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A phase I trial of recombinant human interleukin-1β (OCT-43) following high-dose chemotherapy and autologous bone marrow transplantation

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Summary:

We studied the effects of escalating doses of recombinant human IL-1β in patients receiving high-dose chemotherapy and ABMT for metastatic breast cancer or malignant melanoma. Sixteen patients received IL-1β, 4 to 32 ng/kg/day administered subcutaneously for 7 days beginning 3 h after bone marrow infusion. Three patients at the highest dose level also received G-CSF following completion of IL-1β. All patients completed the 7 days of therapy. The majority of patients experienced chills and fever following one or more injections, and seven had severe pain at the injection site. There was one episode of hypotension and one episode of transient confusion at the highest dose level; other significant toxicity was not identified. Recovery of neutrophils to $>0.5 \times 10^9$/l and platelet transfusion independence occurred at a median of 23 and 22 days, respectively, which was comparable to historical controls. The mean number of bone marrow colony-forming unit granulocyte–macrophage (CFU-GM) per 10^5 mononuclear cells on day +21 post-ABMT was more than twice that of control patients or patients receiving G-CSF or GM-CSF. A linear correlation was found between the dose of IL-1β and endogenous concentrations of several cytokines. These patients also displayed significantly higher concentrations of endogenous G-CSF compared to historical controls receiving GM-CSF. While IL-1β was moderately toxic and had no effect on recovery of peripheral blood counts after ABMT, the increased number of bone marrow CFU-GM suggests that the addition of G- or GM-CSF to a short course of IL-1β may accelerate hematologic recovery.

Keywords: IL-1β; ABMT; high-dose chemotherapy; breast cancer, cytokine

The use of lineage-specific hematopoietic growth factors such as GM-CSF and G-CSF shortens the duration of neutropenia following high-dose chemotherapy and ABMT.[1–3] Recently with molecular cloning much interest has emerged in the use of growth factors, which have multilineage proliferative effects, to shorten the duration of neutropenia and platelet transfusion-dependence and to decrease the number of red blood cell transfusions.[4]

IL-1β is a polypeptide which exerts effects on hematopoiesis through several mechanisms, and produces a broad spectrum of metabolic, immunologic and inflammatory effects.[5,6] In addition to having a direct stimulatory effect on early myeloid precursors,[7] IL-1β stimulates the production of other cytokines by accessory cells, including G-CSF, GM-CSF, M-CSF, IL-6, IL-1 receptor antagonist molecule (IL-1ra) and soluble receptors for tumor necrosis factor (sTNF-r)[8–11] all of which appear important in the growth of both early and late hematopoietic progenitor cells. IL-1 increases the proliferation and differentiation of bone marrow progenitor cells in the presence of G-CSF.[12] IL-1β also enhances the resistance of normal and neutropenic mice to gram-negative infections and enhances hematopoietic recovery after lethal irradiation or treatment with 5-fluorouracil.[13] Enhancement of natural killer (NK) cell activity by IL-1 and direct cytotoxic activity against tumor cells has also been reported.[5] Preclinical studies in non-human primates have demonstrated enhanced neutrophil recovery following chemotherapy in animals receiving IL-1β.[14] Phase I studies in humans with normal bone marrow function have shown that intravenous IL-1β is tolerable, with side-effects that included fever, rigors, headache and reversible hypotension.[15,16] Of considerable interest to the area of bone marrow transplantation is the report of a delayed, sustained increase in platelet count following two or five daily intravenous infusions of IL-1β,[15,16] suggesting that IL-1β could improve platelet recovery post-ABMT.

This study was undertaken to determine the maximum tolerated dose (MTD) of human recombinant IL-1β given subcutaneously following high-dose chemotherapy and autologous bone marrow rescue, and to examine its effect on bone marrow recovery.

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Patients and methods

Patients

Sixteen patients were enrolled on the study. Patients receiving high dose alkylating agent chemotherapy (cyclophosphamide, cisplatin and carmustine (BCNU)) with ABMT for high risk or metastatic breast cancer or malignant melanoma were eligible. All patients had histologic confirmation of malignancy, normal performance status (Karnofsky score $\geqslant$60), normal pulmonary function (diffusion capacity for carbon monoxide, forced expiratory volume in 1 s and forced vital capacity $\geqslant$60% of predicted), cardiac ejection fraction $\geqslant$45%, liver enzymes $\leqslant$2.5 times the upper limit of the normal range and creatinine clearance $\geqslant$60 ml/min. Exclusion criteria consisted of the presence of tumor cells in bone marrow aspiration or biopsy, central nervous system metastases, prior therapy with cytokines $<$1 month before transplant, hematocrit $<$0.30, positive pregnancy test, clinically significant thyroid disease or comorbid illness precluding general anesthesia for bone marrow harvesting. The study was approved by the Duke University Medical Center Institutional Review Board and all patients provided written informed consent.

