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A Phase I Study of Immunostimulation and Toxicity in Patients with Colorectal Carcinoma Using the Immunomodulator 3,6-Bis(2-piperidinoethoxy) Acridine Trihydrochloride (CL 246738)

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Abstract—*Seventeen patients with residual or recurrent colorectal carcinoma were given a new synthetic immunomodulator* [3,6-*bis*(2-*piperidinoethoxy*) *acridine trihydrochloride CL*246738) *as part of a phase I clinical trial. No patients had undergone previous immunotherapy or chemotherapy. Detailed immunological studies including interferon levels, interleukin 2 levels, natural killer cell function, mitogen responses of lymphocytes, immunoglobulin levels and lymphocyte subpopulation levels were analysed in the patients who received this drug in an attempt to find out whether there was any biological activity identifiable in humans. None of the subjects showed any significant increases in post treatment values of the immunological parameters studied. Toxic effects of the drug at high doses included nausea, diarrhoea and decreased levels of consciousness.*

In conclusion, no immunological effects were identified following the administration of CL 246738 *in human subjects with recurrent or residual colorectal cancer.*

INTRODUCTION

3,6-BIS(2-PIPERIDINOETHOXY) ACRIDINE TRIHYDROCHLORIDE (CL 246738) is a novel synthetic immunomodulator which has been shown to have a variety of immunological activities *in vitro* and in animal models. These actions include induction of murine tumour inhibitory macrophages [1], restoration of antibody production and mitogen responses in immunosuppressed animals [2], augmentation of natural killer (NK) cell activity in mice [3], increased survival time of mice bearing implanted tumours and induction of interferon in mice [2].

This paper reports the results of an ascending dose phase I trial using CL 246738 in 17 patients with residual or recurrent colorectal carcinoma. We administered CL 246738 to the patients to find the maximum tolerated dose and study the effect of the drug on components of the immune system.

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PATIENTS, MATERIALS AND METHODS

Seventeen patients with residual or recurrent colorectal adenocarcinoma were entered into the trial with informed consent. The mean age of the patients was 65 years (range 42–77). There were eight males and nine females. No patient had received previous chemotherapy, immunotherapy or radiotherapy to more than two marrow bearing sites. No patient had undergone surgery in the 6 weeks prior to or after the study because of the effects of operation on the immune system [4] and no patient had received a blood transfusion in the month prior to or after entry into the study [5]. No patient had significant cardiovascular or hepatic disorder as judged by electrocardiogram and standard liver function tests (serum bilirubin, alakaline phosphatase and SGOT).

One patient (No. 10) had impaired renal function due to pelvic recurrence of her tumour causing ureteric obstruction and was withdrawn from the study. All other patients completed the study as planned and had normal renal function as judged by serum urea and electrolyte levels.

There were no deaths during the period of the study.

DRUG DOSAGE

CL 246738 (Cyanamid, Pearl River, New York) was given orally as a single dose. The dose was given according to a predetermined escalating schedule. Patients 1 and 2 received 1 mg/kg; patients 3, 4 and 5 5 mg/kg; patients 6, 7 and 8 10 mg/kg; patients 9, 10, 11 and 12 15 mg/kg; and patients 13, 14, 15, 16 and 17 20 mg/kg. Blood levels of the drug were estimated at time 0 and 2, 4, 6, 8, 12 and 24 h and at 4, 7, 9, 16, 23 and 30 days after drug administration. Following drug ingestion vital signs were monitored every 6 h for 48 h and additional untoward effects also noted.

DELAYED TYPE HYPERSENSITIVITY SKIN TESTING

This was performed prior to and on days 1 and 31 after drug administration using the 'multitest C.M.I.' battery of antigens (Institut Merieux, 17 rue Bourgelat, 69002 Lyon, France). Briefly, this disposable plastic applicator is composed of eight sterile heads with seven skin-test antigens and a glycerin negative control. Synchronous intradermal multipuncture application of antigens and control is carried out on healthy skin on the flexor aspect of the forearm and 48 h after application induration diameters are measured providing a score of reactivity.

IMMUNOLOGICAL STUDIES

1. *Interferon levels (alpha YOK, alpha NK2 and gamma)*

These were measured prior to and on days 1, 2, 5, 8, 16, 22 and 29 after drug administration. This was performed by Boots-Celltech Diagnostics Ltd, Slough, Berkshire, U.K., using a two site immunoradiometric assay (IRMA). Results are expressed as units of activity/ml serum. Typically, these assays had a minimum detection limit of 1 μ/ml and an intra assay coefficient of variation of 10%.

