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Original Paper

An Open Phase I Study to Assess the Biological Effects of a Continuous Intravenous Infusion of Interleukin-3 Followed by Granulocyte Macrophage-Colony Stimulating Factor

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To assess any synergistic stimulatory effect *in vivo* of Interleukin 3 (IL-3) and Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) upon white cell and platelet counts, toxicity and antitumour effect, we conducted this phase I study. IL-3 0.25, 0.5 or 5 μg/kg/day for 1, 4 or 7 days was given by continuous intravenous (i.v.) infusion to 35 patients with advanced malignancy. 21 of the 35 patients also received sequential or overlapping treatment with continuous i.v. infusion of GM-CSF 1 or 3 μg/kg/day for up to 10 days. Monotherapy with IL-3 producted significant dose related increases in platelets and white cell counts. Combinations of IL-3 and GM-CSF also produced increases in white cell counts, but these were no greater than would be expected following GM-CSF treatment alone. There was a trend for platelets to increase more in patients receiving IL-3 and GM-CSF than those receiving IL-3 alone, but this did not reach statistical significance. In general, IL-3 and combinations of IL-3 and GM-CSF were well tolerated and the most common side-effect was fever. A maximum tolerated dose was not reached and antitumour effects were not seen. Future studies using combinations of IL-3 5 μg/kg/day and GM-CSF 3 μg/kg/day may help to define the optimal therapeutic regimen.

Key words: GM-CSF, IL-3, phase I clinical trial
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INTRODUCTION

INTERLEUKIN-3 (IL-3) and Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) are haemopoietic growth factors whose target cells range from primitive multipotential haemopoietic cells to those progenitor cells that are committed to specific haemopoietic lineages [1–5]. IL-3 supports the growth of very early haemopoietic progenitors *in vitro* and acts synergistically with other cytokines such as erythropoietin (Epo), GM-CSF and Granulocyte-Colony Stimulating Factor (G-CSF) in the formation of erythroid, myeloid, mixed and megakaryocyte colonies [6, 7]. GM-CSF also interacts with early multipotent haemopoietic progenitor cells to promote growth, differentiation and proliferation of megakaryocyte, eosinophil and erythroid progenitors when acting in concert with Epo, Macrophage-Colony Stimulating Factor (M-CSF) and G-CSF [8–16] but, in comparison to IL-3, its target cells appear to be a less primitive stage of development [2, 5, 8, 17, 18]. Both GM-CSF and IL-3 increase proliferative capacity by increasing the cycling rates of Granulocyte Macrophage-Colony Forming Cells [5, 19, 20]. Additionally, GM-CSF enhances the functional capacity of mature myeloid cells, including antibody dependent cell cytotoxicity in neutrophils and eosinophils [21–23], the responses of neutrophils to chemotactic stimuli [24, 25], oxidative metabolism, phagocytosis and cytotoxicity in mononuclear phagocytes [26] and enhancement of cytotoxicity towards tumour cells [27]. GM-CSF has anti-tumour activity *in vitro* and *in vivo* [28], and, in a phase I trial of GM-CSF, stabilisation of malignant disease was observed in 7 patients and a significant tumour response in 1 out of 20 patients [29].

Primate studies have provided *in vivo* evidence that IL-3 expands an early progenitor cell population that may then be stimulated to proliferate further by GM-CSF [30–33]. In

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humans, GM-CSF with IL-3 may be used to augment the potential of harvested bone marrow *ex vivo* [34]. Thus, application of a combination regimen *in vivo* might accelerate haemopoietic recovery following myelotoxic therapy [34, 35] and enhance the antitumour effects of GM-CSF [36, 37].

We describe here the results of a phase I study of sequential IL-3 and GM-CSF given as a continuous intravenous (i.v.) infusion in order to observe the haematological effects and toxicity of this combination. A secondary aim was to assess any antitumour effect of the combination of IL-3 and GM-CSF.

