A Phase I Study of Recombinant Human Leukemia Inhibitory Factor in Patients with Advanced Cancer

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ABSTRACT

Purpose: Leukemia inhibitory factor (LIF) is a pleiotropic molecule of the interleukin 6 family of cytokines. We aimed to examine the safety, pharmaco kinetics, and biological effects of recombinant human LIF (rhLIF, emfilermin) in patients with advanced cancer.

Experimental Design: In stage 1 of the study, 34 patients received rhLIF or placebo (3:1 ratio) at doses of 0.25–16.0 μg/kg/day or 4.0 μg/kg three times daily for 7 days. In stage 2, 40 patients received rhLIF or placebo, either once daily for 14 days commencing the day after chemotherapy (0.25–8.0 μg/kg/day) or for 7 days commencing the day before chemotherapy (4.0 μg/kg three times daily). The chemotherapy was cisplatin 75 mg/m² and paclitaxel 135 mg/m².

Results: In stage 1, platelet counts increased in most patients, including those who received placebo. Blood progenitor cells increased in response to rhLIF. In stage 2, platelet recovery to baseline levels was earlier for patients receiving higher doses of rhLIF (≥4.0 μg/kg/day; P = 0.02). The neutrophil nadir after chemotherapy was less severe in patients receiving ≥4.0 μg/kg/day of rhLIF. In stages 1 and 2, increases in C reactive protein were seen at higher doses. Several patients developed evidence of autonomic dysfunction, in particular impotence and episodic hypotension. The dose-limiting toxicities were hypotension and rigors. Pharmacokinetic studies demonstrated a short half-life (1–5 h) independent of dose.

Conclusions: We demonstrated a biological effect of rhLIF on blood progenitor cells, C reactive protein levels, and hematopoietic recovery after chemotherapy.

INTRODUCTION

LIF is a cytokine with a broad range of in vitro and in vivo biological effects. The molecule was first described as an inducer of monocytic differentiation in the leukemic cell line M1 (1, 2). LIF is a member of a cytokine family that also includes IL-6, IL-11, oncostatin M, ciliary neurotrophic factor, and cardiotrophin 1. These cytokines have overlapping biological activities and act through receptors that share a common signaling molecule, the gp130 subunit (3). The other subunit of the LIFR complex is the LIFRβ chain. Expression of LIFRβ determines which cells respond to LIF, because gp130 is expressed ubiquitously (4). Ligand binding to the receptor complex results in activation of intracellular signaling via the Janus-activated kinase/signal transducers and activators of transcription pathway.

Receptors for LIF are expressed on hematopoietic cells (macrophages and megakaryocytes), hepatocytes, osteoblasts, preadipocytes, embryonic stem cells, myoblasts, and neuronal cells (S, 6). However, the in vitro biological effects of LIF vary depending on the cell type, so that for example LIF stimulates differentiation in M1 cells and inhibits differentiation in embryonic stem cells. In vivo studies in mice have shown that LIF produces a 2-fold increase in bone marrow megakaryocytes with a dose-dependent increase in platelet numbers (7). Increased...
platelet levels have also been observed in primates (8). The action of LIF on nerve cells has also been examined in animal models. Direct application of LIF to sites of nerve transection improved survival of both sensory and motor neurons (9, 10). Nerve transection has been shown to increase expression of LIF and IL-6, and is associated with retrograde axonal transport of LIF (11). LIF has also been shown to retard progression of motor neuron disease in a murine model of this disorder (12).

Other biological effects of LIF include induction of acute phase proteins (8), reduced lipoprotein lipase activity, and osteoblast stimulation (13).

Emfilermin is rhLIF produced in Escherichia coli. Administration of a single s.c. dose in healthy volunteers found that rhLIF was safe and well tolerated up to doses of 4 μg/kg.5

Here we report results of a randomized, blinded, placebo-controlled, Phase I dose escalation study to test the safety and pharmacokinetics of rhLIF administered before and after chemotherapy in patients with advanced cancer.