IL-1β

OCT-43 is a modified IL-1β molecule with the substitution of cysteine by serine at amino acid 71, which increases the stability of the molecule *in vivo*. The protein was expressed in *E. coli* and provided for clinical use by Otsuka America Pharmaceutical, Rockville, MD, USA. The specific activity of OCT-43 using the human A375 cell line is 45 000 units of activity per milligram.

Study design

This was an open-labelled, non-randomized dose escalation study in which patients received a single course of IL-1β by subcutaneous injection for 7 days immediately following bone marrow infusion. This short course of therapy was chosen since the anticipated therapeutic benefit of IL-1β was stimulation of early progenitor cells and other, more terminally acting growth factors were becoming available. Cohorts of three patients were treated at each dose level from 4 ng/kg to 32 ng/kg. This dose range has previously been found to be well tolerated in a phase I study of OCT-43 in patients with advanced cancer (Otsuka Pharmaceuticals, data on file). Common toxicity criteria were used to determine the MTD, which was defined as the dose level below which grade 4 toxicity was seen in any single patient, or grade 3 toxicity seen in two or more patients.

Table 1 Patient characteristics

Number	16
Median age (years) (range)	44 (31–48)
Male:Female	2:14
Diagnosis:	
Breast cancer	13
Melanoma	3
Karnofsky performance status, median (range)	90 (70–90)
Patients with previous adjuvant chemotherapy	10

Bone marrow harvesting and processing were performed prior to high-dose therapy as described previously.[17] Purging of the bone marrow *ex vivo* was not performed. The median nucleated cell number returned to the patients was 2.02×10^8/kg (range 1.18 to 2.73) and the median number of colony forming units (CFU)-granulocyte–macrophage (CFU-GM) was 5.6×10^4/kg (range 1.1 to 14.9). All patients received cyclophosphamide 1875 mg/m^2 days −6 to −4 over 1 h (total dose 5625 mg/m^2); cisplatin 55 mg/m^2/day as a continuous intravenous infusion day −6 to −4 (total dose 165 mg/m^2) and BCNU 600 mg/m^2 at 5 mg/m^2/min on day −3.[17] Bone marrow infusion was performed on day +1. The first dose of IL-1β was given 3 h after marrow infusion and then daily for a total of seven doses. The three patients treated at the highest dose level (32 ng/kg) also received intravenous G-CSF (filgrastim; Amgen, Thousand Oaks, CA, USA) starting between day 8 and 16 and post-transplant until the absolute neutrophil count was above 2.5×10^9/l for 3 consecutive days. G-CSF was used here as it had just become commercially available and it was felt to be unethical at that time to withhold it.

Supportive care

All patients remained in reverse isolation in high efficiency particle aeration (HEPA) filtered rooms until the absolute neutrophil count was $>0.5 \times 10^9$/l. Packed red blood cells were transfused to maintain the hematocrit $>$0.42 and single donor platelets were given to maintain the platelet count $>25 \times 10^9$/l. All blood products were irradiated to 25 Gy. Fever during neutropenia was managed according to standard antibiotic protocols.

Laboratory evaluation

Bone marrow biopsies and aspirations were obtained prior to entry, on day +1 and every 5 days thereafter until day +21 to assess marrow cellularity and for assay of committed hematopoietic progenitor cells as previously described.[18] Briefly, 10^5–10^6 light density mononuclear cells were cultured in a base mixture of 0.9% methylcellulose (Sigma, St Louis, MO, USA) containing equal amounts of minimal essential alpha medium and Iscove's modified Dulbecco's medium (Gibco, Grand Island, NY, USA), 12.5% horse serum and 12.5% heat-inactivated bovine serum. To each 35-ml plate was added 1% deionized bovine serum albumin (Sigma), 10 μm 2-mercaptoethanol, 10% 5637 conditioned medium and 2 U erythropoietin (Amgen). Day +14 CFU-GM, CFU-granulocyte erythroid monocyte megakaryocyte (CFU-GEMM) and burst forming unit-erythroid (BFU-E) were scored using an inverted microscope.

