Cancer Chemother Pharmacol (1999) 43: 141–144 

ORIGINAL ARTICLE

**Steven H. Bernstein · Joseph Fay · Stanley Frankel
Neal Christiansen · Maria R. Baer · Cindy Jacobs
Consuelo Blosch · Roberta Hanna · Geoffrey Herzig**

# A phase I study of recombinant human soluble interleukin-1 receptor (rhu IL-1R) in patients with relapsed and refractory acute myeloid leukemia

Received: 6 October 1997 / Accepted: 1 April 1998

**Abstract** *Purpose*: The recombinant human interleukin-1 receptor (rhu IL-1R) is a soluble truncated form of the type 1 full-length membrane-bound receptor that binds IL-1 with identical affinity to that of the membrane form. As such, it may have clinical potential by sequestering IL-1, thereby preventing it from binding to its membrane-bound receptor and eliciting a biological effect. As IL-1 has been shown to regulate leukemic cell proliferation in an autocrine fashion, a phase I trial of rhu IL-1R was conducted in patients with relapsed and refractory acute myeloid leukemia (AML). *Methods*: The study group comprised 11 patients who were sequentially treated on one of three dose levels, receiving a single intravenous (i.v.) bolus dose on day 1 followed by 13 days of daily subcutaneous (s.c.) injections with the option of an additional 14 days of treatment if a response of stable disease or better was achieved. *Dose level 1* i.v. bolus 500 $\mu g/m^2$, s.c. dose 250 $\mu g/m^2$ per day (five patients); *dose level 2* i.v. bolus 1000 $\mu g/m^2$, s.c. dose 500 $\mu g/m^2$ per day (three patients); *dose level 3* i.v. bolus 2000 $\mu g/m^2$, s.c. dose 1000 $\mu g/m^2$ per day (three patients). Owing to limited drug availability, the study was designed to only examine these three dose levels. *Results*: rhu IL-1R was well tolerated. There was no grade 3 or 4 non-hematological toxicity related to the study drug and the maximum tolerated dose was not reached. No IL-1R-blocking antibodies developed during the course of the study. Serum levels of IL-1β, IL-6 and TNF were undetectable before, during and after rhu IL-1R administration. The terminal half-life after i.v. dosing was at least 7–12 h, and after s.c. dosing 2–4 days. Serum levels of rhu IL-1R up to 360- and 25-fold those of pretreatment levels were achieved after i.v. and s.c. dosing respectively. No patient had a complete, partial or minor response to treatment; four had stable disease and seven had progressive disease. *Conclusions*: rhu IL-1R therapy was safe but did not have any apparent antileukemic effect at the doses administered.

**Key words** Interleukin-1 · Interleukin-1 receptor · Acute myeloid leukemia · Phase I

Supported by a grant from the Immunex Corporation

S.H. Bernstein · S. Frankel · N. Christiansen · M.R. Baer · G. Herzig
Roswell Park Cancer Institute, Elm and Carlton Street, Buffalo, Ny 14263, USA
Tel.: +1-716-845-7611; Fax: +1-716-845-8446

J. Fay
Baylor/Saammons Cancer Center, Dallas, TX, USA

C. Jacobs · C. Blosch · R. Hanna
Immunex Corporation, Seattle, WA, USA

## Introduction

Progenitor cells from patients with acute myeloid leukemia (AML) have been shown to spontaneously proliferate in vitro. This is thought to be due to the autocrine production of growth factors by leukemic cells [9]. The interruption of such autocrine pathways could potentially be exploited as a novel treatment for AML.

Interleukin-1 (IL-1) appears to play a critical role in regulating leukemic cell proliferation. Human leukemic blasts express the IL-1 gene and secrete the IL-1 protein [2, 6, 11]. In addition, these cells express functional IL-1 receptors that bind and internalize IL-1 [2]. Exposure of leukemic blasts to IL-1 induces significant proliferation [2]. In contrast, the spontaneous proliferation of such cells is inhibited by anti-IL-1 antibodies [2]. Therefore, IL-1 satisfies the requirements for an autocrine growth factor for AML; thus, it may play a role in its pathogenesis.

One potential strategy for the treatment of AML is to use a soluble IL-1 receptor (sIL-1R) to block this IL-1 autocrine loop. The sIL-1R is a truncated form of the type 1 full-length membrane-associated receptor that has identical binding affinity to that of the membrane form [4]. As such, it may act as a competitive inhibitor of IL-1, binding the protein before it binds to the membrane receptor and elicits its biological effects. Indeed, the in vitro exposure of human leukemic progenitors to recombinant

human IL-1 receptor (rhu IL-1R) inhibits leukemic blast colony proliferation in a dose-dependent fashion [5]. This inhibition is partially reversed by exposure to IL-1β. In contrast, normal bone marrow progenitor proliferation is not inhibited by rhu IL-1R exposure. Taken together, these findings suggest that rhu IL-1R could be used as a novel agent for the treatment of AML. To this end, we conducted a phase I study of rhu IL-1R for patients with relapsed and refractory AML.