PATIENTS AND METHODS

Patients. Eligible patients were those with advanced cancer at least 18 years of age, ECOG performance status of 0–2, absolute neutrophil count of ≥1.5 × 10⁹/liter, hemoglobin level of ≥90 g/liter, and platelet count of 120–500 × 10⁹/liter. Adequate renal (creatinine Cl ≥1.2 ml/sec) and hepatic (bilirubin ≤25 μmol/liter) function were also required. Exclusion criteria included surgery, radiotherapy, or chemotherapy within 4 weeks of study entry, prior irradiation to >30% of estimated red marrow volume, and uncontrolled brain metastases.

The study was approved by the Institutional Ethics Committees of the participating hospitals. Each patient gave informed consent before treatment.

Study Design

The study design is shown in Fig. 1.

Stage 1. The study was double-blinded with respect to rhLIF (emfilermin is the International Nonproprietary Name issued by the WHO for rhLIF produced in E. coli). Patients were randomized to rhLIF or placebo in a 3:1 ratio. A computerized randomization schedule was used. Patients received study drug alone given s.c. once daily for 7 days. At least 4 patients were treated in each dose cohort. Dosing was escalated in the following manner: 0.25, 0.50, 1.0, 2.0, 4.0, 8.0 and 16.0 μg rhLIF per kilogram body weight per day. An additional group of patients was treated with rhLIF 4.0 μg/kg tds to determine whether more frequent dosing enhanced the biological effects of rhLIF. Patients were observed for at least 7 days after finishing the study drug.

Stage 2. After completion of stage 1, patients were able to receive chemotherapy provided no longer than 28 days had elapsed after the last dose of study drug. Patients that did not complete stage 1, or that completed stage 1 but did not want to continue, were replaced for stage 2. Patients were also allowed to enter stage 2 directly without treatment in stage 1 to expand the 4.0 μg/kg tds cohort. Patients continuing from stage 1 to stage 2 remained on the same dose of rhLIF, with the exception that patients who received 16.0 μg/kg/day in stage 1 were given 8.0 μg/kg/day in stage 2.

Chemotherapy consisted of paclitaxel 135 mg/m² given over 3 h by i.v. infusion, followed by cisplatin 75 mg/m² i.v. over 1 h, with mannitol 10% i.v. over 15 min. Premedication for chemotherapy was 20 mg of dexamethasone given p.o. the night before chemotherapy and again on the morning of chemotherapy. 50 mg ranitidine i.v., 12.5 mg promethazine i.v. or equivalent, and 24 mg ondansetron i.v.

The study drug was commenced the day after chemotherapy for patients in the once daily cohorts and given for a total of 14 days. Patients receiving 4.0 μg/kg tds began treatment with rhLIF or placebo the day before chemotherapy, and continued for a total of 7 days. Study involvement was completed after one cycle of chemotherapy, but patients were able to receive additional chemotherapy cycles (but without rhLIF) at the discretion of the investigator. During the study, the use of cytokines other than rhLIF such as filgrastim, sargramostim, or Epo was not permitted.

Monitoring and Laboratory Studies

Stage 1. Patients were monitored at least daily during administration of study drug for adverse events. Formal clinical assessments were performed at screening and day 15. All of the patients were observed for at least 7 days after the last dose of study drug.

Full blood counts and ESR were performed at screening and daily for 15 days from the start of administration of study drug. Platelet aggreometry and PBPC analyses were performed in the 4.0 μg/kg tds cohort only. Progenitor cell assays were performed as described previously (14–16). In brief blood samples for progenitor cell assays were separated using Ficoll-Paque. Mononuclear cells were examined in triplicate cultures using 10⁴ and 10⁵ viable cells/ml. The cultures were examined at 14 days using a dissection microscope. GM-CFCs were stimulated with G-CSF, GM-CSF, and IL-3.
and SCF. Erythroid colonies (BFU-E) were stimulated with
GM-CSF, IL-3, IL-6, SCF, and Epo. Meg-CFCs were stimu-
lated with G-CSF, GM-CSF, SCF, IL-3, IL-6, Epo, and
megakaryocyte growth and development factor, and stained
for quantitation of MEG-CFC as described previously (14–
16). CD 34 assays were performed as described previously
(17). Briefly, mononuclear cells were separated and analyzed
on Coulter profile II flow cytometer (Hialeah, FL). A two-
color method was used in which lineage-specific antibodies
(CD3, CD2, CD14, CD19, and CD20; all from Coulter) were
labeled with tricolor dye (Caltag, San Francisco, CA) and
used to separate these cells from lineage-negative CD 34+
cells. The cutoff for positive cells was determined from the
negative control antibody profiles, and percentages and ab-
solute numbers of cells were determined as described (17).