Serum electrolytes and complete blood counts were performed daily in all patients, and liver chemistry, serum protein and plasma coagulation studies (PT and PTT) were done every 4 days until discharge from the bone marrow transplant unit. Plasma was collected for evaluation of endogenous cytokine concentrations from morning blood

Table 2 Hematologic recovery following ABMT according to dose of IL-1β

No. patients	*IL-1 dose ng/kg/day*	*Day to PMN $>0.5\times10^9$/l median (range)*	*Day to plts $>25\times10^9$/l median (range)*
3	4	24 (22–30)	20 (18–31)
3	8	27 (23–28)	26 (23–26)
4	16	25 (20–42)	24 (18–60)
3	24	21 (21–26)	21 (19–24)
3	32[a]	15[a] (13–18)	22 (13–27)
Control[b] ($n=23$)	none	19	21
GM-CSF ($n=19$)	none	15	NA

[a]Patients at this dose also received G-CSF 5 μg/kg/day intravenously starting day +8 (two patients) or day +16 (one patient) until the PMN count was $>2.5\times10^9$/l.
[b]Historic control patients were treated with exactly the same high-dose chemotherapy regimen and autologous bone marrow; however, did not receive growth factors post-transplant.
PMN = polymorphonuclear cell count; plts = platelets; NA = not available.

samples obtained on days −6, −1, +1, +3, +8, +12 and +16. Immunoreactive TNF-α, IL-6, M-CSF, G-CSF, GM-CSF, sTNF-r, and IL-1ra were measured using double-antibody sandwich techniques. Assay kits for the sTNF-R assay were obtained from Bender MedSystems (Vienna, Austria), GM-CSF from Endogen (Boston, MA, USA), whereas materials for the M-CSF assay were kindly provided by Genetics Institute (Boston, MA, USA). All remaining cytokines were analyzed by ELISA kits obtained from R&D Systems (Minneapolis, MN, USA).

Results

The characteristics of the 16 patients enrolled in this study are summarized in Table 1. Three had metastatic malignant melanoma and 13 had stage IV breast cancer. Ten of the 13 patients with breast cancer had received prior adjuvant chemotherapy; none of the patients had received chemotherapy for metastatic disease. Three patients received 7 days of subcutaneous IL-1β at each dose level of 4, 8, 24 and 32 ng/kg; four patients received 16 ng/kg (Table 2). All patients were able to complete the full 7-day course of treatment at the prescribed dose.

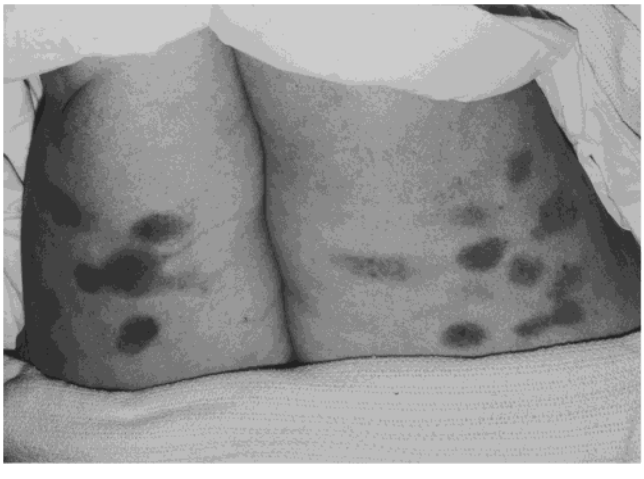

Figure 1 Injection site erythema and induration in a patient receiving subcutaneous IL-1β 8 ng/kg/day.

Toxicity

Subcutaneous IL-1β was well tolerated and no patient required discontinuation of the study drug because of side-effects. The majority of patients experienced chills (15/16) and fever >38.5°C (15/16) following one or more injections. Erythema at the injection site was seen in all patients (Figure 1) and was accompanied by pain in 7/16. Local pain was ameliorated in two patients by infiltration of the site with local anesthetics prior to injection of IL-1β. Three patients also experienced nausea thought to be due to IL-1β.

Hypotension has been dose-limiting following intravenous administration of IL-1β[15] but was observed in only one patient in our study, occurring at the highest dose level (32 ng/kg). This event was managed with intravenous fluids and brief use of intravenous inotropic support. A second patient at the 32 ng/kg level experienced mild confusion (grade 3) which resolved totally after completion of the study drug. No other neurologic toxicity was noted.

Five patients had positive blood cultures during the transplant period. In two patients fungal organisms were isolated (*Candida albicans* and *Torulopsis glabrata*); the former patient died of complications of candida septicemia on day +32. Three patients had bacteremia and were successfully treated with intravenous antibiotics.

There were no episodes of cardiac, pulmonary, renal or hepatic toxicity attributable to IL-1β during this study. One patient developed hyperbilirubinemia and right upper quadrant tenderness beginning day +23 post-transplant consistent with hepatic veno-occlusive disease. Tests of thyroid function measured on day +10 in all patients were unchanged compared to baseline values.