PATIENTS AND METHODS

Patients

Adult patients (aged 18–80 years) with locally advanced inoperable or unresponsive metastatic adenocarcinoma of the colon, rectum or pancreas, proven on biopsy, were eligible for entry. Patients had a Karnofsky performance status of >70, creatinine, urea and liver function tests within twice normal limits, haemoglobin >8 g/dl, white cell count (WCC) $>2 \times 10^9$/l and platelets $>100 \times 10^9$/l. Patients with known allergic diseases and fertile women not using contraception were excluded as were patients who had received any other investigational drug within the preceding month. All patients gave written informed consent and the protocol was approved by South Manchester Ethics committee.

IL-3 and GM-CSF

Recombinant IL-3 (*E. coli*, non-glycosylated, Sandoz) was supplied in vials containing 150 μg of IL-3 as a sterile lyophilised powder. These were reconstituted for infusion in 2 ml of sterile water and the total daily dose made up to 48 ml in 0.9% sodium chloride containing 0.1 mg/ml of human serum albumin (HSA). This was administered via a continuous ambulatory delivery device (Pharmacia Deltac) and central venous line at a rate of 2 ml/h. Six weeks after the start of the study, the concentration of HSA was increased from 0.1 to 2 mg/ml for the low doses of IL-3 following the result of adsorption studies.

Recombinant GM-CSF (*E. coli*, non-glycosylated, Sandox/Schering Plough) was supplied in vials containing 50, 100 or 400 μg of GM-CSF as a sterile lyophilised powder formulated with mannitol, HSA, polyethylene glycol and phosphate buffer that was reconstituted with 1 ml of sterile water. The total daily dose was made up to 48 ml in 0.9% sodium chloride and administered via a continuous ambulatory delivery device (Pharmacia Deltac) and central venous line at a rate of 2 ml/h.

Study design

This was an open non-randomised study of patients receiving IL-3 therapy of varying daily dosage and duration with or without consecutive or overlapping treatment with GM-CSF (Table 1). Therapy was followed by a 21 day post-infusion observation period. Patients were allocated to treatment groups, each containing a minimum of 3 patients. Groups 1, 2 and 6 received IL-3 alone at a dose of 0.25, 0.5 or 5 μg/kg/day for 7 days. Groups 3, 4 and 5 received IL-3 0.25 μg/kg/day for 1, 4 or 7 days followed by 3 μg/kg/day of GM-CSF. GM-CSF was given for 10 days or until a WCC of 20×10^9/l or a platelet count of 600×10^9/l was reached. However, after the study was underway, it became apparent that a WCC of 20×10^9/l was reached too rapidly to allow the platelet response to be assessed. Hence, groups 7, 8 and 9 received IL-3 5.0 μg/kg/day for 1, 4 or 7 days followed by 1 μg/kg/day of GM-CSF given for 10 days or until a WCC of 30×10^9/l or a platelet count of 600×10^9/l was reached. Finally, a group of 3 patients was given IL-3 5.0 μg/kg/day for 4 days with GM-CSF given concurrently on days 3 and 4 and continued for up to 10 days or until a WCC of 30×10^9/l or a platelet count 600×10^9/l was reached (group 11) while another group received only IL-3 5 μg/kg/day for 4 days (group 10).

Table 1. Treatment allocation of the 35 patients who entered the phase I trial of IL-3 plus GM-CSF

		IL-3		GM-CSF	
			Study		
Group	*n*	μg/kg/day	days	μg/kg/day	Start day
1	3	0.25	1–7	—	—
2	4‡	0.5	1–7	—	—
3*	3	0.25	1	3	2
4*	3	0.25	1–4	3	5
5*	3	0.25	1–7	3	8
6	4‡	5.0	1–7	—	—
7†	3	5.0	1	1	2
8†	3	5.0	1–4	1	5
9†	3	5.0	1–7	1	8
10	3	5.0	1–4	—	—
11†	3	5.0	1–4	1	3
Total	35				

Patients in groups 3, 4, 5, 7, 8 and 9 received GM-CSF for a maximum of 10 consecutive days after IL-3 therapy; patients in group 11 received GM-CSF for a maximum of 10 days starting on the third day of IL-3 therapy.