2. *Interleukin II levels*

Maintenance of mouse cytotoxic T lymphocyte line cells (CTLL). Continuously proliferating CTLL cells were maintained in growth medium [RPMI/10% FCS supplemented with 5% (v/v) of 10 × concentrates of BC-IL preparations (as a source of IL-2)] by incubation (37°C, 100% humidity, 5% carbon dioxide in air).

Assays. Serial two- or four-fold dilutions of the Laboratory Standard of IL-2 (BC 6A) and the unknown BC-IL preparations were made in RPMI/10% FCS. Replicate (3–4) aliquots (100 μl) of the samples, or RPMI/10% FCS alone as control, were distributed into individual wells of round-bottomed 96-well culture plates (Costar No. 3796: Northumbria Biologicals Ltd). Target cells—CTLL cells at 2×10^6 viable cells/ml in RPMI/10% FCS—were added as 100 μl volumes to each well.

Tritiated thymidine ([methyl-^{3}H]thymidine, 185 GBq/mmol: Amersham Int.)—50 μl aliquots at 74 kBq/ml (CTLL cells) in RPMI/10% FCS—was then added to each well, and re-incubated for 18 h.

Radioisotopic incorporation by the cultured target cells was determined by liquid scintillation spectrometry (1216 Rackbeta: LKB Instruments Ltd) and expressed as disintegrations per minute (dpm) per culture.

Qantification of IL-2. Potency estimation. The results were plotted (log dose/linear response) and data from the steepest, most linear and most parallel parts of the dose–response curves were chosen by inspection for estimation of potencies. The value for the laboratory standard was used to estimate the concentration of IL-2 units/ml in the unknown BC-IL preparations.

3. *Lymphocyte subpopulations*

These were analysed using flow cytometry (Beckton Dickinson, FACS 420, California) as previously described [4]. A panel of monoclonal antibodies directed against T (CD 3), B (CD 19), helper inducer (CD 4), suppressor cytotoxic (CD 8) and natural killer (CD 16) lymphocytes was used. Subpopulations were measured prior to and on days 1, 2, 5, 7, 14, 21 and 30 after drug administration.

4. *Natural killer (NK) cell function*

This was carried out using K562 cells as targets for human NK cells as previously described [6] prior to and on days 1, 2, 5, 7, 14, 21 and 30 after drug administration. Healthy volunteers were tested on the same day and time as the patients and the same control was used throughout each patients period of study. Specific cytotoxicity was calculated using the formula:

$$\text{Percentage cytotoxicity} = \frac{E - S}{M - S} \times 100$$

where E = experimental release in counts/mins, S = spontaneous release in counts/min and M = maximum release in counts/min.

The results from the NK cell assays using an effector:target cell ratio of 50:1 were used for statistical analysis.

The two sample t test was used to analyse pairs of data from normals and patients tested on the same day.

5. *Immunoglobulin levels*

IgM, IgA and IgE levels were measured using immunoelectrophoresis prior to and on weeks 1, 2, 3 and 4 after drug administration.

6. *Mitogen responses of lymphocytes*

This was performed using phytohaemagglutinin and concanavalin A in concentrations of 1 and 10 μg/ml respectively. The methods used were as previously described [7].

7. *Statistical analysis*

Comparisons of data sets before and after treatment in the patients were performed using the Wilcoxon rank sum test for paired non-parametric data.

RESULTS

1. *Clinical*

Clinically apparent side-effects were noticed in 13 patients (Table 3). The commonest side-effect was vomiting, usually within 2 h of drug ingestion (patients 6, 7, 9, 10, 11, 12, 13, 14 and 15). Diarrhoea also occurred within 6 h of drug ingestion in patients 3, 4, 11, 12, 13 and 15. Two patients developed drowsiness shortly after taking the drug (patients 14 and 16). The frequency and severity of the side-effects appeared to be dose related. There were no other clinical effects of the drug, and all of the above were self-limiting and lasted for no more than 60 min. There was no evidence of marrow suppression, liver toxicity or renal toxicity in any patient judged by standard laboratory tests over 1 month after drug administration. No patient showed evidence of static disease or tumour regression during the study.

