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Ex vivo assessment of drug response by differential staining cytotoxicity (DiSC) assay suggests a biological basis for equality of chemotherapy irrespective of age for patients with chronic lymphocytic leukaemia

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With a mean age at diagnosis for chronic lymphocytic leukaemia (CLL) of 65 years, development of optimal therapeutic regimens has been hampered by the advanced age of patients. In general, because of comorbidity older patients are not treated with the intent of achieving a complete response and so do not attain the quality of response of younger patients and do not survive as long. We have investigated whether or not *ex vivo* cellular sensitivity to cytotoxic drugs could be an underlying biological basis for this age differential in response and survival by comparing *ex vivo* drug response with age in untreated CLL patients. Cells from 365 untreated CLL patients aged 31.1–87.1 years (average 65.3 years) were tested for drug response by differential staining cytotoxicity (DiSC) assay with a panel of 10 drugs. An average of 280 results (range 196–361) obtained for each drug was compared with patient age. For chlorambucil, cyclophosphamide, prednisolone, vincristine, doxorubicin, epirubicin, fludarabine, cladribine and methylprednisolone, no relationship was found between *ex vivo* drug response and age ($r < 0.12$). For pentostatin, a possible but very weak relationship ($r = 0.18$; $n = 210$; $P = 0.06$) was found. We conclude that cellular sensitivity to cytotoxic drugs does not support the differential treatment of older and younger CLL patients. *Leukemia* (2000) **14,** 712–715.
Keywords: cellular sensitivity; cytotoxic; DiSC assay; chronic lymphocytic leukaemia; age

Introduction

Traditionally, older chronic lymphocytic leukaemia (CLL) patients are treated less aggressively than their younger counterparts, as evidenced by bone marrow transplants and intensive chemotherapy being restricted to younger (<50 years) patients. It is likely that concurrent disease or comorbidity foster a cautious approach to administering chemotherapy to older patients (>65 years). Where this is not the case, it is debatable whether older patients should automatically be treated less aggressively that younger.[1] Almost without exception, the behaviour of common cancers is no more or less aggressive in older patients.[1]

Studies have found a biological basis for a difference in response to chemotherapy with respect to age in ALL,[2] childhood ALL[3] and AML.[4] This raises the question of whether there is a similar biological basis to support age-related treatment schedules in CLL, which, unlike other malignancies, is genetically very stable.[5]

CLL is a markedly heterogeneous disease with respect to prognosis and clinical course. Treatment decisions are guided by stage of disease and it is generally agreed that only patients with advanced Binet or Rai stages require chemotherapy.[6] CLL is predominantly a disease of affluent, western populations and, as with the majority of cancers, a disease associated with old age. With the increase of life expectancy in these populations, increasing numbers of people will be diagnosed with CLL. However, treatment choices for CLL patients, traditionally derived from results of controlled randomised clinical trials, may be based on unrepresentative populations: elderly people are more likely to be excluded from clinical trials.[7]

Results from the differential staining cytotoxicity (DiSC) assay, an *ex vivo* drug response assay where drug-induced apoptotic cell death is observed, correlate well with subsequent patient response in CLL.[8–10] In this work, therefore, we investigated the relationship between *ex vivo* drug response and age in untreated CLL patients.

Methods

Cells from untreated CLL patients, many of whom were entered into the MRC CLLIII trial running from 1990 to 1997, were sent to Bath Cancer Research for drug response testing. Diagnosis was according to standard MRC criteria: $\geqslant 10 \times 10^9$/l mature lymphocytes (<10% atypical); with markers of SmIg+/–, CD5+, CD19+, CD20+, CD23+, FMC7–/+, CD22+/–; and ⩾40% bone marrow infiltration.

The DiSC assay has been described in detail.[11] Briefly, mononuclear cells were isolated from blood over Ficoll-Hypaque, washed, counted and incubated for 94 h with seven to 10 standard CLL drugs: chlorambucil, cyclophosphamide (as mafosfamide *in vitro*), prednisolone, vincristine, doxorubicin, epirubicin, fludarabine, cladribine, pentostatin and methylprednisolone. At the end of incubation, fixed duck erythrocytes (as an internal standard) and fast green/nigrosin (to stain dead cells black) were added and the cells cytocentrifuged. Counterstaining with a Romanowsky stain allowed identification of remaining live cells which were evaluated morphologically to determine cell survival. LC_{90}s, the concentration of drug to produce a 90% reduction in tumour cell survival compared with control cells, were then calculated by fitting logistic regression curves to the cell survival data.[12] Results were analysed using SPSS version 8.0 for Windows 95 to determine whether drug response by DiSC assay might be affected by age.