*GM-CSF was stopped before 10 days of therapy if WCC $>20 \times 10^9$/l or platelets $>600 \times 10^9$/l in groups 3–5. †GM-CSF was stopped before 10 days of therapy if WCC $>30 \times 10^9$/l or platelets $>600 \times 10^9$/l in groups 7–9 and 11. ‡In groups 2 and 6 the fourth patient replaced patients who were withdrawn prematurely.

Patients were monitored closely for haematological and biochemical changes and for clinical symptoms and signs of toxicity during the infusion period and for 21 days after completion. Patients discontinuing treatment for any reason were replaced, but all patients were included in the assessment of toxicity. Electrocardiograms and dipstick urinalysis (Ames Multistix) were included in these assessments. The observation period was extended if any parameter had not returned to prestudy level by 21 days. Studies in primates using a range of doses of IL-3 equivalent to those used in this study have shown a 10–100 fold increase in intracellular histamine levels (Sandoz Pharmaceutical Ltd, company data, not shown). Therefore, blood histamine levels (total and intracellular) were also measured daily during the infusions and on each of the 5 days, the 10th day and the 21st day following infusion in the first patient in treatment groups 1–5. Bone marrow aspirates and trephines were taken pre-study, at the end of IL-3 and at the end of GM-CSF treatment. Tumour measurements were made before treatment and after the 21 day period of follow-up.

Toxicity was assessed using WHO criteria [38]. If 2 of the 3 patients exhibited grade 3 or 4 toxicity, then the previous dosage and duration was to be specified as the maximum tolerated dose (MTD).

Statistical analysis

Data from groups 1–11 were pooled where possible and the statistical procedures used were the non-parametric Mann–Whitney *U*-test to compare changes in WCC and platelet counts in groups receiving monotherapy with IL-3 versus groups receiving combination therapy with IL-3 and GM-CSF and the Wilcoxon matched-pairs signed rank test to detect differences between the groups receiving IL-3 only.

RESULTS

Patients

33 patients were evaluable for assessment of biological activity of IL-3 and GM-CSF in 11 groups. 2 other patients were withdrawn because of adverse events and were replaced, and 35 patients were therefore available for evaluation of toxicity. There were 22 males and 13 females with a median age of 55 years (range 33–71 years) and median Karnofsky performance status 80 (range 70–100). 30 had colorectal carcinoma and 3 had pancreatic carcinoma, while in 2 patients the site of the primary malignancy was unknown. One patient had evidence of malignant infiltration in the bone marrow prior to study treatment; previously 31 patients had undergone surgery and 1 had received limited field radiotherapy.

Haematological changes

The effect of IL-3 monotherapy on white cell and platelet counts. Treatment with IL-3 caused a rise in WCC, and this was significantly related to dosage when measured at day 7 ($P = 0.04$ at day 7). Thus the mean leucocyte count increased from 7.6 before treatment to a peak of 9.0×10^9/l in group 1 and from 8.0 to 18.9×10^9/l in group 6 (Table 2). An absolute WCC exceeding 20×10^9/l was only seen in patients receiving IL-3 5 μg/kg/day and almost all of the increase was due to dose related increments in neutrophil counts (Figure 1), evident within 48 h of the start of therapy. The eosinophil count rose in all patients but remained below 2×10^9/l, except for 1 patient receiving IL-3 5 μg/kg/day for 7 days, in whom eosinophils reached 6×10^9/l by day 6. There were no consistent changes in lymphocyte, monocyte or basophil counts and white cell counts returned to baseline values during the observation period with a rapid decline in the first 48 h after IL-3 treatment was completed (Figure 1).