2. *Delayed type hypersensitivity (DTH)*

This is summarized in Table 1. It can be seen that some patients showed an increase in DTH response at both 24 h and at 1 month after drug administration, but this was not consistently related to the drug dose given, nor did it achieve statistical significance.

Table 1. Delayed type hypersensitivity (DTH) scores prior to 24 h and 30 days after administration of CL 246738.

Patient	Dose(mg/kg)		DTH score Pre	24 h	30 days
1	1		0	8	0
2	1		5	13	6
3	5		4	7	5
4	5		3	7	6
5	5		3	7	4
6	10		4	5	2
7	10		5	3	—
8	10		8	9	5
9	15		7	11	19
10	15	withdrawn	—	—	—
11	15		5	5	10
12	15		10	6	15
13	20		6	5	5
14	20		7	7	9
15	20		11	10	9
16	20		6	4	0
17	20		5	5	9

Table 2. Significance of serial NK function assays compared to healthy volunteers

Concentration of drug	Patient No.	*P* value of control versus patient results using the two sample *t* test	NK cell activity
1 mg/kg	1	0.70	
	2	0.86	
5 mg/kg	3	0.15	
	4	0.19	
	5	0.017*	<Normal control
10 mg/kg	6	0.45	
	7	0.001*	<Normal control
	8	0.04*	>Normal control
15mg/kg	9	0.045*	
	10†		
	11	0.32	
	12	0.6	
20 mg/kg	13	0.27	
	14	0.001*	<Normal control
	15	0.3	
	16	0.15	

*P value (NK cell activity of the patient significantly different from the normal control).

†Patient No. 10 was withdrawn from the study due to impaired renal function.

Table 3. Patient data and side-effects

Patient No.	Age	Sex	CL 246738 dose (mg/kg)	Vomiting (V), diarrhoea (D), sleepy (S)
1	69	♀	1	
2	59	♂	1	
3	76	♂	5	D
4	73	♀	5	D
5	68	♀	5	
6	66	♀	10	V
7	66	♀	10	V
8	70	♀	10	
9	72	♂	15	V
10	53	♀	15	V
11	53	♂	15	VD
12	50	♂	15	VD
13	73	♀	20	VD
14	66	♀	20	VS
15	41	♂	20	VD
16	72	♂	20	S
17	48	♂	20	Nausea/D

3. *Interferon levels (alpha YOK, alpha NK and gamma)*

These are shown in Fig. 1. There was no significant or consistent change in any of the three interferons assayed.

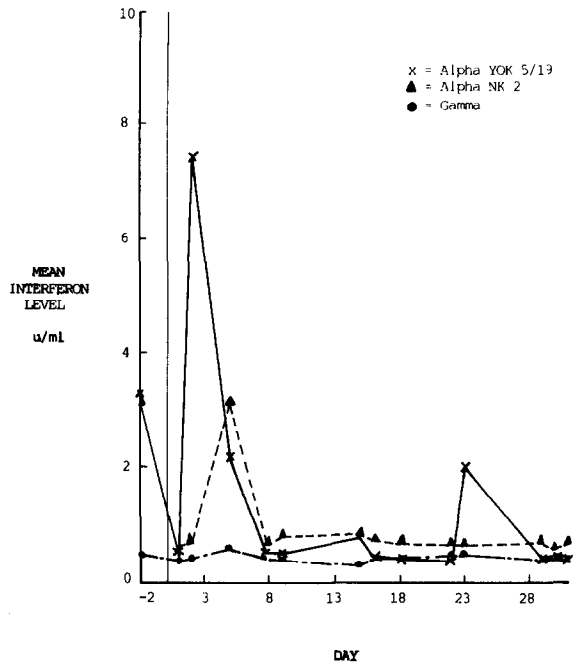

Fig. 1. Interferon levels in patients receiving CL 246738.

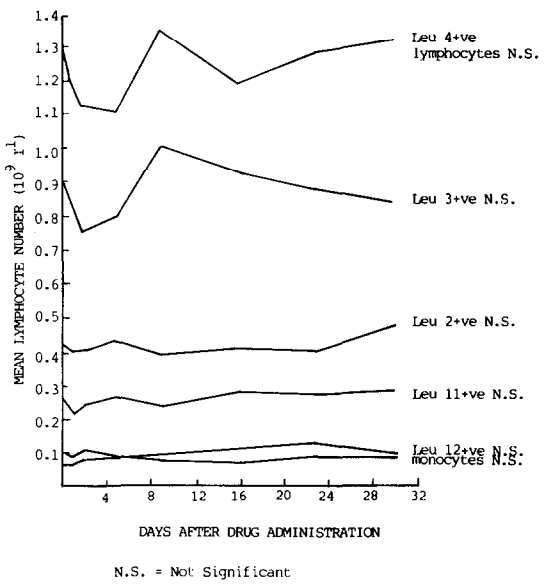

Fig. 3. Mean lymphocyte subset counts for 16 patients before drug administration and up to 30 days thereafter.

