffectiveness of targeted screening. *Sex Transm Dis* 1990; **17:** 122–26.
11. Landesman SH, Minkoff HL, Holman S, et al. Serosurvey of human immunodeficiency virus infections in parturients: implications for human immunodeficiency virus testing programs of pregnant women. *JAMA* 1987; **258:** 2701–03.
12. Ascher MS, Burke MD, Dodd RY, Saah AJ, Rapoza NP. Serologic tests for HIV in AIDS: information for the practising physician. Chicago: American Medical Association, 1987.
13. Jonas MM, Schiff ER, O'Sullivan MJ, et al. Failure of Centers for Disease Control criteria to identify hepatitis B infection in a large municipal obstetrical population. *Am Intern Med* 1987; **107:** 335–37.

Correlations between therapeutic response of leukaemias and in-vitro drug-sensitivity assay

ANDREW G. BOSANQUET

To develop the differential staining cytotoxicity (DiSC) assay, an in-vitro drug sensitivity test designed specifically for use with fresh human haematological tumour cells, into a predictive test for response to therapy as well as a tool for identifying new treatment strategies, test results have been correlated with response in patients with haematological malignancies. 22 of 119 tests indicated extreme drug resistance (tumour cell survival >55%) in vitro. None of these patients responded to chemotherapy. The proportion of patients responding to therapy and 50% patient survival rose with in-vitro drug sensitivity. 11 specimens showing extreme drug resistance to the agents prescribed for the patient were subjected to drug sensitivity tests of drugs not prescribed; patients showing some sensitivity to these drugs had better survival than did those with extreme drug resistance to all drugs tested. The DiSC assay can also be used to identify new agents worth testing in clinical trials, and cross resistance profiles of new agents may indicate what combinations might be useful.

Lancet 1991; **337**: 711–14.

Introduction

The differential staining cytotoxicity (DiSC) assay is one of several in-vitro drug sensitivity assays using fresh human tumour cells.[1-7] These tests are similar in their ability to predict drug sensitivity or resistance and have test sensitivities and specificities of around 0·8.[8] The DiSC assay, originally pioneered by Weisenthal,[9,10] has been developed so that it is now a method particularly well suited to the testing of leukaemias and other haematological malignancies such as myeloma and non-Hodgkin lymphoma.[1,11] Unlike the colony-forming assay, the DiSC assay can be done with cells that are not dividing. Although more labour intensive than the tetrazolium colorimetric (MTT) assay, the DiSC assay has an advantage of having as its end-point cells that can be identified morphologically. This feature enables the testing of malignancies with impure cell populations such as myeloma, where the response of only the plasma cells is required; the effect on the tumour cells is thus distinguishable from any effect on normal cells.[11]

Until recently, in-vitro drug sensitivity assays had not been widely accepted. However, they can identify drug sensitivity with around 50–70% accuracy and resistance with >90% accuracy.[8] Recently a ^{3}H-thymidine assay using solid tumours has been described as a drug resistance assay;[2] it has a predictive accuracy of >99%. Use of in-vitro assays can prevent patients from being treated with drugs to which they are very unlikely to respond clinically. Tests that use fresh human tumour cells can also be of considerable benefit in helping to identify new treatment regimens.

This paper describes the past 9 years' work on the DiSC assay to relate in-vitro test results to patient response and to test new anticancer drugs against fresh human tumour cells.

Methods

Drugs

Drugs were frozen at −70°C at 10 times the final concentrations required. In-vitro drug concentrations and radiation dose used to calculate the minimum tumour cell survival (TCS) values for correlation with patient response were as published[3] except for the following: chlorambucil, 1000 ng/ml; doxorubicin, 50 ng/ml; epirubicin, 50 ng/ml; mitoxantrone, 10 ng/ml; and 2·56 Gy irradiation on the 6MV linear accelerator (Hinkley HJ, Bosanquet AG, unpublished). 4-hydroperoxycyclophosphamide was used in vitro in place of cyclophosphamide.

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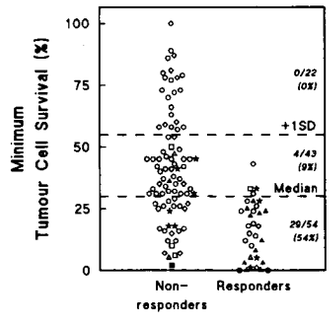

Fig 1—Minimum tumour cell survival of cells isolated from treatment responders and non-responders.