Hematologic recovery

Myeloid engraftment occurred in all 16 patients, with recovery of neutrophils to $>0.5\times10^9$/l developing at a

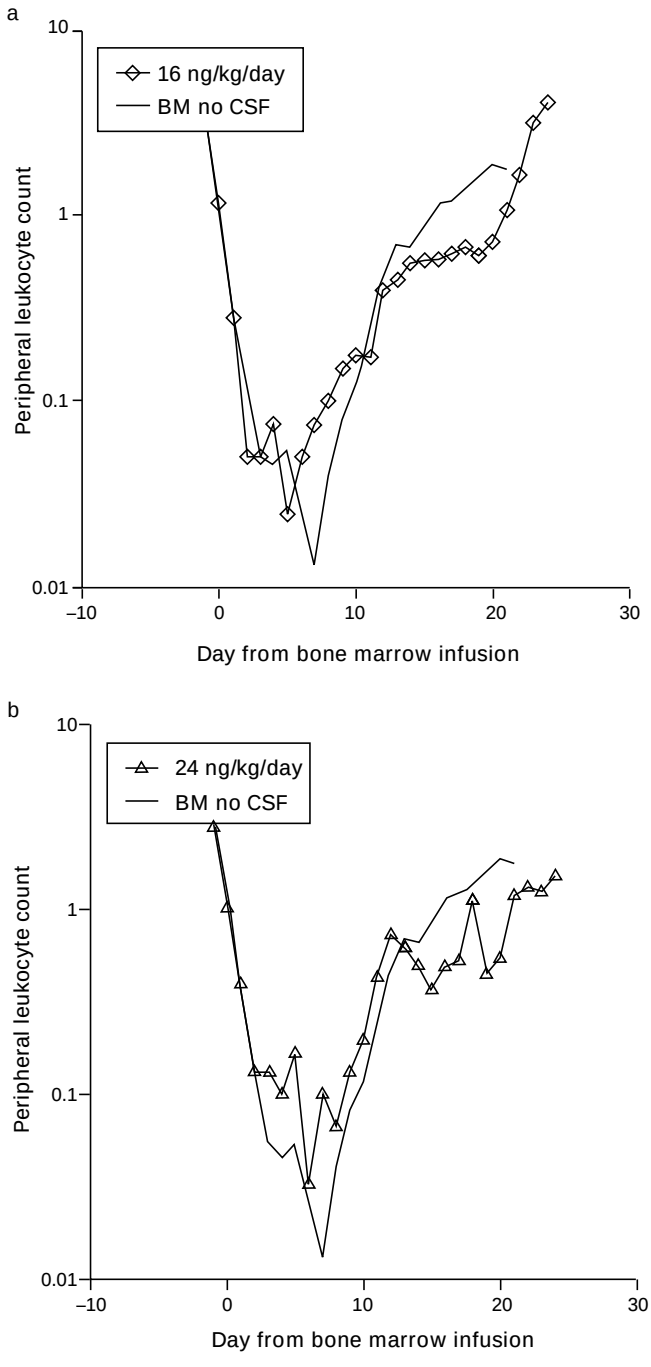

Figure 2 Recovery of peripheral blood leukocyte count in patients receiving IL-1β 16 ng/kg (**a**) or 24 ng/kg (**b**) day +1 to +7 following ABMT.

median of 23 days after marrow infusion (range 13 to 42 days). Fifteen patients were evaluable for platelet engraftment (stable platelet count $>25\times10^9$/l without transfusion), which occurred after a median of 22 days (range 13 to 60 days). Data on hematologic recovery at each dose level is shown in Table 2, along with data of 23 historical controls not receiving growth factors and 19 patients enrolled in a phase I study of GM-CSF.[1] The time course of recovery of peripheral blood leukocytes for the four patients treated with 16 ng/kg and the three patients treated at 24 ng/kg is shown in Figure 2.

The majority of patients had bone marrow biopsies evaluable for cellularity and morphologic assessment of hematologic recovery. Bone marrow cellularity remained low following high-dose therapy (<20%) to day +16 in most patients and myeloid maturation was slower compared to similar patients receiving GM-CSF.[1] There was morphologic evidence of recovery of all three cell lineages by day +21 in 14 of 14 patients studied. One or more biopsy samples obtained between day +1 and +21 from 11 of 16 patients showed increased fibrosis, a feature not present in any of the patients pre-transplant.

Progenitor cell assays

Table 3 shows the bone marrow colony data for each dose level of IL-1β. Although the number of patients at each dose level is small, the number of hematopoietic colonies present at day +16 and day +21 is increased at higher doses of IL-1β and the number of bone marrow CFU-GM present on day +21 was higher compared to historical control patients receiving no growth factor.[19] The observed increase in day +21 CFU-GM colonies in the group receiving 32 ng/kg/day IL-1β followed by intravenous G-CSF compared to G-CSF-treated and untreated controls may be a result of the relatively high dose of IL-1β; however, it could also suggest that sequential administration of these cytokines may have an enhanced stimulatory effect.