Table 2. The effects of monotherapy with IL-3 0.25, 0.5 or 5.0 μg/kg/day on platelet and white blood cell counts

Group	IL-3 μg/kg/day	IL-3 Study days	Platelet increment* ×10⁹/l	Platelet increment* AUC	WCC increment ×10⁹/l†
1	0.25	1–7	71	552	0.5
			49	693	2.4
			33	247	1.3
2	0.5	1–7	49	33	4.0
			76	180	5.9
			41	890	2.7
6	5.0	1–7	262	2595	5.3
			277	317	17.0
			224	2802	10.5
10	5.0	1–4	58	847	6.9
			160	429	16.6
			164	1653	9.1

*Platelet increment is expressed as the peak count during the first 21 days minus the baseline value and as the area under the curve (AUC) of platelet count plotted against time during the first 21 days of the study. †WCC increment is the peak count during IL-3 therapy minus the baseline value.

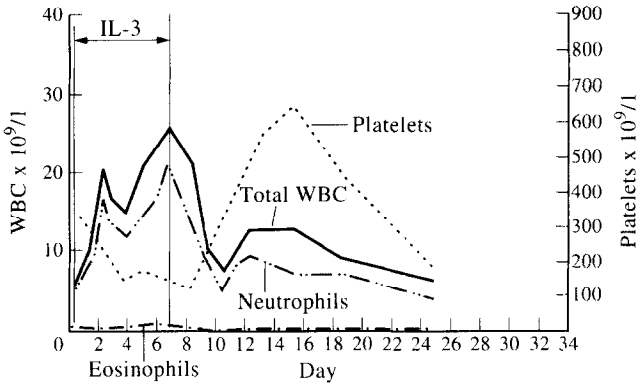

Figure 1. The effect of monotherapy with IL-3 at 5 μg/kg/day i.v. for 7 days on total WCC, neutrophils, eosinophils and platelets in one representative patient.

IL-3 also caused an increase in platelet counts (Table 2 and Figure 1). The platelet count did not start to rise until at least 5 days after the start of the treatment but then continued to rise when IL-3 was discontinued. Maximum values were reached at a median of 13.5 days (range 12–15 days) after the start of treatment, and at day 14 there was evidence of a significant dose related effect of IL-3 upon platelet counts ($P = 0.028$ at day 14). There was then a decline to pretreatment values over the next 14 days.

The effect of combination therapy with IL-3 and GM-CSF on white cell and platelet counts. Treatment with IL-3 and GM-CSF combinations was associated with increases in WCC, predominantly in neutrophil counts (Figure 2). Eosinophil counts rose as expected [29] when IL-3 treatment was followed by GM-CSF but fell towards baseline within 48 h of withdrawal of GM-CSF. There were no consistent changes in lymphocytes, monocytes or basophils. Overall, the rises in WCC in patients on combination regimens were greater than those in patients receiving monotherapy with IL-3 (Tables 3 and 4). This was statistically significant when the WCC were

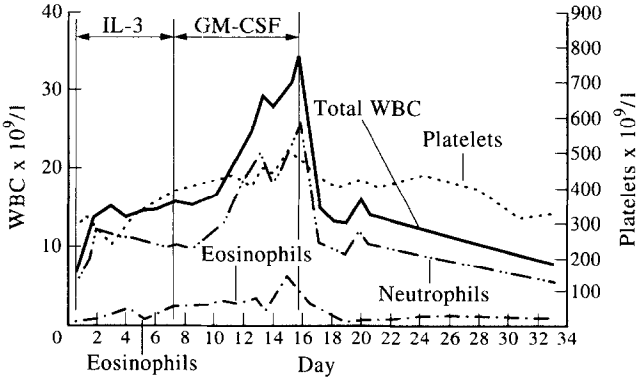

Figure 2. The effect of combination therapy with IL-3 at 5 μg/kg/day i.v. for 7 days followed by GM-CSF 1 μg/kg/day i.v. on total WCC, neutrophils, eosinophils and platelet counts in one representative patient.