Numbers (and percentages) shown are of responders classified by TCS values—ie, TCS < median TCS value; TCS between median and median + 1 SD; and TCS > median + 1 SD. Data points include the 61 clinical correlations published previously.[1,11,12,21]

Symbols: ○ = CLL; △ = acute lymphocytic leukaemia; □ = acute myelogenous leukaemia; * = non-Hodgkin lymphoma; ◇ = myeloma; solid symbols = specimens from childhood leukaemias.

DiSC assay

Blood (3–10 ml into edetic acid) and bone marrow samples (0·2–1·0 ml into heparin) were collected between November, 1981, and June, 1990, mainly from outpatient clinics. Normal lymphocytes were obtained from healthy volunteers and normal bone marrow from bone marrow donors. Cells were isolated, and DiSC assays were done as previously described.[11–13] Briefly, isolated cells were incubated in RPMI 1640 medium with or without drugs. After 4 days, dead cells were stained with fast green/nigrosin, and an internal standard of fixed duck erythrocytes (DRBC) was added. The cells were cytocentrifuged onto microscope slides and the live cells counterstained with a Romanowsky stain ('Diff Quik', Merz and Dade, Dudingen, Switzerland) for identification. For each disc of cells, the ratio of live tumour cells to DRBCs was counted; when expressed as a percentage of control the ratio was termed the TCS value.

Analysis

The tumour LC_{90} (the lowest concentration of drug required to kill 90% of the cells compared with control) was calculated from the TCS value. Occasionally, when the maximum drug concentration tested turned out to be less than the LC_{90}, the LC_{90} was taken as the maximum concentration multiplied by the dilution factor. LC_{90}s for normal cells were determined with 2–8 normal bone marrow mononuclear cells (from bone marrow donors) ($bmLC_{90}$) and 3–20 normal mixed peripheral blood lymphocytes ($lyLC_{90}$). The therapeutic index was calculated as $\frac{1}{2}$(mean $bmLC_{90}$ + mean $lyLC_{90}$)/mean tumour LC_{90}. All means and SDs were calculated on log LC_{90}s.

DiSC assay results to March, 1989, were compared with patient response. The in-vitro result used was the minimum TCS value of the drugs given in vivo. Patient response was defined according to published criteria of response for the various tumour types. Both complete and partial responses were accepted as responses.

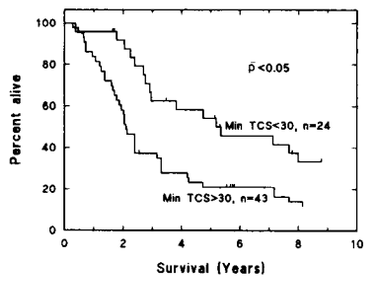

Fig 2—Survival of CLL patients after DiSC assay.

Survival was calculated from DiSC assay date to Oct 1, 1990.

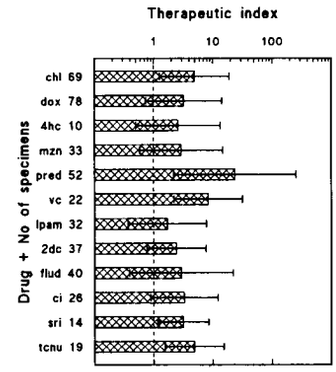

Fig 3—In-vitro drug efficacy of CLL.

A therapeutic index of greater than 1·0 suggests that the drug has killed more tumour cells than normal cells.

Abbreviations: chl, chlorambucil; dox, doxorubicin; 4hc, 4-hydroperoxycyclophosphamide; mzn, mitoxantrone; pred, prednisolone; vc, vincristine; lpam, melphalan; 2dc, pentostatin; flud, fludarabine; ci, anthrapyrazolone (CI 941); sri, SRI 62-834; tcnu, tauromustine.

Results

From November, 1981, to June, 1990, 834 DiSC assays were done on haematological samples obtained mainly from patients attending the Royal United Hospital's outpatients clinics. Patients were treated according to local protocols, without reference to DiSC assay results except on a few occasions (< 10) in early years. By March, 1989, 119 correlations could be made between the minimum TCS in vitro and patient response in vivo (fig 1).

From these data three groups of patients have been identified, similar to those suggested recently by Kern, who used a radioactive precursor uptake assay.[2] For patients whose minimum TCS was below the median TCS of all samples of 30% (fig 1), patient response was 54% or approximately double that of the whole group (28%). For patients with TCS of 30–55%, patient response was 9%. None of the 22 patients with TCS values above 55% responded to treatment. Kern termed the third group an extreme drug resistance (EDR) group on the basis of the absence of drug cytotoxicity in vitro;[2] their patients in this group had an in-vivo response rate of < 1% (1/127).