## Patients and methods

### Patients

Patients with a diagnosis of AML that had either relapsed from or failed to respond to conventional primary therapy were eligible. Patients needed to be >18 years of age, have an ECOG performance status of 0–2 and an expected survival >4 weeks from the start of treatment. Prior chemotherapy, other than hydroxyurea, was not allowed within 3 weeks of initiation of treatment; hydroxyurea was not allowed within 3 days of the study. Patients could not have received any investigational agent within 4 weeks of study entry. Patients were required to have an absolute neutrophil count (ANC) >250/μl and a leukemic blast count <40 000/μl. A screening complete blood count (CBC) was required 1 week prior to study drug administration. If the peripheral blood blast count was >5000/μl on this screening CBC, the patient was excluded if the blast count 2 days prior to study drug administration was greater than twice that of the screening CBC. Patients with active infection, concurrent chemotherapy or corticosteroid therapy were excluded. Patients were required to have adequate renal (BUN <50 mg/dl, creatinine <2.0 mg/dl), hepatic (bilirubin less than twice the upper limit of normal, SGOT or SGPT less than three times the upper limit of normal) and cardiac function (cardiac class III and IV were excluded). This study was approved by the Institutional Review Boards at both Roswell Park Cancer Institute and Baylor/Sammons Cancer Center and all patients were required to sign informed consent.

### Study design

This was a multicenter, phase I, open label, dose- finding study to evaluate the safety and tolerability of rhu IL-1R administration (Immunex Corporation, Seattle, Wash). Patients received one of three subcutaneous (s.c.) doses of rhu IL-1R for 13 days, beginning 24 h after a single intravenous (i.v.) loading dose that was given over 30 minutes on day 1 of the study. Patients with stable disease or evidence of a response by day 14 could receive an additional 14 days of therapy.

Three dose levels were studied: *level 1* (day 1) i.v. bolus of 500 μg/m$^2$, (days 2–14) s.c. dose of 250 μg/m$^2$, per day; *level 2* (day 1) i.v. bolus of 1000 μg/m$^2$, (day's 2–14) s.c. dose of 500 μg/m$^2$ per day; *level 3* (day 1) i.v. bolus of 2000 μg/m$^2$ (day's 2–14) s.c. dose of 1000 μg/m$^2$ per day. Dose escalation proceeded if three patients successfully completed treatment at the prior dose level without dose-limiting toxicity (grade 3 nonhematological toxicity not relieved by symptomatic treatment or any grade 4 nonhematological toxicity). Owing to limited drug availability, the study was designed to only examine these three dose levels.

### Cytokine assays

Peripheral blood was collected for determinations of IL-1β, tumor necrosis factor α (TNF-α) and IL-6 levels prior to, twice weekly during and once upon completion of therapy. Cytokine levels were determined using an enzyme-linked immunosorbent assay (ELISA).

**Table 1** Clinical, laboratory and response data (*WBC* white blood count, *ANC* absolute neutrophil count, *ABC* absolute blast count, *ND* not done, *PD* progressive disease, *SD* stable disease)