Table 3. The effects of combination therapy with IL-3 0.25 or 0.5 μg/kg/day followed by GM-CSF 3.0 μg/kg/day on platelet and white blood cell counts

	IL-3		GM-CSF	Platelet increment*		WCC increment during IL-3
Group	μg/kg/day	Study days	μg/kg/day	$\times 10^9$/l	AUC	$\times 10^9$/l†
1	0.25	1–7	—	71	552	0.5
				49	693	2.4
				33	247	1.3
2	0.5	1–7	—	49	33	4.0
				76	180	5.9
				41	890	2.7
3	0.25	1	3	971	15479	0
				83	839	0
				165	520	0.1
4	0.25	1–4	3	255	1094	0.8
				93	558	1.4
				27	0	2.3
5	0.25	1–7	3	84	526	3.7
				556	676	5.3
				119	324	4.9
			Median (Range)	83 (27–971)	552 (0–15479)	2.3 (0–59)

*Platelet increment is expressed as the peak count during the first 21 days minus the baseline value and the area under the curve (AUC) of platelet count plotted against time during the first 21 days of the study. †WCC increment is the peak count during IL-3 therapy minus the baseline value.

Table 4. The effects of combination therapy with IL-3 5 μg/kg/day followed by or overlapping with GM-CSF 1.0 μg/kg/day on platelet and white blood cell counts

	IL-3		GM-CSF	Platelet increment		WCC increment during IL-3
Group	μg/kg/day	Study days	μg/kg/day	$\times 10^9$/l†	AUC*	$\times 10^9$/l†
10	5.0	1–4	—	58	847	6.9
				160	429	16.6
				164	1652	9.1
6	5.0	1–7	—	262	2595	5.3
				277	317	17.0
				224	2802	10.5
7	5.0	1	1	55	79	3.8
				87	1343	4.2
				84	0	1.6
8	5.0	1–4	1	160	1563	11.5
				326	1495	5.2
				186	2087	8.0
9	5.0	1–7	1	187	1636	11.4
				369	2564	4.8
				202	2049	8.0
11	5.0	1–4 (overlap)	1	217	2251	5.2
				28	0	8.3
				252	2945	9.6
			Median (range)	186 (28–367)	1643 (0–2945)	7.6 (1.6–17)

*AUC, area under the curve of platelet count plotted against time during the first 21 days of the study. †WCC increment is the peak count during IL-3 therapy minus the baseline value.

compared on days 7, 14 and 21 of the study ($P = 0.01$ at day 7, $P = 0.0015$ at day 14, and $P = 0.018$ at day 21). The rises in WCC that occurred during IL-3 treatment tended to be greater in those patients receiving IL-3 5 μg/kg/day than in those receiving 0.25 or 0.5 μg/kg/day but there was no evidence that prior treatment with IL-3 accelerated the WCC response to GM-CSF (Table 5).

In a historical group of 6 patients ([39] and Table 5) receiving GM-CSF 3 μg/kg/day by continuous infusion, 5 achieved a WCC $>20 \times 10^9$/l within 48 h. We found that

Table 5. The effect of IL-3 0.25 or 5.0 μg/kg/day given for 1, 4 or 7 days upon the rise in WCC achieved by day 2 of treatment with GM-CSF 1 or 3 μg/kg/day

IL-3 μg/kg/day	GM-CSF μg/kg/day	Number of days treatment with IL-3 0	1	4	7	4†	Total
0	3	**5/6***	—	—	—	—	**5/6**
0.25	3	—	**2/3**	**1/3**	**2/3**	—	**5/9**
5.0	1	—	**1/3**	**0/3**	**1/3**	**1/3**	**3/12**

Number of patients achieving WCC $>20 \times 10^9$/l within 48 hours of starting GM-CSF are shown in bold.
*Historical control [39]. †IL-3 treatment overlapping with GM-CSF.

treatment with combinations of IL-3 and GM-CSF, whether sequential or overlapping, conferred no apparent benefit over GM-CSF alone in causing the WCC to rise; the overall effect was additive rather than synergistic.