The survival of the 67 patients who had chronic lymphatic leukaemia (CLL) and were tested in vitro with drugs (rather than radiotherapy) is shown in fig 2. The survival for those with EDR was similar to that for those with TCS 30–55%, so the results have been pooled. 50% survival of the group resistant in vitro (TCS > 30) was 2·1 years, whereas for the sensitive group (TCS < 30) it was 5·3 years. The difference in the survival curves was significant ($\chi^2 = 4{\cdot}47$, $p < 0{\cdot}05$).

11 EDR CLL specimens were also subjected to sensitivity tests with drugs that were not prescribed for the patients. 4 of these specimens showed EDR (TCS > 55%) to all the drugs tested in vitro (median = 5), and the patients survived 0·5, 0·7, 0·9, and 2·0 years from DiSC assay. The other 7 specimens, although showing EDR to the drugs given in vivo, showed some sensitivity to the other drugs tested (TCS < 48%), and these patients survived a median of 3·3 years.

Results from specimens tested in the DiSC assay between June, 1987 (at which point in-vitro drug concentration

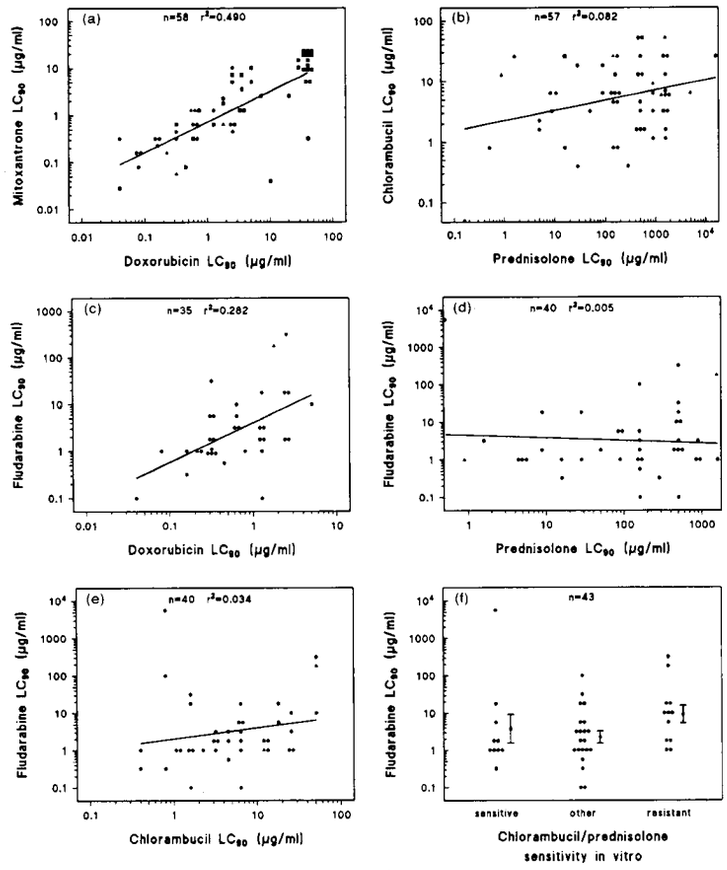

Fig 4—Drug cross-resistance patterns.

Data points show results from specimens where two (or three for fig 4f) relevant drugs were tested. The statistical significance of the regression line was (a) $p < <0{\cdot}001$; (b) $p = 0{\cdot}05$ (but removal of the one point in the bottom left corner makes this not significant); (c) $p = 0{\cdot}001$; (d and e) $p > 0{\cdot}1$.

Symbols: □ = solid tumours; ○ = CLL; △ = other leukaemias. Drug LC_{90}s were determined as described for fig 2. Repeat data were scattered to ease visualisation.

ranges were increased to obtain LC_{90}s), and June, 1990, have been used to identify potentially useful therapeutic agents for the patients. The therapeutic indices for several known and potential anticancer drugs against CLL lymphocytes (fig 3) indicate good activity in vitro for all drugs known to be active in CLL and less activity for melphalan, as occurs in clinical practice. The new drugs anthrapyrazolone, tauromustine, and the ether lipid SRI 62-834 also show good activity in vitro against CLL.

Many in-vitro drug cross-resistance patterns have been identified among the 20 or so drugs tested in the DiSC assay (fig 4)—eg, the expected cross-resistance of doxorubicin and mitoxantrone, which are similar drugs ($p < <0{\cdot}001$); the expected lack of cross-resistance between chlorambucil and prednisolone (dissimilar drugs with different modes of action); and some cross resistance between fludarabine and doxorubicin ($p = 0{\cdot}001$) but not between fludarabine and prednisolone or chlorambucil. Little difference is seen in fludarabine sensitivity whether specimens are sensitive to both chlorambucil and prednisolone, or resistant to both these drugs.