Neurological assessment included a standardized neurologi-
cal symptom questionnaire on motor, sensory, and autonomic
disturbances (18). Patients also had neurological examinations,
creatine kinase measurements, and assessments of autonomic
function including postural changes in blood pressure and heart
rate response. Assessments were performed at baseline, at com-
pletion of rhLIF treatment, and again 1 week later. Neurological
assessment was performed independently of adverse event re-
porting.

Assays for antibodies to rhLIF were performed weekly.
Each assay required 5 ml of blood that was collected in a
heparinized tube. The blood was centrifuged, and the aliquot
of plasma was frozen and transported to Amrad Operations, where
a validated ELISA assay that detected both endogenous and
recombinant LIF.5 Five-ml of blood were collected in a hepa-
rinized tube, centrifuged within 3 h of collection, and the aliquot
of plasma was then frozen at −20°C until the time of assay.
Samples were analyzed in ascending dose order, as each cohort
completed the study. The limit of detection using this method
was 0.05 ng/ml.

Statistical Analysis
The data were analyzed using SigmaStat (Jandel Scientific
Software, San Rafael, CA) or Prism (GraphPad Software, San
Diego, CA). Data are expressed as medians and ranges for
continuous data, and frequency and percentage for categorical
data, unless otherwise specified. Dose cohort size was deter-
mined empirically to allow efficient dose escalation and not to
produce sufficient statistical power to allow comparisons be-
tween individual dose cohorts.

RESULTS

Patients
Fifty-two patients in total were enrolled in stages 1 and 2.
The most common tumor types were non-small cell lung cancer
and carcinoma of unknown primary. In each of stages 1 and 2,
2 patients were withdrawn after enrolment before receiving
study drug. The reasons for withdrawal were inadequate major
organ function (therefore, that patient was deemed ineligible),
acute myocardial infarction, reaction to paclitaxel chemotherapy
in stage 2, and withdrawal of consent. These 4 patients are not
included in the safety or biological activity analyses. The base-
line characteristics of the patients that received at least one dose
of study drug are shown in Table 1. There were no significant
differences between the placebo and rhLIF cohorts in terms of
age, sex, performance status, and extent of prior treatment.

The number of patients in each dose cohort is shown in
Table 2. Twenty-six patients who completed stage 1 were
treated in stage 2. An additional 16 patients were enrolled
directly into stage 2, of whom 14 received treatment.

Peripheral Blood Counts
Stage 1 (rhLIF/Placebo Alone before Chemotherapy).
Platelet levels rose over the period of monitoring in most pa-
inients, including those treated with placebo. We have observed
this effect on platelets in patients receiving placebo in previous
studies (19). Fig. 2 shows the peak level of platelets versus
baseline for patients receiving placebo, lower doses of rhLIF
(0.25–2.0 μg/kg/day), doses of 4.0–16.0 μg/kg/d rhLIF and 4.0
μg/kg tds rhLIF. There were no significant differences between
these four groups (P = 0.60; ANOVA). There were also no
significant changes in platelet aggregometry, hemoglobin, or
neutrophil counts (data not shown).

Stage 2 (rhLIF with Chemotherapy). The median time
of platelet recovery to baseline after administration of cho-
motherapy was shorter in the patients receiving “high” doses
of rhLIF (with ≥4.0 μg/kg/day arbitrarily defined as the high dose
and including the 4.0 μg/kg tds cohort; Fig. 3). Recovery to
baseline levels was 3 days earlier in patients that received high
doses of rhLIF when compared with patients that received doses
≤2.0 μg/kg/day or placebo. This grouping of patients was
justified, because there was no significant difference in either
the time to platelet recovery or the depth of the platelet nadir between patients who received placebo or ≤2.0 μg/kg/day rhLIF. The baseline characteristics of the “low” and high dose groups are shown in Table 3. There were some differences with regard to age, sex, and prior chemotherapy. The median baseline platelet count of the high dose group was higher than that of the low dose group, although this difference did not reach statistical significance (P = 0.09; t test). The median time of platelet recovery to baseline was 12 days after chemotherapy in the high dose rhLIF group. On this day, platelet levels in patients receiving high doses of rhLIF were significantly higher than those in the combined low dose plus placebo group (P = 0.02; t test). The levels remained significantly higher for the next 2 days. The median time to platelet recovery in the other group was 15 days. No patients required platelet transfusion during stage 2.