Combination treatment with IL-3 and GM-CSF was associated with an increase in the platelet count (Tables 3 and 4). In general, the platelet count started to rise 5 days after IL-3 treatment began and peaked between days 8 and 26, median day 19, for low doses of IL-3 and between days 7 and 20, median day 14, for high doses of IL-3. Once cytokine treatment was complete, the platelet count gradually fell and had reached pretreatment values by the end of the 21 day observation period. When all patients who had received monotherapy with IL-3 were compared to all patients receiving IL-3 and GM-CSF, there were no significant differences in platelet counts until day 21 of the study. Patients receiving combination treatment then had a significantly greater platelet count relative to pretreatment values than those receiving monotherapy ($P = 0.028$). GM-CSF was stopped prematurely because the WCC had reached 20×10^9/l in 8 patients, but in all patients the peak platelet count occurred after GM-CSF was stopped. A 50-year-old female patient with metastatic carcinoma of the rectum developed thrombocythaemia following IL-3 0.25 μg/kg/day 1 day + GM-CSF 3 μg/kg/day. The baseline platelet count was 119×10^9/l and this increased to 671×10^9/l after IL-3. Following a single dose of GM-CSF, the platelet count was 675×10^9/l and no further GM-CSF was given, in accordance with the protocol. The platelet count continued to rise, reaching a maximum of 1070×10^9/l at 11 days post infusion.

Haemoglobin Haemoglobin decreased slightly in most groups without a clear relationship to cytokine dosage. 4 patients required transfusion during the study.

Bone marrow. Bone marrow aspirates and trephines were examined before treatment started, after IL-3 treatment and again after GM-CSF treatment was completed. All but 2 patients had normal bone marrow cellularity throughout the study; 1 of these (patient 20, IL-3 5.0 μg/kg/day 1 day and GM-CSF 1.0 μg/kg/day) had evidence of malignant infiltration before starting treatment and died of progressive disease 13 days after the last dose of GM-CSF, while the other had hypocellular marrow prior to treatment (IL-3 5.0 μg/kg/day 4 days and GM-CSF 1.0 μg/kg/day). There were no consistent changes in numbers of megakaryocytes, degree of myeloid or erythroid maturation or in myeloid: erythroid ratios.

Clinical toxicity

IL-3 infusion 0.25 or 0.5 μg/kg/day. Low doses of IL-3 given at 0.25 or 0.5 μg/kg/day were generally very well tolerated ($n = 16$). 3 patients had mild fever (one grade 1, two grade 2). Somnolence was reported by 3 patients (2 grade 2, 1 grade 3). Patient 14 (IL-3 0.25 μg/kg/day 7 days and GM-CSF 3 μg/kg/day), a 69-year-old man with adenocarcinoma who developed grade 3 somnolence, had a history of similar episodes prior to the study and treatment was not stopped. Patient 4, a 49-year-old woman with metastatic adenocarcinoma receiving IL-3 0.5 μg/kg/day and concomitant therapy with amitryptiline, fluphenazine and nortryptiline, complained of blurred vision and headache lasting 1 h on day 1 of the IL-3 infusion and was withdrawn from the study. These symptoms recurred twice more in the following 12 h causing a residual visual deficit which improved over the following 11 days. Computerised tomography and electroencephalography of the brain did not identify a cause, but it is possible that IL-3 or her other medication were contributory.

IL-3 infusion 5 μg/kg/day. 19 patients received high doses of IL-3 at 5.0 μg/kg/day (Table 6). Grade 3 toxicity was reported in patient 19 (IL-3 5 μg/kg/day 7 days), a 65-year-old woman with metastatic adenocarcinoma of the caecum who had had a myocardial infarction 2 years previously, and was receiving concomitant treatment with morphine, cyclizine, metoclopramide, prochlorperazine, verapamil, spironolactone and isosorbide mononitrate. She developed atrial flutter on day 7 of treatment with IL-3 and was withdrawn from the study. Ventricular failure and cardiac arrest followed and the patient died that day. However, these events were considered unlikely to be secondary to treatment with IL-3.