Discussion

In-vitro drug sensitivity assays for individual testing of chemotherapy have been investigated for decades, most intensively since Salmon popularised the colony-forming assay in 1978.[14] Until recently, these tests had not gained widespread acceptance and have seldom been used to guide patient treatment, but now there is increasing evidence of the usefulness of some in-vitro assays, including the DiSC assay[15] that "stands out as [an assay] with particular promise".[16]

In-vitro drug sensitivity tests can predict drug resistance with near 100% accuracy,[2,8] a point that has only recently been recognised as being of benefit to the patient. Knowledge of in-vitro resistance means that patients need not receive drugs to which they are very likely to be resistant. The option to give them alternative and possibly more effective therapy might increase recruitment to clinical trials of new agents.[2] Identification of drug sensitivity may also help in the choosing of the best drug combination for a patient, a point that also has benefits for hospital budgets. However, as pointed out by Calvert et al,[17] financial considerations should not colour interpretation of the clinical data.

In the series of patient correlations described here is a group of patients showing extreme drug resistance in vitro to the drugs that they were treated with. All these patients did not respond at all to their drug treatment. Although CLL patients within this EDR group who were resistant to all the drugs tested in vitro survived only an average of 1·0 year, those showing sensitivity to some of the drugs survived 3·3

years (even though the drugs to which they were sensitive were not used in their treatment). These results suggest that there is a group of CLL patients who can be accurately identified as being resistant to some regimens (those in whom all the drugs of the combination show TCS values of >55%) and will not respond; such patients would be ideal candidates for trials of drug resistance modifiers or new agents.

Of the 43 CLL patients in the TCS>30 groups, 18 of them (42%) showed sensitivity (TCS<30) to at least one other drug. More generally, if patients had been treated on the basis of the best three in-vitro drugs results, 37/54 (69%) of patients who did not respond would have had different treatment, with greater probability of entering a remission and increased life-expectancy.

Many drug resistance modifiers, the best known of which is verapamil, are being investigated in vitro, and clinical trials have been set up. Preliminary results with verapamil have been disappointing because of cardiotoxicity and a low response rate. We have found, by using fresh CLL cells,[7] that amiodarone, reserpine, verapamil/lignocaine, and ethacrynic acid act as drug resistance modifiers on CLL lymphocytes in vitro (Boullier BA, Bosanquet AG, Nagourney RA, Weisenthal LM, unpublished). Of particular interest was the observation that samples differ in the drug resistance modifiers to which they respond. Thus, although 71% of specimens had their resistance reduced by one agent or another, each drug resistance modifier sensitised an average of only 25% of specimens, which suggests that drug resistance modifiers should be chosen for each patient individually by in-vitro drug sensitivity assay.

We have investigated the in-vitro drug sensitivity of CLL specimens to several drugs and have found that anthrapyrazolone, tauromustine, and SRI 62-834 have a high therapeutic index. These agents are being developed for use in solid tumours, but the findings reported here suggest that phase II trials in CLL are warranted. In general, the phase II testing of new anticancer drugs could be conducted more effectively by targeting the tumour types that respond well in in-vitro tests.

Fludarabine has been shown by DiSC assay to be not only active but also not cross resistant with both chlorambucil and prednisolone. This finding suggests that the drug should be active in end-stage CLL patients, who would be expected to be resistant to both chlorambucil and prednisolone, since these two drugs form the basic therapy for CLL;[18] this prediction has been borne out clinically.[19,20] DiSC assays have also indicated that addition of fludarabine to a chlorambucil and prednisolone regimen should increase the likelihood of remission; phase III trials of this combination are already underway.[18]

6 results in figure 1 are from CLL samples irradiated in vitro when the patients underwent splenic irradiation (Hinkley HJ, Bosanquet AG, unpublished). Samples from 5 patients showed extreme radio-resistance at 2·56 Gy (ie, TCS>55%), and these patients did not respond to splenic irradiation. The sixth showed sensitivity in vitro (TCS=0% at 2·56 Gy) and obtained a complete response. In 41 specimens (including the 6 above), a TCS value of ≤5% was observed at 2·56 Gy in 8 (19%), which suggests that radiotherapy could be of considerable benefit to this group of patients.

In conclusion, the treatment of many patients with leukaemia (especially those with resistant disease) could be improved by use of the DiSC assay. Drug sensitivities and their cross-resistance patterns as determined by DiSC assay may also help in the selection of agents for phase II and phase III trials in leukaemias.