Most patients had detectable concentrations of cytokines at all time points analyzed with the exception of TNF-α (Table 4). In general, concentrations were highest at or following post-transplant day +3. A linear relationship was noted when a dose of IL-1β was compared to either G-CSF, M-CSF, IL-1ra, or sTNF-r concentrations on the day of maximal cytokine secretion during IL-1β administration (Figure 3). Endogenous concentrations of G-CSF were very high as early as day +3 and declined over the next two sampling times (Figure 4). Treatment with IL-1β resulted in significantly higher G-CSF concentrations on both days +3 and +8 compared to a group of historical control patients who received recombinant GM-CSF and also had absolute neutropenia on days +2 to +8.[20] Similarly, day +3 endogenous IL-6 concentrations were higher in patients treated with IL-1β compared to historical controls treated with either recombinant G-CSF or GM-CSF (Figure 5). By day +8 GM-CSF and IL-1β groups displayed similar IL-6 concentrations and were higher than those in the G-CSF group.

Discussion

The evaluation of therapy with new cytokines or hematopoietic growth factors in the setting of high-dose chemotherapy and ABMT is often difficult because of the associated regimen-related toxicity. In this study we have shown that recombinant human IL-1β (OCT-43) can be administered safely to such patients subcutaneously for 7 days. The major toxicities encountered were hypotension and con-

Table 3 Bone marrow progenitor assay results

IL-1 dose ng/kg/day	*CFU-GM[a] post-ABMT day*			*CFU-GEMM[a] post-ABMT day*			*BFU-E[a] post-ABMT day*		
	+11	*+16*	*+21*	*+11*	*+16*	*+21*	*+11*	*+16*	*+21*
4	0	21	12	0	7	2	0	23	34
8	12	10	7	2	5	0.5	10	100	1.5
16	21	19	57	1	0	1	3	0	21
24	7	54	95	0	8	4	0	7	95
32[b]	3	20	140	0	0	0	0	0.5	10
Control	10	26	23						
G-CSF	4	5	5						
GM-CSF	40	33	32						

[a]Mean number of colonies per 10^5 mononuclear cells plated.
[b]Patients at this dose level received G-CSF 5 μg/kg/day i.v. starting day +8 (two patients) or day +16 (one patient) until polymophonuclear cell count $>2.5 \times 10^9$/l.