Table 6. Adverse events during administration of IL-3 at 5 μg/kg/day i.v. in 19 patients

	Toxicity (WHO grade) 0	1	2	3/4
Fever	2	4	13	0
Headache	10	6	3	0
Bone pain	9	7	3	0
Nausea	17	2	0	0
Somnolence	17	0	2	0
Cardiac	18	0	0	1
Vision	19	0	0	0
'Flu' symptoms	15	1	3	0
Totals	—	20	24	1

Fever was more frequent in those who received IL-3 5 μg/kg/day compared to those who received 0.25 or 0.5 μg/kg/day. The highest mean increase in body temperature (1.1°C) was observed during concurrent administration of IL-3 and GM-CSF (group 11) and all 3 of this group reported 'flu' like symptoms (one grade 1, two grade 2). Overall, the various dosing regimens were well tolerated while toxicity due to GM-CSF was as anticipated and very mild [29, 40].

Prior to treatment, 26/35 patients had elevated liver enzymes (grade 1–3) and subsequent changes in liver enzymes were not dose related, but instead probably reflected the underlying malignancy. There were no significant changes in renal function, electrolytes, plasma proteins, blood glucose, histamine or urinalysis attributable to treatment with IL-3 or GM-CSF.

Tumour responses

There were no tumour responses; 14 of the 34 evaluable patients had stable disease and 20 progressive disease. One patient, patient 20 (IL-3 5 μg/kg/day 1 day and GM-CSF 1 μg/kg/day) was withdrawn on day 24 because of tumour progression causing ureteric obstruction.

DISCUSSION

Clinical phase I/II studies with IL-3 alone suggest that it causes a significant dose dependent increase in peripheral platelet counts by day 15 after the start of therapy [41], in addition to increases in bone marrow progenitor cells [42]. Increases in neutrophils have been reported in some patients together with small increases in circulating haemopoietic progenitor cells [43]. Fever, headache, rash and 'flu' like symptoms are common side-effects, but appear to be tolerated at doses lower than 16 μg/kg/day subcutaneous (s.c.) [39, 41, 44]. These reports are in agreement with our observations of an IL-3 induced dose dependent increase in platelet counts, starting within 5 days of treatment and peaking at 12–15 days, together with small dose related increases in neutrophils which occurred during IL-3 therapy. Toxicity attributable to IL-3 was generally mild, although, in addition to fever and 'flu' like symptoms, bony pain was also repeated. WHO grade 3 symptoms of somnolence and cardiac arrythmias during the study were not clearly attributable to IL-3 treatment, but instead appeared to be exacerbations of pre-existing clinical syndromes. Concomitant therapy with anticholinergics may have contributed to the visual disturbance occuring in one patient. Although we found no evidence of IL-3 induced increases in total or intracellular venous blood histamine levels, it should be noted that there is rapid sample decay which may give falsely low results.

The use of GM-CSF in clinical trials, in contrast to IL-3, is associated with a marked elevation in circulating neutrophils, eosinophils and monocytes with an early rise, particularly in neutrophils, in the first 4 days, followed by a plateau with further elevation at 8 days while the bone marrow cellularity increases with an increase in the myeloid:erythroid ratio [5, 39, 45–50]. However, there is little consistent effect on thrombopoiesis [40, 51], although some patients achieve an increase in platelet counts on doses of 120–500 μg/kg/day [52]. While the increases in white cells following GM-CSF treatment are dose dependent, the route of administration is also important since equivalent effects are seen when low doses of GM-CSF given by s.c. or continuous i.v. schedules are compared to higher doses given as i.v. boluses [46]. Toxicity, however, is seen most frequently when i.v. boluses are used and the most often reported side-effects are fever, bone pain, myalgia, lethargy, skin rashes and dyspnoea. Bony pain is severe at doses over 15 μg/kg, but skin rashes are largely associated with s.c. use [53]. However, at doses less than 10 μg/kg, only 5% of patients have WHO grade 3 or 4 toxicity. At the doses of GM-CSF used in this study, toxicity would be predicted to be mild [29, 40], and, in fact, most patients experienced few toxic symptoms. Symptomatic treatment of IL-3 induced toxicity with simple analgesia may have masked further symptoms due to GM-CSF, and it is of note that the concurrent administration of IL-3 and GM-CSF was associated with the greatest rises in temperature.