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REFERENCES

1. Bird MC, Bosanquet AG, Forskitt S, Gilby ED. Long-term comparison of results of a drug sensitivity assay in vitro with patient response in lymphatic neoplasms. *Cancer* 1988; **61:** 1104–09.
2. Kern DH, Weisenthal LM. Highly specific prediction of antineoplastic drug resistance with an in vitro assay using suprapharmacologic drug exposures. *J Natl Cancer Inst* 1990; **82:** 582–88.
3. Efferth T, Volm M. Rapid detection assays for multidrug resistance. *Arzneim-Forsch/Drug Res* 1988; **38:** 1771–74.
4. Von Hoff DD, Sandbach JF, Clark GM, et al. Selection of cancer chemotherapy for a patient by an in vitro assay versus a clinician. *J Natl Cancer Inst* 1990; **82:** 110–16.
5. Sanfilippo O, Silvestrini R, Salvioni R, Pizzocaro G. Relation of in vitro drug activity to clinical response in a prospective trial for advanced germ cell testicular tumours. *Eur Urol* 1989; **16:** 450–55.
6. Tidefelt U, Sundman-Enberg B, Rhedin A-S, Paul C. In vitro drug testing in patients with acute leukaemia with incubations mimicking in vivo intracellular drug concentrations. *Eur J Haematol* 1989; **43:** 374–84.
7. Weisenthal LM, Nagourney RA, Kern DH, et al. Approach to the clinical circumvention of drug resistance utilizing a non-clonogenic in vitro assay measuring the effects of drugs, radiation, and interleukin II on largely non-dividing cells. *Adv Clin Oncol* 1989; **1:** 91–111.
8. Weisenthal LM, Lippman ME. Clonogenic and nonclonogenic in vitro chemosensitivity assays. *Cancer Treat Rep* 1985; **69:** 615–32.
9. Weisenthal LM, Marsden JA, Dill PL, Macaluso CK. A novel dye exclusion method for testing in vitro chemosensitivity of human tumours. *Cancer Res* 1983; **43:** 749–57.
10. Weisenthal LM, Dill PL, Kurnick NB, Lippman ME. Comparison of dye exclusion assays with a clonogenic assay in the determination of drug-induced cytotoxicity. *Cancer Res* 1983; **43:** 258–64.
11. Bird MC, Bosanquet AG, Gilby ED. In vitro determination of tumour chemosensitivity in haematological malignancies. *Hematol Oncol* 1985; **3:** 1–9.
12. Bosanquet AG, Bird MC, Price WJP, Gilby ED. An assessment of a short-term tumour chemosensitivity assay in chronic lymphocytic leukaemia. *Br J Cancer* 1983; **47:** 781–89.
13. Bird MC, Bosanquet AG, Forskitt S, Gilby ED. Semi-micro adaptation of a 4-day differential staining cytotoxicity (DiSC) assay for determining the in vitro chemosensitivity of haematological malignancies. *Leuk Res* 1986; **10:** 445–49.
14. Salmon SE, Hamburger AW, Soehnlen B, Durie BGM, Alberts DS, Moon TE. Quantitation of differential sensitivity of human-tumor stem cells to anticancer drugs. *N Engl J Med* 1978; **298:** 1321–27.
15. Veerman AJP, Pieters R. Drug sensitivity assays in leukaemia and lymphoma. *Br J Haematol* 1990; **74:** 381–84.
16. Shoemaker RH. New approaches to antitumor drug screening: the colony-forming assay. *Cancer Treat Rep* 1986; **70:** 9–12.
17. Calvert AH, Horwich A, Newlands ES, et al. Carboplatin or cisplatin? *Lancet* 1988; ii: 577–78.
18. Cheson BD. Current approaches to the chemotherapy of B-cell chronic lymphocytic leukemia: a review. *Am J Hematol* 1989; **32:** 72–77.
19. Grever MR, Kopecky KJ, Coltman CA, et al. Fludarabine monophosphate: a potentially useful agent in chronic lymphocytic leukemia. *Nouv Rev Fr Hematol* 1988; **30:** 457–59.
20. Keating MJ, Kantarjian H, Talpaz M, Redman J, McCredie KB. Fludarabine therapy in chronic lymphocytic leukemia (CLL). *Nouv Rev Fr Hematol* 1988; **30:** 461–66.
21. Bird MC, Forskitt S, Gilby ED, Bosanquet AG. The influence of sample source and cell concentration on the in vitro chemosensitivity of haematological tumours. *Br J Cancer* 1986; **53:** 539–45.