Consistent with the effect on platelet recovery, rhLIF also hastened neutrophil recovery. After chemotherapy, neutrophil counts fell additionally in patients who received placebo or low dose rhLIF when compared with those who received ≥4.0 μg/kg/day (Fig. 4). The median neutrophil count remained above 2.0 × 10^9/ml in patients receiving the high doses of rhLIF. At day 14 after chemotherapy, the difference between the high dose group and the group that received either low dose or placebo was significantly different (P = 0.02; t test). This significant difference was maintained on day 15 (P = 0.01; t test). Only a few patients had neutrophil counts measured on day 16, and neutrophil counts recovered to ≥2.0 × 10^9/ml in the placebo group by day 17. Episodes of febrile neutropenia were infrequent with this chemotherapy regimen and did not allow a comparison of this end point for the different groups.

There was no significant difference in hemoglobin levels at different doses of rhLIF versus placebo.

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* Non-small cell lung cancer.
* Includes ovary, bladder, colon, endometrium, and prostate.

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* ( ), patients enrolled directly into stage 2, who did not receive treatment in stage 1.

Peripheral Blood Progenitor Cells

The results of CD34 and PBPC assays are shown in Fig. 5. A >10-fold increase in GM-CFC and BFU-E levels was seen in 2 of the patients receiving rhLIF 4.0 μg/kg tds. This was associated with an 8-fold and 3-fold increase in Meg-CFC levels, and a 10-fold and 1.2-fold increase in CD34-positive cells. Consistent with the increases in progenitor cell counts, the platelet levels in these 2 patients increased ~2-fold and 1.5-fold, respectively, during stage 1 (see Fig. 2D). These 2 patients were relatively young (52 and 57) and had a good performance status (0 and 1). Neither patient had received chemotherapy, radiotherapy, or hematologic growth factors before the study.

Biochemical Assays

No significant changes in renal or liver function tests (including lactate dehydrogenase) were demonstrated.
Fibrinogen and ESR were not altered significantly by rhLIF administration (data not shown).

CRP levels were elevated during stages 1 and 2 for patients receiving doses of rhLIF ≥4.0 μg/kg/day. Whereas baseline CRP levels were highest in patients receiving ≥4.0 μg/kg/day rhLIF, these patients also had the largest median rise in CRP. CRP levels in patients receiving ≥4.0 μg/kg/day rhLIF were significantly higher than those in the placebo group over the course of stage 1 (Fig. 6; P = 0.008; Wilcoxon test) and stage 2 (data not shown; P < 0.001). In stages 1 and 2, CRP levels in patients receiving ≥4.0 μg/kg/day rhLIF rose to a peak 24 h after the first dose. No atherosclerotic complications of elevated CRP were observed during the study period.

Neurological Evaluation

There were no consistent motor or sensory changes found. However, some changes in autonomic function were documented. Seven male patients reported impotence in response to the neurological symptom questionnaire, but it was a pre-existing symptom in 3 of these patients. No one receiving placebo reported this symptom in stage 1. Two patients that received active rhLIF reported onset of impotence in stage 1 that persisted throughout stage 2. An additional 3 patients reported it developing in stage 2, and 1 of these patients received placebo. The impotence had not resolved by the completion of stage 2 in any of the patients. Generally these patients received higher doses of rhLIF: it was reported by patients receiving ≥4.0 μg/kg/day rhLIF. Because there were in total 25 males in the active therapy group and 6 in the placebo group, the percentage developing impotence during the study was similar for the two groups (16% in active group and 17% in the placebo group). The impotence was not consistently associated with hypotension. However, 1 patient, who received 8.0 μg/kg/day of rhLIF developed both impotence and postural dizziness. Other symptoms consistent with autonomic dysfunction were reported: postural dizziness was noted in 6 patients, 2 of whom received placebo, and nocturnal diarrhea occurred as a new symptom in 1 patient who also developed postural dizziness and received 1.0 μg/kg/day rhLIF.