4. *Interleukin 2 activity*

The data for IL-2 measurements are shown in Fig. 2. Again, no significant augmentation of IL-2 activity was demonstrated for any or all doses.

5. *Lymphocyte subpopulation analysis*

This is shown graphically in Fig. 3. Although there was a trend suggesting a fall in the number of circulating Leu 4 +ve and Leu 3 +ve in the first week after drug administration, this failed to reach statistical significance.

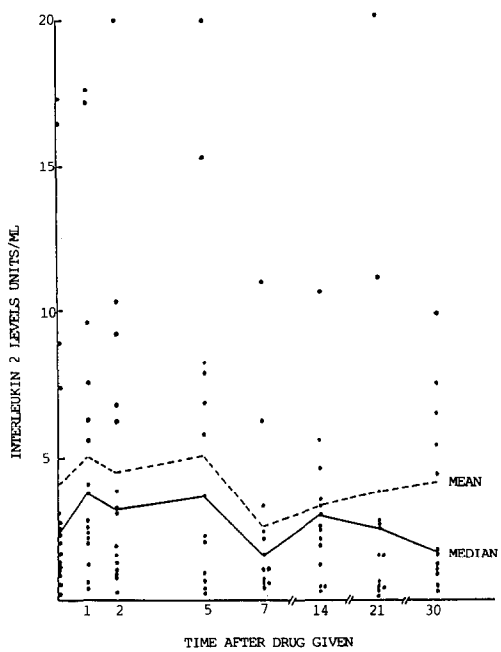

Fig. 2. Interleukin 2 levels after administration of CL 246738.

6. *Natural killer (NK) cell function*

The results are summarized in Table 2. NK cell activity of healthy volunteers and patients was compared after drug administration. The majority of patients and controls followed the same pattern of variation. These data suggested that the variation in NK cell activity observed was related to the assay [8] and not an effect of CL 246738. The same healthy volunteer was used throughout a patient's time of study.

In the majority (66%) of the patients the drug CL 246738 had no effect on *in vitro* NK cell activity compared to normal control values. However, in 26% of patients (4/15) CL 246738 appeared to have an *inhibitory* effect on *in vitro* NK cell activity and this effect was seen irrespective of drug concentration administered to the patient. When these functional results were correlated with the data showing numbers of Leu 11 positive cells obtained from flow cytometry, the numbers of Leu 11 positive cells were unchanged irrespective of NK cell activity. It can be seen that the drug had a direct inhibitory effect on NK cell activity on 4/15 patients (patients 5, 7, 9 and 14) and a direct stimulatory effect on one patient (patient 8).

7. *Immunoglobulins*

Results of immunoglobulin levels are shown in Fig. 4; no significant changes occurred in these over the trial period.

8. *Mitogen stimulation of peripheral blood lymphocytes*

These results are shown graphically in Fig. 5. They are expressed as a proportion of normal con-

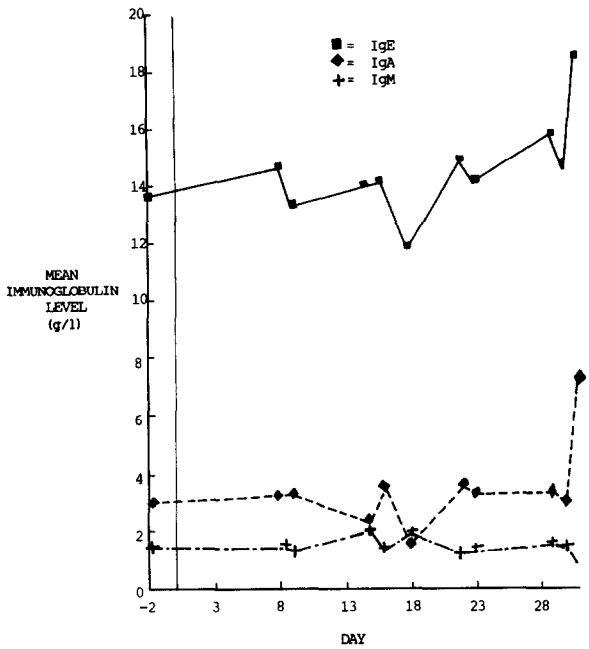

Fig. 4. Immunoglobulin levels in patients receiving CL 246738.