| Patient no. | Age/Sex (years) | Disease status | Dose level | No. of doses of rhu IL-IR | Pretreatment bone marrow | | Posttreatment bone marrow | | Pretreatment blood | | | Posttreatment blood | | | Response |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Cellularity (%) | Myeloblasts (%) | Cellularity (%) | Myeloblasts (%) | WBC (×10$^9$/l) | ANC (×10$^9$/l) | ABC (×10$^9$/l) | WBC (×10$^9$/l) | ANC (×10$^9$/l) | ABC (×10$^9$/l) | |
| 101 | 73/M | Relapse (2) | 1 | 5 | 50 | 65 | ND | ND | 23.3 | 1.2 | 12.4 | 36.5 | 1.1 | 22.6 | PD |
| 102 | 58/M | Relapse (1) | 1 | 8 | 35 | 95 | ND | ND | 39.9 | 1.2 | 37.1 | 92.1 | 0.0 | 86.6 | PD |
| 103 | 71/M | Relapse (1) | 1 | 16 | 40 | 24 | 95 | 31 | 2.4 | 0.4 | 0.1 | 11.4 | 2.9 | 0.8 | PD |
| 104 | 74/F | Relapse (2) | 1 | 14 | 95 | 70 | 100 | 76 | 10.3 | 1.3 | 0.0 | 25.8 | 1.3 | 0.0 | SD |
| 105 | 67/M | Relapse (1) | 1 | 28 | 25 | 20 | 35[a] | 30 | 3.4 | 1.8 | 0.0 | 2.5 | 1.2 | 0.1 | PD |
| 201 | 61/F | Relapse (1) | 2 | 14 | 55 | 20 | 95 | 70 | 9.5 | 0.7 | 5.0 | 55.8 | 7.8 | 30.1 | PD |
| 202 | 69/M | Refractory | 2 | 14 | 30 | 25 | 55 | 80 | 2.3 | 0.4 | 0.0 | 4.7 | 0.5 | 0.2 | PD |
| 203 | 56/M | Relapse (1) | 2 | 14 | 50 | 34 | 35 | 36 | 3.0 | 1.9 | 0.0 | 1.8 | 0.6 | 0.1 | SD |
| 301 | 62/M | Relapse (1) | 3 | 14 | 25 | 18 | 25 | 22 | 1.2 | 0.3 | 0.0 | 0.9 | 0.4 | 0.0 | SD |
| 302 | 52/F | Relapse (1) | 3 | 14 | 45 | 58 | 35 | 70 | 1.4 | 0.6 | 0.0 | 2.0 | 0.4 | 0.4 | SD |
| 303 | 77/F | Releapse (1) | 3 | 11 | 50 | 73 | 50 | 90 | 10.6 | 3.9 | 4.7 | 87.4 | 10.5 | 72.5 | PD |

[a] Bone marrow test done on day 14, not at the end of treatment

### Antibody determinations

Peripheral blood was collected prior to and upon completion of therapy for determination of antibodies to rhu IL-1R. Anti-IL-1R antibodies were determined by ELISA. For the single patient who developed an anti-IL-1R antibody, the serum was further evaluated in the sIL-1R inhibition assay.

### Pharmacokinetic studies

Peripheral blood was collected for pharmacokinetic analysis at 0.5, 1, 3, 6, 10 and 24 h after the i.v. bolus dose. During s.c. dosing, samples were collected twice weekly prior to the injections, prior to the last injection; 2, 4, 8, 10 and 24 h after the last injection, then daily for 5 consecutive days. Rhu IL-1R was assayed using an ELISA developed at Immunex as previously described [8]. This assay did not distinguish between endogenous sIL-1R and rhu IL-1R and measured both free and bound sIL-1R. The limit of detection of this assay was 125 pg/ml.

### Response definitions

A complete response (CR) was defined as recovery of normal marrow elements with $< 5\%$ blasts as well as recovery of peripheral counts with an ANC $\geq 1500/\mu l$, hematocrit (Hct) $\geq 30\%$, platelet count $\geq 100\,000/\mu l$ and no peripheral blasts. A partial response (PR) was defined as recovery of normal peripheral blood counts (ANC $\geq 1500/\mu l$, Hct $\geq 30\%$, platelet count $\geq 100\,000/\mu l$) with a bone marrow that contained 5–24% leukemic blasts. A minor response was defined as any decrease in the number of leukemic blasts in the peripheral blood and bone marrow with no decline in the neutrophil and platelet counts compared to pretreatment values. Stable disease was defined as a less than a twofold increase in the number of leukemic blasts in the bone marrow and blood compared to pretreatment values while maintaining an ANC $> 200/\mu l$ and a stable platelet count. Progressive disease was defined as a response less than that of stable disease.

## Results

### Patients

Patient demographic, clinical and laboratory data are shown in Table 1. The median age was 62 years (range 52–77 years). All patients had received prior cytarabine- and anthracycline-based chemotherapy regimens. Eight patients were treated at first relapse, two at second relapse and one had primary refractory disease. Of 11 patients enrolled in this study, all were evaluable for toxicity. Eight patients completed at least one course of therapy (six patients received 14 days, one received 16 days and one received 28 days of rhu IL-1R). Three patients were withdrawn from study because of progressive disease after 5, 8 and 11 days of rhu IL-1R, respectively.

### Toxicity

The rhu IL-1R was well tolerated. There were no grade 3 or 4 toxicities felt possibly, probably or definitely related to rhu IL-1R. Grade I/II toxicities felt possibly, probably or definitely related to rhu IL-1R included, at level 1, elevated alkaline phosphatase (one patient), fatigue (one patient), injection site pain (one patient) and fever (one patient), and at level 2, anorexia (one patient) and hypomagnesemia (one patient). There were no grade I/II toxcities in patients treated at level 3.