Adverse Events

Stage 1. The adverse events shown in Table 4 are those attributed, by the blinded investigators, to study drug in patients receiving ≥4.0 μg/kg/day rhLIF, whereas baseline CRP levels were highest in patients receiving ≥4.0 μg/kg/day rhLIF, these patients also had the largest median rise in CRP. CRP levels in patients receiving ≥4.0 μg/kg/day rhLIF were significantly higher than those in the placebo group over the course of stage 1 (Fig. 6; P = 0.008; Wilcoxon test) and stage 2 (data not shown; P < 0.001). In stages 1 and 2, CRP levels in patients receiving ≥4.0 μg/kg/day rhLIF rose to a peak 24 h after the first dose. No atherosclerotic complications of elevated CRP were observed during the study period.

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Adverse Events

Stage 1. The adverse events shown in Table 4 are those attributed, by the blinded investigators, to study drug in ≥2 patients, or that were deemed severe. The dose-limiting toxicities at 16.0 μg/kg/day were hypotension and rigors. The most common adverse events were fevers and rigors, hypotension, headache, dizziness, and local reactions at the rhLIF injection sites. The fevers and rigors occurred within 2 h of rhLIF administration and usually resolved spontaneously within 1 h. Musculoskeletal pain occurred on the day of rhLIF administration and in some cases persisted throughout the day.

Hypotension occurred in 5 patients receiving rhLIF. It was not noted in any placebo patients. There were 3 episodes of severe hypotension. These occurred in patients receiving 2.0, 4.0, and 16.0 μg/kg/day of rhLIF. The episodes occurred within 1 h of administration of study drug. Two patients were treated with i.v. normal saline with resolution of the event within a few
hours. The hypotension in the third patient resolved on lying down for 10 min.

Five patients did not complete stage 1. The reasons were withdrawal of consent, disease progression, severe hypotension (16.0 µg/kg/day cohort), and intolerable rigors (2 patients in 4.0 µg/kg tds cohort).

Stage 2. The adverse events attributed to rhLIF were similar in type, frequency, and severity to the events in stage 1. One patient receiving rhLIF developed deep vein thrombosis. The platelet count at the time was 333 × 10^9/liter.

Three patients did not complete stage 2. The reasons were withdrawal of consent by 1 patient, sepsis, and renal impairment unrelated to the study drug and hypotension (2.0 µg/kg/day cohort).

There were no deaths related to the study drug.

Pharmacokinetics

Absorption of rhLIF from the s.c. injection site was rapid, with maximum plasma concentration reached within 2 h and within 10–20 min in some patients. The other major pharmacokinetic parameters measured were C_max, AUC, Cl, V_d, and t_1/2. These are shown in Table 5 for stage 1. The parameters measured in stage 2 were similar to those in stage 1. Cl and V_d appeared to be inversely related to the dose, with results at the 4.0 µg/kg/day dose being similar to the 4.0 µg/kg tds dose. However, calculation of these two parameters assumed that absorption from the s.c. injection site was complete, which may not have been the case. t_1/2 appeared to be independent of dose and was relatively short at ~2 h. The relationship between dose of rhLIF and plasma concentration is shown in Fig. 7. This demonstrates that, in stage 1, when the 16.0 µg/kg/day cohort (Fig. 7B) was compared with the 8.0 µg/kg/day cohort (Fig. 7A), the C_max was 10-fold higher and the AUC >4-fold higher. Thus, for this dose increment, a doubling of dose more than doubled the resulting concentration.

DISCUSSION

This study examined the safety and pharmacokinetics of emfilermin (rhLIF) when given before and after chemotherapy. A number of parameters were monitored to determine the biological activity of rhLIF.