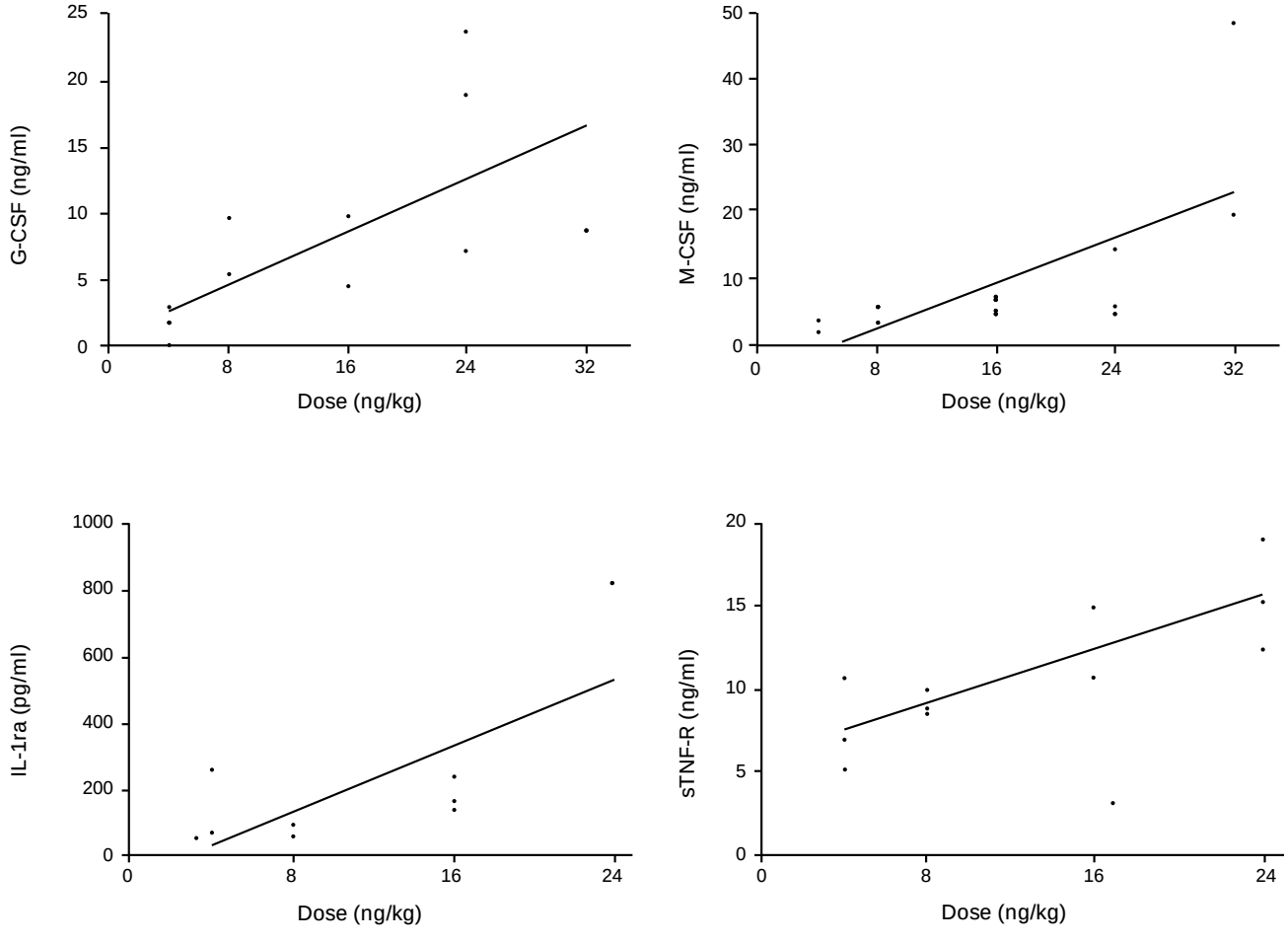

Figure 3 Linear regression of G-CSF, M-CSF, IL-1ra, or TNF-α plasma concentrations *vs* dose of IL-1 (r^2/P values 0.44/0.01, 0.48/0.01, 0.57/0.02, 0.70/0.001, respectively). Day of comparison was selected as the day during IL-1 administration in which the highest percent of samples had measurable concentrations or the day with maximal median concentration (if 100% were measurable).

fusion at the highest dose level, and severe local reactions at the injection site. The MTD of IL-1β given subcutaneously for 7 days following ABMT in this study was 24 ng/kg/day. Toxicity at 32 ng/kg/day was not acceptable. Fever and chills were experienced by the majority of patients. No other significant organ toxicity was identified in our patients that could be attributed to IL-1β.

We did not observe any effect of IL-1β administered by this schedule on hematopoietic recovery compared to historical control patients treated with the same preparative

Table 4 Median serum cytokine concentration, inter-quartile range, and percent of serum samples with detectable concentrations segregated on transplant day in all 16 patients

Day of therapy	*−6*	*−1*	*+1*	*+3*	*+8*	*+12*	*+16*
G-CSF	16.0	66.8	133	7820	3707	301	253
(pg/ml)	2.0–49.0	19.8–82.1	79.0–423	2615–9679	2563–6579	183–845	150–639
	69%	100%	100%	100%	100%	100%	100%
M-CSF	1.15	3.56	2.56	4.81	5.29	7.39	4.10
(ng/ml)	0.952–1.40	2.15–3.98	1.92–3.09	3.31–6.04	3.53–8.77	3.27–12.7	3.20–6.80
	100%	95%	100%	100%	100%	100%	100%
GM-CSF	7.98	8.38	7.24	2.64	6.40	9.89	9.44
(pg/ml)	0–21.3	5.65–17.6	0–16.0	0–11.0	0–11.7	0–22.0	6.55–13.3
	73%	83%	63%	50%	64%	69%	100%
IL-6	4.07	8.51	11.0	80.1	71.4	44.0	47.0
(pg/ml)	0.997–7.04	5.76–11.4	2.4–12.6	23.9–128	54.6–175	24.0–130	21.0–145
	100%	100%	100%	100%	71.4	100%	100%
TNF-α	0	0	0	0	0	0	0
(pg/ml)	0–0	0–0	0–0	0–0	0–0	0–0	0–19.0
	0%	10%	0%	0%	0%	14%	44%
sTNFr	4.36	7.85	6.96	10.6	9.10	10.7	13.8
(ng/ml)	3.30–6.06	6.26–9.12	5.66–8.11	8.38–14.8	5.91–12.7	8.48–17.2	9.84–14.8
	100%	92%	100%	100%	100%	100%	100%
IL-1ra	239	202	95.3	232	163	363	718
(pg/ml)	140–546	133–293	72.9–142	114–516	81.8–389	115–810	447–754
	100%	100%	100%	91%	100%	100%	100%

Bone marrow readministered on day +1, subcutaneous IL-1 given on days +1 to +7.