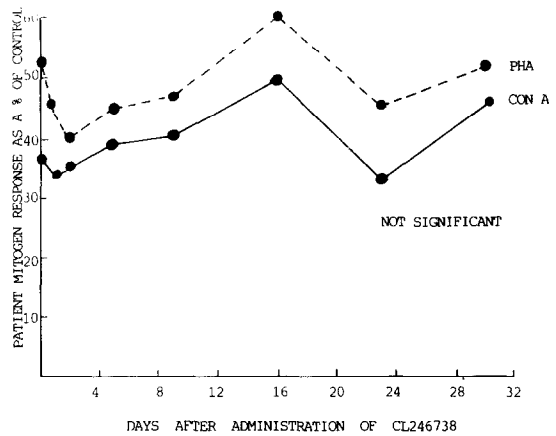

Fig. 5. Mean mitogen responses of 16 patients.

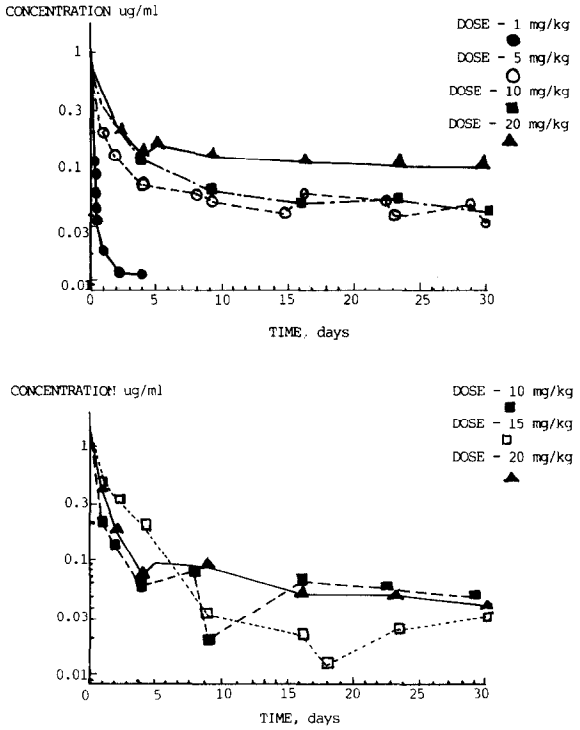

Fig. 6 Pharmacokinetic studies. Mean concentration–time profile for non-vomiting subjects (top) and subjects who did vomit (bottom).

trols. No significant changes were seen in the response of the lymphocytes to concanavalin A (Con A) or phytohaemagglutinin (PHA) at any dose or time period in the study.

9. *Biochemical tests*

Changes in liver function tests and haemoglobin were observed in patients. These were thought to be related to developing liver metastases in every case and not to a toxic effect of CL 246738.

10. *Drug blood concentrations*

Mean whole blood drug concentrations in subjects who did and did not vomit are shown in Fig. 6. These were measured by Cyanamid using high pressure liquid chromatography (HPLC). The half life of the drug was confirmed to be similar to that found in pre-clinical studies (unpublished data, American Cyanamid), reflecting the fact that the drug is concentrated largely in circulating leukocytes.

Absorption would appear to be rapid and probably from the stomach since even in those patients with vomiting, blood levels similar to those who did *not* vomit were achieved.

The maximum absorption capacity was probably reached by 5 mg/kg since after this dose all blood levels were similar. Pre-clinical data did not suggest that parenteral administration would result in higher blood levels or greater immunological effect (data unpublished).

DISCUSSION

CL 2465738 is an agent which has been shown to have active immunomodifying properties in a series of animal studies [1–3]. This phase I trial reports a detailed study of components of the immune system over a 1 month period in patients with recurrent or residual colorectal cancer. There were short-term clinical side-effects, predominantly nausea, vomiting and diarrhoea, on the day of drug ingestion and in two patients drowsiness.