On the basis of previous studies (7, 8), rhLIF was predicted to influence the platelet count. When rhLIF was administered alone, platelet counts increased over time in the majority of patients. However, a similar increase was also seen in patients receiving placebo, and the difference between the placebo and active drug groups was not significant. The rise in platelets in all of the groups was probably a result of the regular venules that was required, a response we have seen before (19) and illustrating the importance of placebo cohorts in these studies. Of more interest were the results after chemotherapy. In stage 2, recovery of platelets to baseline levels after chemotherapy was significantly more rapid in patients receiving high doses of rhLIF. For this analysis, patients were divided into a low (including placebo) and a high dose group. This division was based on the lack of evidence of a biological effect of rhLIF in any of the parameters tested at the lower doses. Furthermore, the comparison of patients who received low (apparently inactive) doses of rhLIF with those receiving active doses would only serve to reduce the significance of a genuine effect of rhLIF. However, there were differences in the baseline characteristics of the low and high dose groups, particularly with regard to age and prior chemotherapy that would tend to favor the high dose group. A significant advantage in terms of platelet recovery was demonstrated for those patients who received high doses of rhLIF. Thus, although when administered alone rhLIF did not have a detectable effect on platelet counts, the thrombopoietic action was more apparent after the hematopoietic stress imposed by chemotherapy. It is noteworthy that the effects seen with rhLIF are quite consistent with those seen with the related cytokines IL-6 (20) and IL-11 (21), as well as others. In particular, IL-11 has been demonstrated to be useful clinically despite a relatively modest effect on platelets, comparable with the effect seen with rhLIF here, and in contrast to the dramatic rise in platelets after administration of thrombopoietin (22–25). Given the small numbers of patients in the study and the differences between patient...
groups, a randomized study designed to look specifically at the clinical benefit of rhLIF after chemotherapy would be required to confirm the effect on platelet recovery.

When administered alone rhLIF produced no significant change in neutrophil counts. However, the neutrophil nadir after chemotherapy was significantly less severe in patients receiving 4.0 μg/kg/day of rhLIF. This effect on neutrophil recovery was not seen with other thrombopoietic agents and was not a prominent feature of the preclinical studies. It may be related to the fact that fewer patients had received prior chemotherapy in the group that received 4.0 μg/kg/day of rhLIF.

Peripheral blood progenitor cell levels were measured in the 4.0 μg/kg tds cohort in stage 1. Two of the 5 patients receiving active rhLIF at this dose had >10-fold increases in levels of megakaryocyte, granulocyte-macrophage, and erythroid precursors, and this was associated with an increase in platelet counts. Two other patients in this cohort did not receive a full course of rhLIF therapy, which was ceased because of rigors. An increase in blood progenitor cell levels has been seen previously with numerous cytokines including G-CSF (26, 27), GM-CSF (28), megakaryocyte growth and development factor (15), and SCF (14). The kinetics of PBPC mobilization seen with rhLIF were similar to those seen with SCF (rather than G-CSF), although the mechanism is unknown.

Because LIF and related cytokines are known to be involved in the acute phase response, several markers of this response were tested. There was a significant increase in CRP in response to rhLIF. This was evident although the baseline levels of CRP were higher in the high dose cohorts. However, no

**Table 4  Adverse events in 34 patients in stage 1**

<table>
<thead>
<tr>
<th></th>
<th>rhLIF (n = 26)</th>
<th>Placebo (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection site reaction/rash/pain</td>
<td>8 (31)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Systemic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>4 (15)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fever</td>
<td>6 (23)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rigors</td>
<td>9 (35)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>5 (19)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dermatological</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pruritus</td>
<td>2 (8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Neurological</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>6 (23)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Headache</td>
<td>5 (19)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>4 (15)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Impotence</td>
<td>3 (12)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>4 (15)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other gastrointestinal</td>
<td>8 (31) [1]</td>
<td>4 (50)</td>
</tr>
</tbody>
</table>

* (), percentage of patients.

[^1]: no. of patients experiencing severe adverse events.

[^2]: One life-threatening episode included in severe group.

[^3]: Severe episode of constipation.

---

**Fig. 5** CD34 assays and progenitor cell numbers in patients in 4.0 μg/kg tds cohort from stage 1. Four patients received rhLIF and 1 patient received placebo. The 2 patients referred to in the text are shown A, CD34 counts; B, GM-CFC (note logarithmic axis); C, BFU-E; D, Meg-CFC.