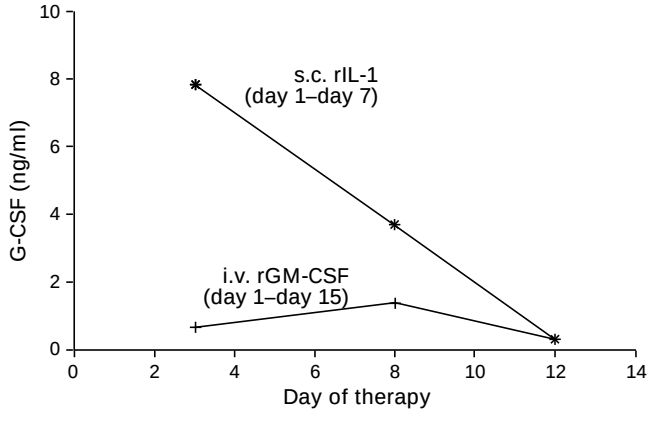

Figure 4 Comparison of median endogenous G-CSF concentrations in 14 patients receiving IL-1β and 20 historical control ABMT patients receiving recombinant GM-CSF.[20] Values were significantly different on days 3 and 8 (*P* values <0.02 on each day).

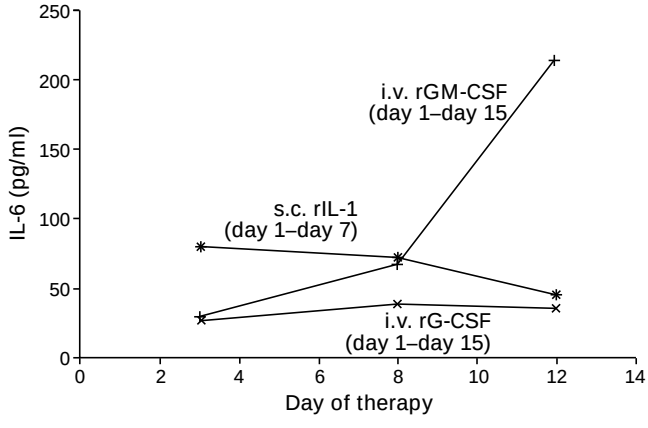

Figure 5 Comparison of median endogenous IL-6 concentrations in 16 patients receiving IL-1β, 20 receiving GM-CSF, and 17 receiving G-CSF.[20] Concentrations were significantly lower in the G-CSF and GM-CSF groups on day 3; whereas the G-CSF group was also lower on day 8 (*P* values <0.05). By day 12 the GM-CSF-treated patients displayed substantially higher IL-6 concentrations (*P* 0.021).

regimen. There appeared to be, however, a dose-related increase in day +21 bone marrow CFU-GM. In addition, the number of day +21 CFU-GM was greater than that seen in historical controls receiving no growth factor post-ABMT. This may be due to an increase by IL-1β in the number of myeloid progenitor cells entering into cell cycle, as suggested by the data of Neta *et al*.[21] The three patients who received IL-1β for 7 days followed by G-CSF until neutrophil recovery had an average number of bone marrow CFU-GM which was greater than that seen in patients we have treated previously from day +1 with G-CSF alone. Although it is tempting to speculate that the high numbers of bone marrow CFU-GM seen in these patients is due to synergy with G-CSF (in agreement with animal models), we do not have data on patients treated at 32 ng/kg of IL-1β alone.

Nemunaitis *et al*[22] treated 17 patients undergoing ABMT for AML with 5 days of 10, 20 or 50 ng/kg/day recombinant human IL-1β given as daily 30-min intravenous infusions. The time required to achieve an absolute neutrophil count greater than 0.5×10^9/l was 25 days compared to 34 days among historical controls who did not receive IL-1β post-ABMT; however, moderate toxicity was observed in all patients. Hypotension was evident in 14 of 17 patients 5 to 8 h after the infusion which required intervention in five patients, only one of which received the highest dose. In comparison, only one patient on our study

required therapeutic intervention for hypotension, despite a 7-day treatment duration. The reason for this difference may be related to differences in patient population (leukemia *vs* solid tumors) or method of drug administration (i.v. *vs* s.c.).