We found that combinations of IL-3 and GM-CSF given as consecutive or overlapping continuous infusions caused peripheral granulocyte and platelet counts to rise significantly above baseline values, but were little better than GM-CSF alone in elevating the peripheral white cell count (Table 5 and [39]). The time taken to reach a WCC of 20×10^9/l was similar to historical controls treated with GM-CSF 3 μg/kg/day and there was thus no evidence of synergy. However, cell kinetics data reported previously [54, 55] reveal that the elevation in neutrophil counts associated with GM-CSF therapy is accompanied by prolongation of the half life of peripheral blood neutrophils and by a considerable degree of ineffective haemopoiesis in the bone marrow. This is in contrast to our study described in this communication from which Lord and associates [56] have derived *in vitro* data that show that the half life of neutrophils appearing in the blood in response to combination therapy is, at 9.3 h, near normal and that there is no evidence of an increase in ineffective haemopoiesis in bone marrow. Hence, combination therapy with IL-3 and GM-CSF approximates more closely to normal physiology than does monotherapy with GM-CSF.

Bruno and associates report that administration of IL-3 followed by GM-CSF increases thrombopoiesis by sequentially increasing megakaryocyte numbers (IL-3) and maturation (GM-CSF) [57]. This is a synergistic effect that appears to be limited to the amplitude of the platelet response and does not affect the duration of the response after treatment stops. We found no evidence that combinations of IL-3 0.25 μg/kg/day and GM-CSF 3 μg/kg/day were more effective at increasing the platelet count response than IL-3 alone. High dose IL-3, 5 μg/kg/day, and low dose GM-CSF, 1 μg/kg/day, tended to produce a greater platelet response than high dose IL-3 alone although, due to the small numbers of patients, this did not reach statistical significance. Direct comparison between the high and the low IL-3 dosages was not possible because of the varying dosage and duration of GM-CSF treatment, in part a consequence of the altered WCC endpoint. It is noteworthy that in 1 patient receiving IL-3 0.25 μg/kg/day followed by GM-CSF 5 μg/kg/day, the platelet count rose to 1070×10^9/l and this may have been due to extreme sensitivity to the administered growth factors, perhaps due to enhanced IL-3 binding to its receptor, to hypersensitivity of the second messenger system or the lack of production *in vivo* of a normal inhibitor, or it may have represented a synergistic effect with some other unidentified growth factor *in vivo*.

Fay and associates have recently reported the results of administration of sequential IL-3 and GM-CSF following autologous bone marrow rescue in myeloablated heavily pretreated patients with lymphoma [58]. They showed that this

regimen caused significant improvements in thrombopoietic recovery compared to GM-CSF, G-CSF or IL-3 monotherapy and was comparable to G-CSF primed peripheral blood progenitor cell rescue in similar patients.

Interpretation of the data in this study is constrained by the small numbers of patients within individual groups. However, it was possible to draw meaningful conclusions by considering overall trends and subjecting these to statistical analysis as described in Materials and Methods. We found that combination treatment with IL-3 and GM-CSF may be used to produce increases in neutrophil counts which are comparable to those observed when GM-CSF is used alone, but which appear to be produced in a more physiologically normal way. Combination therapy with IL-3 and GM-CSF also produces a rise in platelet counts which, at the higher dose of IL-3, 5 μg/kg/day, may be greater than that seen when IL-3 alone is given. Since this dose of IL-3 was well tolerated and there was no severe toxicity attributable to combination therapy, further studies should be done using IL-3 5 μg/kg/day and GM-CSF 3 μg/kg/day.

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