The interest in immunomodulating agents is increasing for two reasons—firstly, there has been little progress in improving the prognosis for patients with solid malignant tumours of the gastrointestinal tract in the past 20 years, despite chemotherapy, advances in surgery and radiotherapy.

Recently the association between recurrence of some malignant tumours and a perioperative blood transfusion has been observed [5]. Whether this is due to an immunological effect or not is still open to debate but animal studies would certainly support this hypothesis, as does the observation that in renal transplant recipients blood transfusions enhance allograft survival.

An oral immunomodulator given after surgery may help reverse this effect in those who require blood transfusions.

In many studies of biological response modifiers, e.g. interferons, tumour necrosis factor, the drug dosage is increased to maximum toxicity with no assessment of the biological end-point required. Since the dose–response curve may not be linear for stimulation of various components of the immune systems [9], we chose to study a range of immunological functions as end points. The aim was to select a dose of drug that produced a defined biological effect, for phase II studies, rather than use a dose selected on toxicity criteria.

However, although preclinical data showed that CL 246738 was active as an immunomodulator, no effects of a single oral dose were detectable in patients and unexpected drowsiness as a side-effect plus vomiting and diarrhoea were the reasons for halting dose escalation.

In many previous studies of biological response modifiers, patients who had been heavily pretreated with radiotherapy or chemotherapy were studied, so immunological monitoring as a trial end-point would have been difficult to achieve.

Although all our patients had residual or recurrent disease, they had no marrow compromising therapy or immune suppressive therapy and this would have optimized the possibility of detecting a biological end-point in this study. The failure of the drug to stimulate immune function was not due to poor absorption, since levels of drug similar to those achieved in preclinical evaluations were achieved (data unpublished; American Cyanamid).

Multiple dose regimens using CL 246738 in patients with widespread malignancy are being carried out at other centres, and results will be reported elsewhere.

This study shows the need to carry out concomitant immunological as well as tolerance studies in phase I to obtain optimal information for subsequent clinical development of biological response modifiers.

REFERENCES

1. Wang BS, Lumanglas AL, Ruszala-Mallon VM, Durr FE. Induction of tumour inhibitory macrophages with a novel synthetic immunomodulator 3,6-bis(2-piperidinoethoxy)acridine trihydrochloride (CL 246738). *J Immunol* 1985, **135**, 679–683.
2. Durr FE, Citarella RV, Wallace RE, Wang BS, Lin FV. Biological effects of CL 246738, a new immunomodulating agent. In: Spitzy KH, Karrer K, eds. *Proc. 13th International Congress on Chemotherapy*. Vienna, Egermann, 1983, Vol. 17, 287.
3. Wang BS, Lumanglas AL, Silva J, Ruszala-Mallon VM, Durr FE. The mechanism of action of 3,6-bis(2-piperidinoethoxy)acridine trihydrochloride (CL 246738) in the potentiation of natural killer cells. *J Immunol* 1986, **137**, 2640–2645.
4. Lennard TWJ, Shenton BK, Barzotta A *et al.* The influence of surgical operations on components of the human immune system. *Br J Surg* 1985, **72**, 771–776.
5. Parrott NR, Lennard TWJ, Taylor RMR *et al.* Effect of perioperative blood transfusion on recurrence of colorectal cancer. *Br J Surg* 1986, **73**, 970–973.
6. Dickinson AM, Shenton BK, Alomran AH, Donnelly PK, Proctor SJ. Inhibition of natural killing and antibody-dependent cell-mediated cytotoxicity by the plasma protease inhibitor alpha 2-macroglobulin (alpha 2-M) and alpha 2-M protease complexes. *Clin Immunol Immunopathol* 1985, **36**, 259–265.
7. Allen EG, Lennard TWJ, Shenton BK *et al.* Circulating leukocyte subpopulations and their function in benign and malignant disease. *J Exp Clin Cancer Res* 1985, **4**, 293–301.
8. Levin AC, Massey RJ, Deinhardt F. Spontaneous human mononuclear cell cytotoxicity to cultured tumour cell: reproductivity of serial measurements with the use of a chromium-51-release microcytotoxicity assay. *J Nat Cancer Inst* 1978, **60**, 1283–1294.
9. Maluish AE, Ortaldo JR, Conlon JC *et al.* Depression of N.K. cytotoxicity after *in vivo* administration of recombinant leukocyte interferon. *J Immunol* 1983, **131**, 503–507.