### Serum cytokine levels

As IL-1 has been shown to induce the production of both IL-6 [14] and TNF-$\alpha$ [1], cytokines that affect leukemic cell proliferation [3, 7], we evaluated the effect of rhu-IL-1R on the serum levels of these cytokines. There were no detectable levels of IL-6 or TNF in the serum assayed from ten patients before, during or after rhu IL-1R administration. In addition, there were also no detectable levels of IL-1$\beta$ in these patients.

### Anti-rhu IL-1R antibodies

Serum samples from ten patients were assayed for IgG antibodies to rhu IL-1R. Antibody was detected in one patient, but this antibody was shown not to block IL-1 binding to the receptor in a receptor inhibition assay.

### Pharmacokinetics (Table 2)

Serum levels of sIL-1R range between 0 and 1 ng/ml in normal individuals. In this study, most patients had pretreatment sIL-1R levels higher than this, with a median of 2.29 ng/ml (range 0.55–6.21 mg/ml). The mean

**Table 2** Pharmacokinetic analysis (*AUC* area under the concentration curve, $C_{max}$ maximum concentration of drug, $t_{1/2}$ half-life of drug). Values are means ± SE

| Dose ($\mu g/m^2$) | No. of patients | AUC[a] ($\mu g \cdot h/ml$) | $C_{max}$ (ng/ml) | $t_{1/2}$ (min) |
|---|---|---|---|---|
| Intravenous dosing | | | | |
| 500 | 3 | 37 690 ± 6724 | 84.64 ± 18.94 | 566.02 ± 92.55 |
| 1000 | 3 | 115 922 ± 15 446 | 237.85 ± 50.68 | 710.45 ± 177.45 |
| 2000 | 5 | 254 834 ± 24 301 | 835.73 ± 221.28 | 474.42 ± 10.11 |
| Subcutaneous dosing | | | | |
| 250 | 2 | 67 538 ± 15 316 | 18.27 ± 4.29 | 6464.47 ± 995.33 |
| 500 | 3 | 188 261 ± 160 658 | 33.80 ± 9.97 | 3391.93 ± 5448.56 |
| 1000 | 4 | 241 864 ± 542 675 | 57.56 ± 1.55 | 5628.58 ± 512.99 |

[a] For i.v. dosing, $AUC_{(0\text{-}24h)}$, i.e. the AUC from the time of the pre-i.v. dose sample to the 24-h posttreatment i.v. dose sample. For s.c. dosing, $AUC_{(0-t)}$, i.e. the AUC from the time of the pre-s.c. dose sample to the sample taken between 1 and 12 days after the last s.c. dose

maximum concentration of rhu IL-1R achieved after i.v. bolus at a dose of 2000 $\mu g/m^2$ was 835.73 ± 221.28 ng/ml, and after s.c. administration at a dose of 1000 $\mu g/m^2$ was 57.56 ± 1.55 ng/ml. The terminal half-life after a single i.v. bolus was at least 7–12 hours. After s.c. administration, the terminal half-life was at least 2–4 days.

Treatment response

No patient had a CR, PR or minor response. Four patients met criteria for stable disease and seven patients had progressive disease.

## Discussion

Both IL-1α and IL-1β bind to either type I or type II IL-1-specific membrane-associated receptors [12]. The cDNA for the human type I IL-1R has been cloned and the sequences isolated and truncated to form soluble receptors suitable for clinical use [4, 13]. The binding affinity of such soluble receptors is indistinguishable from that of the full-length membrane-associated receptors [4]. The potential clinical utility of such a soluble receptor is in its ability to sequester IL-1α and IL-1β before such cytokines activate the membrane-bound receptor and mediate its biological effects. Indeed, such soluble receptors have shown potential benefit in patients with allergic conditions as well as in those with steroid-resistant graft-versus-host disease (GVHD) [8, 10]. Based upon in vitro studies suggesting an autocrine role for IL-1 in the proliferation of human leukemic blasts, this phase I study was conducted in patients with relapsed and refractory AML.

Rhu IL-1R was well tolerated at i.v. doses up to 2000 $\mu g/m^2$ and s.c. doses up to 1000 $\mu g/m^2$. There was no dose-limiting toxicity seen. Only one patient had a local skin reaction to the s.c. injections which was grade 1 in intensity. No patient developed neutralizing antibodies to the membrane-associated IL-1R.

Endogenous sIL-1R levels were elevated pretreatment in most of the patients studied. Such elevations have been reported in inflammatory conditions such as active GVHD. Levels up to 25- and 360-fold that of baseline were achieved after s.c. and i.v. dosing, respectively. At the dose levels studied there appeared to be linear kinetics with i.v. dosing; however additional patients and dose levels need to be studied to determine if there are linear kinetics after s.c. dosing.