Weisdorf *et al*[23] recently published an evaluation of IL-1α in patients undergoing bone marrow or stem cell transplantation. Daily infusions of IL-1α were given to patients with either Hodgkin's or non-Hodgkin's lymphoma who received total body irradiation and either cyclophosphamide or cyclophosphamide, BCNU, and etoposide with either autologous marrow or peripheral blood progenitor cell support. Doses ranged from 0.1 to 10 mg/m^2/day given for up to 14 days as 6-h infusions. Infusion of IL-α was accompanied by chills and fever ($>$38.3°C) in the majority of patients as was the case in our study of IL-1β. Hypotension (systolic blood pressure $<$90 mm Hg) was also common and dose-limiting. Other toxicities included transient hyperbilirubinemia and renal insufficiency. Patients receiving the maximally tolerated dose of 3.0 μg/m^2/day (approximately twice the maximal dose given on our study) were noted to have acceleration of peripheral myeloid recovery and a shorter time to RBC transfusion independence but no increase in bone marrow cellularity. It is difficult to make direct comparisons of the hematopoiesis induced by IL-1 in the trials mentioned above to the current study since many treatment variables differ. Particularly important is the slower neutrophil recovery in the historic controls of these studies (34 and 24 days to achieve ANC $>$500, respectively) compared to our study (19 days).

Previous studies in humans with normal hematopoiesis have shown that intravenously administered IL-1β can produce a delayed thrombocytosis after 2[15] or 5 days.[16] In a primate ABMT model,[24] subcutaneous injection of IL-1β, 1 to 10 μg/kg twice daily for 5 days after marrow infusion significantly shortened the duration of thrombocytopenia compared to controls. In that study, no shortening of the duration of neutropenia was seen, despite the fact that neutrophilia and a modest increase in bone marrow CFU-GM were seen in normal monkeys given the same doses of IL-1β. One explanation for these variable results may be differences in the cellular composition of the bone marrow in the untreated state and after 5-fluorouracil chemotherapy, total body irradiation or high-dose alkylating agent chemotherapy, in terms of responding cell populations and effector cell secretion of secondary cytokines.[14,22]

Endogenous cytokine concentrations following the administration of IL-1α have also been evaluated by Tilg *et al*.[25] Four patients were treated with IL-1α at a dose of 0.03 μg/kg intravenously over 30 min daily for 5 consecutive days. Both sTNFr as well as IL-1ra levels transiently increased, with maximal concentrations observed at 1 h and 2 h after IL-1α infusion, respectively. The authors note that the levels are much higher than those observed in patients receiving endotoxin or high-dose IL-2.

Secondary production of other cytokines induced by IL-1β may be responsible for some of its observed hematopoietic properties; for example, increased plasma IL-6 is seen in mice after IL-1 treatment.[11] In our patients, plasma concentrations of endogenous G-CSF, M-CSF, IL-1ra, and sTNF-r were linearly correlated to the dose of IL-1β which was administered, thus providing another surrogate marker for a dose-related biologic response. Within 48 h of initiating IL-1β the endogenous concentrations of G-CSF were of the order of those noted following administration of therapeutic doses of recombinant G-CSF[26,27] and significantly higher than those found during administration of recombinant GM-CSF.[20] The synergistic activity obtained when IL-1 and GM-CSF are combined *ex vivo* has been attributed to induction of G-CSF.[28] One interpretation of our data would be that simultaneous combination therapy with IL-1β and G-CSF (in contrast to sequential therapy) would not be of substantial benefit due to the high degree of G-CSF induction noted.

In summary, recombinant human IL-1β can be administered safely to patients following high-dose chemotherapy and ABMT. Our trial confirmed the moderate toxicity observed with IL-1 post-ABMT. No direct effect on recovery of neutrophils or platelets was observed in our patients. However, the presence of increased CFU-GM in the bone marrow is consistent with previous data demonstrating that IL-1β has direct or indirect stimulatory effects on early hematopoietic cells. Dose-dependent increases in endogenous plasma cytokine concentrations were found in these patients, and were significantly higher for molecules such as G-CSF and IL-6 compared to patients receiving GM-CSF. Our data and that of others[14,16,29] suggest that combining brief administration of IL-1β followed by late acting cytokines, such as G-CSF or GM-CSF may produce more rapid recovery of hematopoiesis and possibly affect multiple cell lineages following ABMT. The future role of combination cytokine therapy following high-dose chemotherapy will also be determined by results of the ongoing clinical evaluations of platelet specific factors (IL-11 and thrombopoietin). Should the clinical data from such trials suggest that hematopoiesis remains suboptimal due to a delay in early progenitor maturation, therapeutic combinations which include IL-1β or stem cell factor in addition to a granulocyte and megakaryocyte stimulating molecule may be attempted. Such studies will require detailed evaluation of bone marrow progenitor cell growth and plasma cytokine levels in order to clarify the exact mechanisms operating *in vivo* in man during combination cytokine therapy.

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