**Fig. 6** Stage 1, median CRP levels. The groups shown received placebo (●; n = 8), 0.25–2.0 μg/kg/day (▲; n = 12) and ≥4.0 μg/kg/day rhLIF (■; n = 14; includes 4.0 μg/kg tds cohort).
increase in ESR or fibrinogen was seen. In clinical studies both IL-6 and IL-11 have produced increases in CRP and fibrinogen (20, 21). rhLIF has been shown to increase acute phase proteins IL-6 and IL-11 have produced increases in CRP and fibrinogen increase in ESR or fibrinogen was seen. In clinical studies both IL-6 and IL-11 have produced increases in CRP and fibrinogen (20, 21). rhLIF has been shown to increase acute phase proteins

In the studies, the dose levels tested in the primates were 2, 10, and 50 in rhesus monkeys. However, the dose levels tested in the studies were 2, 10, and 50 μg/kg/day for 14 days, and the increases seen in that study were much smaller at the 2 μg/kg/day dose level than at higher doses (8). Therefore, it may be that the doses tested in our study were high enough to induce an increase in CRP, but not high enough to increase fibrinogen and ESR. CRP is known to activate the complement system (29) and may have a role in the pathogenesis of arterial inflammation leading to atherosclerosis (30). Whereas nonspecific inflammatory effects such as fever and local injection site reactions were seen during the study, no clinical evidence of arterial inflammation was observed. Complement levels were not measured, and there was no long-term follow-up for delayed atherosclerotic events after study completion.

On the basis of published data (9, 10), we anticipated an effect of rhLIF on the nervous system. Despite this, we were surprised when both neurological assessment and adverse event reporting revealed impotence as an effect frequently associated with rhLIF therapy. Additional symptoms consistent with an autonomic effect of rhLIF were hypotension, postural dizziness, and nocturnal diarrhea. The episodes of hypotension were usually noted within the first hour after rhLIF administration, and usually resolved rapidly and spontaneously. There were three episodes of severe hypotension, and 2 patients required therapy with i.v. fluids. Whereas hypotension was documented as a part of adverse event assessment, the independent neurological evaluation showed postural dizziness to be a common symptom. Of the 6 patients affected by postural dizziness, 4 received rhLIF. Three patients reported onset of impotence and 1 patient onset of diarrhea after receiving chemotherapy; 1 of the patients who experienced impotence received placebo. Whereas neurotoxicity is a recognized side effect of both chemotherapy drugs used in this study (31–33), the symptoms in patients receiving rhLIF before chemotherapy suggest that these effects were mediated by rhLIF.

In addition to hypotension, the main adverse effects of rhLIF in this study were fevers and rigors, hypotension, headache, dizziness, and local reactions at the rhLIF injection sites. Whereas 2 of the 5 patients in stage 1 who received 4.0 μg/kg tds were withdrawn because of rigors, all 9 of the patients treated at this dose level in stage 2 completed the study. The local reactions and constitutional effects of rhLIF are similar to those reported for related cytokines (20, 21).

The t1/2 of rhLIF was relatively short, ranging from approximately 1–5 h, and was independent of dose. The observation of the t1/2 in stage 1 led to the 4.0 μg/kg tds schedule being added to the protocol. This schedule resulted in more sustained plasma concentrations, with the biological effects described above, but no accumulation of rhLIF, presumably because of the short t1/2. However, in apparent contrast, there was a disproprionate increase in CRP, but not high enough to increase fibrinogen and ESR.
tionate increase in $C_{\text{max}}$ and a 4-fold increase in AUC with dose escalation from 8.0 $\mu$g/kg/day to 16.0 $\mu$g/kg/day. We assumed this reflected increased bioavailability of rhLIF, although decreased CI could have contributed to this result. The increase in $C_{\text{max}}$ at lower dose levels was more linear.

In conclusion, we demonstrated a biological effect of rhLIF on blood progenitor cells, CRP levels, and on platelet and neutrophil recovery after chemotherapy. Administration was associated with autonomic effects, particularly postural dizziness and impotence. Despite these effects, rhLIF was generally well tolerated in doses up to and including 8.0 $\mu$g/kg/day and 4.0 $\mu$g/kg tds. Its action on neurological cells, particularly nerve regeneration, suggests that additional clinical studies are warranted.

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REFERENCES


A Phase I Study of Recombinant Human Leukemia Inhibitory Factor in Patients with Advanced Cancer

Dishan H. Gunawardana, Russell L. Basser, Ian D. Davis, et al.


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