Although this study was designed as a phase I safety and tolerability trial, we did not see any responders to rhu IL-1R therapy. There are several possible explanations for this. First, the mean highest sustained serum levels of rhu IL-1R achieved after daily sc dosing in this study was approximately 60 ng/ml. Under in vitro conditions, leukemic blasts directly exposed to these concentrations still show less than 50% growth inhibition [5]. In addition, it is likely that the concentration of rhu IL-1R in the marrow would be even less than that in the serum. Given that dose-limiting toxicity was not achieved in this study, it is possible that at higher dosages, an inhibitory effect on leukemic growth may occur. However, there clearly are other mechanisms and cytokines involved in leukemic cell growth. Thus, even if an IL-1 autocrine loop is inhibited, it is possible that leukemic cell proliferation will continue to be driven by these other mechanisms.

## References


1. Billiau A, Van Damme J, Opdenakker G, Fibbe WE, Falkenburg JHF, Conten J (1986) Interleukin-1 as a cytokine inducer. Immunobiology 172: 323
2. Cozzolino F, Rubartelli A, Aldinucci D, Sitia R, Torcia M, Shaw A, Di Guglielmo R (1989) Interleukin 1 as an autocrine growth factor for acute myeloid leukemia. Proc Natl Acad Sci USA 86: 2369
3. Delwel R, Buitenen C van, Lowenberg B, Touw I (1992) Involvement of tumor necrosis factor (TNF) receptors p55 and p75 in TNF responses of acute myeloid leukemia blasts in vitro. Blood 80: 1798
4. Dower SK, Wignall JM, Schooley K, McMahan CJ, Jackson JL, Prickett KS, Lupton S, Cosman D, Sims JE (1989) Retention of ligand binding activity by the extracellular domain of the IL-1 receptor. J Immunol 142: 4314
5. Estrov Z, Kurzrock R, Estey E, Wetzler M, Ferrajoli A, Harris D, Blake M, Gutterman JU, Talpaz M (1992) Inhibition of acute myelogenous leukemia blast proliferation by interleukin-1 (IL-1) receptor antagonist and soluble IL-1 receptor. Blood 79: 1938
6. Griffin JD, Rambaldi A, Vallenga E, Young D, Ostapovicz D, Cannistra SA (1987) Secretion of interleukin-1 by acute myeloblastic leukemia cells in vitro induces endothelial cells to secrete colony stimulating factors. Blood 70: 1218
7. Hoang T, Haman A, Goncalves O, Wong GG, Clark SC (1988) Interelukin-6 enhances growth factor-dependent proliferation of the blast cells of acute myeloblastic leukemia. Blood 72: 823
8. McCarthy PL, Williams L, Harris-Bacile M, Yen J, Przepiorka D, Ippoliti C, Champlin R, Fay J, Blosch C, Jacobs C, Anasetti C (1996) A clinical phase I/II study of recombinant human interleukin-1 receptor in glucocorticoid-resistant graft-versus-host disease. Transplantation 62: 626
9. Metcalf D (1989) The roles of stem cell self-renewal and autocrine growth factor production in the biology of myeloid leukemia. Cancer Res 49: 2305
10. Mullarkey MF, Leiferman KM, Peters MS, Caro I, Roux ER, Hanna RK, Rubin AS, Jacobs CA (1994) Human cutaneous allergic late-phase response is inhibited by soluble IL-1 receptor. J Immunol 152: 2033
11. Rodriguez-Cimadevilla JC, Beauchemin V, Villeneuve L, Letendre F, Shaw A, Hoang T (1990) Coordinate secretion of interleukin-1β and granulocyte-macrophage colony-stimulating factor by the blast cells of acute myeloblastic leukemia; The role of interleukin-1 as an endogenous inducer. Blood 76: 1481
12. Sims JE, Giri JG, Dower SK (1994) The two interleukin-1 receptors play different roles in IL-1 actions. Clin Immunol Immunopathol 74: 9
13. Slack J, McMahan CJ, Waugh S, Schooley K, Spriggs MK, Sims J, Dower SK (1993) Independent binding of interleukin-1α and interleukin-1β to type I and type II interleukin-1 receptors. J Biol Chem 268: 2513
14. Van Damme J, Cayphas S, Opdenakker G, Billiau A, Van Snick J (1987) Interleukin-1 and poly(rI) poly (rC) induce production of a hybridoma growth factor by human fibroblasts. Eur J Immunol 17: 1