Results

Cells from 365 untreated CLL patients aged 31.1–87.1 years (mean ± s.d., 65.3 ± 9.7 years) were tested for *ex vivo* drug response by DiSC assay with a panel of seven to 10 drugs. 295 patients had been entered into the MRC CLLIII and 10 into the MRC CLLII trial, 19 patients were expected to enter MRC CLLIII but were not randomised, 13 patients were from trials of cladribine, 17 patients were accrued locally and 11 specimens were sent for drug sensitivity testing to aid choice of treatment. An average of 280 results (range 196–361) was

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Table 1 Drug LC_{90}s and correlation with age

Drug	Abbreviation	No. of results	Mean LC_{90} (GSD)[a]			Pearson correlation coefficient
			Overall	<65 years	>65 years	
Cladribine	cda	291	0.0378 (2.99)	0.0360	0.0397	0.069
Chlorambucil	chl	361	2.12 (2.85)	2.00	2.24	0.054
Pentostatin	dc	210	14.3 (67.0)	10.2	19.1	0.189
Doxorubicin	dox	268	0.193 (2.33)	0.202	0.185	0.027
Epirubicin	epi	347	0.270 (1.80)	0.260	0.278	0.064
Fludarabine	fl	348	0.335 (3.20)	0.304	0.364	0.118
Mafosfamide	maf	259	1.12 (2.15)	1.09	1.15	0.097
Methylprednisolone	mep	233	15.5 (8.45)	15.1	16.0	0.056
Prednisolone	pr	283	46.9 (9.62)	42.2	51.4	0.108
Vincristine	vc	196	1.34 (5.96)	1.07	1.63	0.081

[a]As LC_{90}s have a log-normal distribution, means are based on log values (ie geometric means) and GSD is the geometric standard deviation.[12]

obtained for each drug (Table 1). Figure 1, the plot of fludarabine LC_{90} against age, is typical of other drug results and illustrates that there is no relationship between LC_{90} and patient age (Pearson correlation coefficients <0.12, Table 1). When mean LC_{90}s are plotted against age groups of <50, 50–60, 60–70 and >70 years (Figure 2) only pentostatin shows any trend. The results for pentostatin are plotted in more detail in Figure 3. This shows the trend is minimal ($r = 0.189$; $n = 210$; $P = 0.06$ after Bonferroni correction) partly due to the very large scatter of pentostatin results (Table 1).

Discussion

Half the newly diagnosed cases of cancer in western Europe will be in people aged over 70 years and 20–30 per 100 000 of this age group are diagnosed with CLL. The median age of CLL patients at diagnosis is 65 years, and only 10–15% are under 50 years of age.

Selecting optimum treatment for elderly CLL patients is problematic. The elderly are often disenfranchised from entry to clinical trials,[7,13] because of worries that death from comorbidity would diminish the potential effect of the therapy being tested. This bias against recruitment of elderly patients means that clinical trial information for this cohort of patients is not readily available. Therefore, selection of therapy for elderly patients, and dosage choices, must be made empirically.

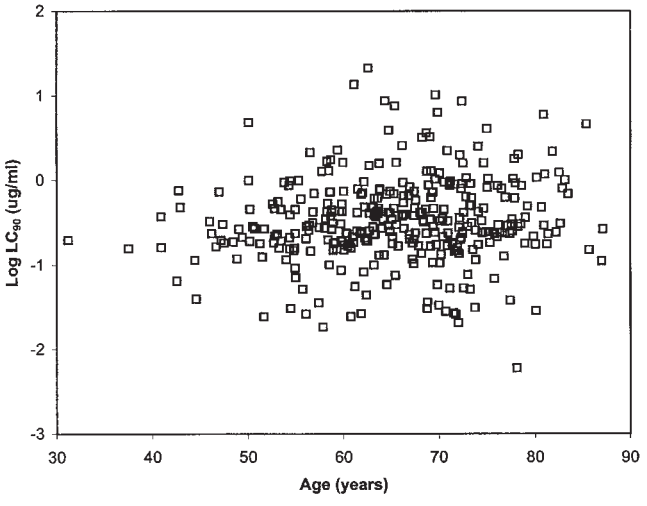

Figure 1 Age *vs* fludarabine LC_{90} (on a log scale).

Clinician belief that the elderly are poorly tolerant of chemotherapy or radiotherapy may result in the under-referral and under-treatment of many elderly patients.[1] Many physicians arbitrarily use single agents (as opposed to combination chemotherapy), decrease the dosage of chemotherapy, or even withhold cytotoxics for the elderly patient. Ironically, elderly patients are then eminently suitable candidates to evaluate new agents for CLL because they have usually received less previous treatment than younger patients.[1]

Some newer anthracyclines and purine analogues seem to have a beneficial therapeutic index particularly for elderly patients.[14] There are other precedents to suggest that age is not a significant factor in cellular response to treatment. One study found that in human T-lymphocytes, age does not affect the cytotoxic and mutagenic effects of X-irradiation.[15] In our laboratories, we found no difference in cellular sensitivity in normal cells from patients older or younger than 65 years of age.[12] Also the neoplastic cells of B-CLL, even when exposed to genotoxic therapeutic agents, seem to have unusual genetic stability compared with CML[16] and other human neoplasms.[5]

With few exceptions, common cancers progress in comparable ways in young or older patients and there are no distinctive features of CLL in younger adults.[17] Older patients may have concurrent health problems, but the malignant disease still exerts greatest influence on the survival of the patient irrespective of age. Since CLL therapy is based on prognosis, inadequate or under-treatment in deference to the age of the patient will often ensure that death is due to leukaemia, not comorbidity. Elderly CLL patients are a heterogeneous group and therefore as much attention must be given to the treatment offered to them as that offered to younger patients. Although they might not accept treatment as readily as younger patients, biologically elderly CLL patients have comparable *ex vivo* cellular sensitivity and may enjoy robust good health. In NHL, a multivariate analysis showed that given doxorubicin-containing chemotherapy, the risk of treatment-related death for elderly patients is associated with poor performance status rather than increasing chronological age.[18]

In some haematological malignancies other than CLL, a biological basis has been found to suggest different treatment is necessary in different age groups. In a study looking at the relationship of age and *ex vivo* drug resistance in children with ALL, there was a biological factor to explain the response

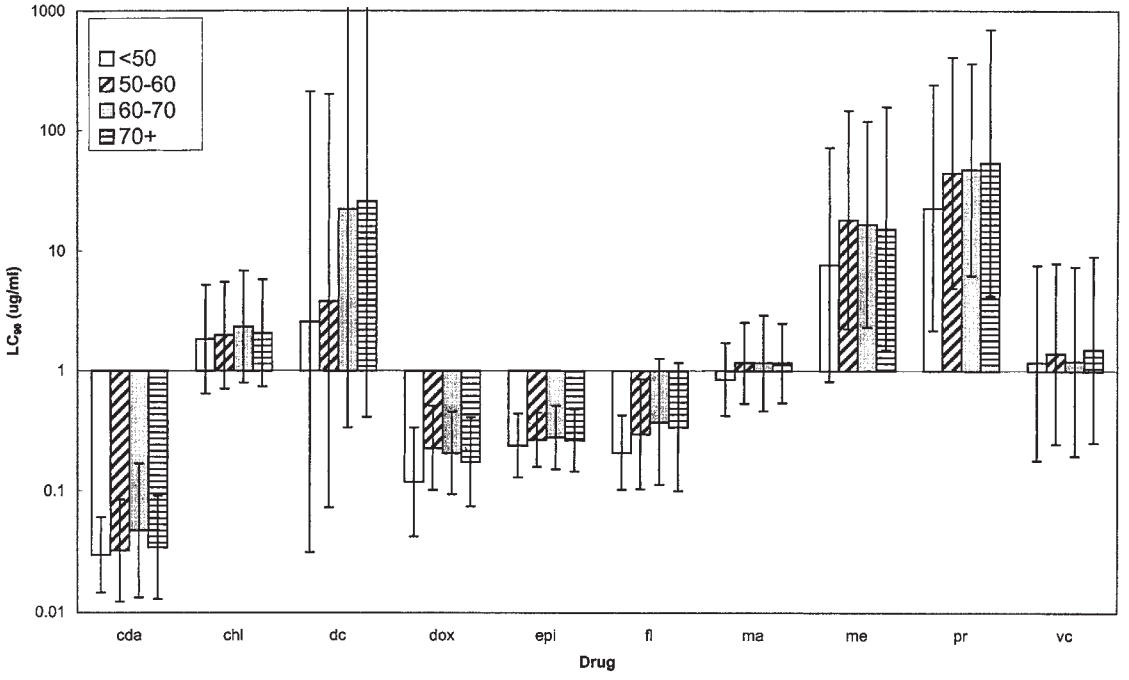

Figure 2 Age *vs* LC_{90}. Drug abbreviations are as in Table 1. Age categories are <50 years, 50–60 years, 60–70 years and 70+ years. Values are mean 1 s.d.

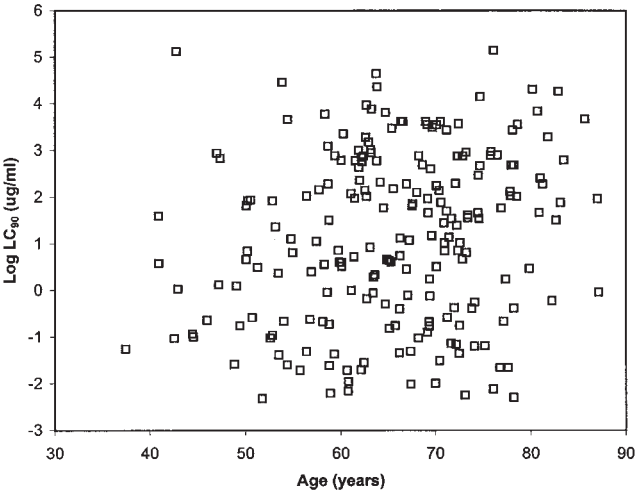

Figure 3 Age *vs* pentostatin LC_{90} (on a log scale).

of infants (<1.5 years) and older children (>10 years): their poor prognosis was associated with *ex vivo* resistance to glucocorticoids and asparaginase.[3] Compared with younger patients, elderly patients with acute myeloid leukaemia (AML) respond poorly to conventional chemotherapy.[4] Whilst elderly MDR1 *de novo* AML patients with favourable cytogenetics had a complete response (CR) rate of 81%, a greater proportion expressed MDR1 with a reduced CR rate, suggesting increased MDR1 expression as a biological basis for this difference. For AML patients aged 65+ years, overall survival when given intensive-induction chemotherapy was longer (median 21 weeks) than when mild cytoreductive chemotherapy was given (11 weeks),[19] indicating that a supportive care approach in treating elderly AML constituted suboptimal treatment.

Although the data are not definitive, several studies have shown that intensive therapy in younger CLL patients with advanced disease may prolong survival.[20] Response to therapy is generally associated with longer survival,[21] but with fludarabine this was not the case.[22] In aiming at palliation or less aggressive therapy in deference to the age of their patient, doctors may be giving suboptimal therapy to older CLL patients. Previous treatment strategies for CLL have been, for the most part, unsuccessful in prolonging survival. The benefits of achieving CR should be balanced with the risks associated with therapy and treatment aimed at achieving the greatest possible response with acceptable toxicity.

Drug response data from DiSC assays facilitate achieving a clinical response.[8–10,23] Patients treated with assay-sensitive drugs are eight times more likely to respond to treatment than those treated with assay-resistant drugs.[10] In a study of breast cancer patients, it was concluded that clinicians managed patients with a highly treatable disease on the basis of age rather than physiological status, thereby worsening the prognosis for older patients.[24] CLL is eminently treatable and DiSC assay guided therapy can make economic sense; life could be extended for £1470 per life year gained,[25] and expensive and toxic therapy that is highly unlikely to be effective can be avoided.[8]

Although studies have shown a significantly greater prevalence of severe and lethal toxicity in elderly lymphoma patients treated with intensive chemotherapy regimens,[26] age alone should not be the determining factor in modifying treatment in CLL. Cancer treatment in the elderly is remarkably safe when the co-morbid conditions are also treated.[27] The elderly suffer more serious myelotoxicity and are more susceptible to cardiotoxicity or neurotoxicity, but age is a poor predictor for complications in other organs or systems.[28] Elderly patients with good performance status may benefit from systemic cancer treatment to the same extent as younger patients.[18,28]

Conclusions

Many older CLL patients have a life expectancy of 10+ years and have robust good health compared with their peers of, say, 30 years ago. The poorer response and survival of older CLL patients is not caused by an intrinsically reduced cellular sensitivity to cytotoxic drugs, but might be influenced by empirical under-treatment. *Ex vivo* drug response by DiSC assay helps to identify optimum treatment for patients with CLL and may give a response and survival advantage. 'Clear information and realistic hopes may enable older patients to assert themselves and not be paternalistically palmed off with suboptimal management'.[13]

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