# Clinical and Biological Effects of Recombinant Human Interleukin-18 Administered by Intravenous Infusion to Patients with Advanced Cancer

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**Abstract**

**Purpose:** Interleukin-18 (IL-18) is an immunostimulatory cytokine with antitumor activity in preclinical animal models. A phase I study of recombinant human IL-18 (rhIL-18) was done to determine the toxicity, pharmacokinetics, and biological activities of rhIL-18 in patients with advanced cancer.

**Experimental Design:** Cohorts of patients were given escalating doses of rhIL-18, each administered as a 2-hour i.v. infusion on 5 consecutive days. Toxicities were graded using standard criteria. Serial blood samples were obtained for pharmacokinetic and pharmacodynamic measurements.

**Results:** Twenty-eight patients (21 with renal cell cancer, 6 with melanoma, and 1 with Hodgkin's lymphoma) were given rhIL-18 in doses ranging from 3 to 1,000 μg/kg. Common side effects included chills, fever, nausea, headache, and hypotension. Common laboratory abnormalities included transient, asymptomatic grade 1 to 2 neutropenia, thrombocytopenia, anemia, hypoalbuminemia, hyponatremia, and elevations in liver transaminases. One patient in the 100 μg/kg cohort experienced transient grade 3 hypotension and grade 2 bradycardia during the first infusion of rhIL-18. No other dose-limiting toxicities were observed. Plasma concentrations of rhIL-18 increased with increasing dose, and 2.5-fold accumulation was observed with repeated dosing. Biological effects of rhIL-18 included transient lymphopenia and increased expression of activation antigens on lymphocytes and monocytes. Increases in serum concentrations of IFN-γ, granulocyte macrophage colony-stimulating factor, IL-18 binding protein, and soluble Fas ligand were observed. Two patients experienced unconfirmed partial responses after rhIL-18 treatment.

**Conclusions:** rhIL-18 can be safely given in biologically active doses to patients with advanced cancer. A maximum tolerated dose of rhIL-18 was not determined. Further clinical studies of rhIL-18 are warranted.

Interleukin (IL)-18 is an immunostimulatory cytokine that regulates both innate and adaptive immune responses (1, 2). The effects of IL-18 are mediated through a specific cell surface receptor complex composed of at least two subunits, an α chain (IL-1Rrp1) and a β chain (AcPL; ref. 3). IL-18 induces synthesis of IFN-γ by T cells and natural killer (NK) cells, augments the cytolytic activity of NK cells and CTLs, and promotes differentiation of activated CD4 T cells into helper effector cells (1, 2). IL-18 and IL-12 show similar activities (4), including synergistic induction of IFN-γ synthesis and stimulation of Th1 immune responses (5–7). However, IL-12 and IL-18 are structurally unrelated, belonging to the hematopoietic cytokine superfamily and the IL-1 superfamily, respectively. The biological activity of IL-18 is modulated in a negative feedback loop by a specific IL-18 binding protein (IL-18 BP) induced through IFN-γ.

IL-18 has antitumor activity in animal models (8–12). Regression of tumors in IL-18-treated animals is not dependent on the presence of IFN-γ or IL-12 but seems to require an intact Fas/Fas ligand pathway (9, 10). The antitumor effects of IL-18 in multiple animal models provided the rationale for investigation of recombinant human (rh) IL-18 in cancer immunotherapy. We describe the results of the first clinical trial of rhIL-18 in patients with cancer.

## Materials and Methods

***Patient selection.*** Eligible patients included adults (ages >18 years) with histologically confirmed, locally advanced, or metastatic solid tumor or lymphoma that was measurable and refractory to standard

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therapy or for which no effective therapy was available. Patients with Hodgkin's lymphoma were required to have had a second recurrence and to be ineligible for autologous hematopoietic stem cell transplantation. Patients were required to have a Karnofsky performance status of at least 70%, an estimated life expectancy of at least 12 weeks, hemoglobin ≥9 g/dL, absolute neutrophil count ≥1,500 per μL, platelet count ≥100,000 per μL, serum bilirubin ≤1.5 mg/dL, serum aspartate aminotransferase and alanine aminotransferase ≤3× upper limit of normal, serum creatinine ≤1.5 mg/dL or estimated creatinine clearance >50 mL/min, prothrombin and partial thromboplastin within normal limits, and no detectable serum antibody to the study drug. Patients with a history of coronary artery disease were required to have a stress test with no clinically significant abnormality; patients with a history of congestive heart failure, myocardial infarction, or prior anthracycline therapy were required to have a left ventricular ejection fraction of at least 40%. Patients were excluded if they were pregnant or breast-feeding or had severe or uncontrolled infection, known leptomeningeal or brain metastases, significant autoimmune disease, or a history of ventricular arrhythmia requiring drug or device therapy.

***Study design.*** This open-label, nonrandomized, dose-escalation phase I clinical study was conducted at two centers. The protocol was approved by the Institutional Review Boards at Indiana University Medical Center (Indianapolis, IN) and Beth Israel Deaconess Medical Center (Boston, MA); written informed consent was obtained from each patient before enrollment on study. rhIL-18 (study drug SB-485232) was supplied by GlaxoSmithKline (Research Triangle Park, NC). rhIL-18 was given as a 2-hour i.v. infusion on each of 5 consecutive days. Premedication with antipyretics was not given before the first dose of rhIL-18. Oral acetaminophen was given for drug-related fever and i.v. meperidine as needed for rigors. Patients returned for follow-up evaluations on days 10, 15, 22, and 29 of the study. Only a single 5-day course of rhIL-18 was given.

Successive cohorts of patients received rhIL-18 in doses ranging from 3 to 1,000 μg/kg daily for 5 consecutive days. Escalation of the rhIL-18 dose for an individual patient was not permitted.

At least three patients were enrolled in each dose cohort, and all patients within a dose cohort were followed for at least 14 days on study before initiating treatment at the next dose level. If any patient experienced dose-limiting toxicity (DLT) at a particular dose level, then an additional three patients were to be enrolled at that dose level and an additional cohort of three patients added at a dose level that was 50% of the next planned dose level. Dose escalation was to be halted if more than two patients within a dose cohort experienced DLT, as the maximum tolerated dose would be exceeded. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria version 2.0. DLT was defined as any grade 3 or 4 toxicity assessed to be related to study drug, except that grade 3 fever, nausea, vomiting, lymphopenia, or leukopenia were not considered DLT.

***Pharmacokinetic measurements.*** Blood samples were collected for determination of rhIL-18 concentration before the dose of rhIL-18 on days 1 to 5 and at 0.67, 1.33, 2, 2.5, 4, 8, 12, and 24 hours after the start of the rhIL-18 infusion on days 1 and 5 of study. Plasma concentrations of rhIL-18 were measured using a specific fluoroimmunoassay.

***Pharmacodynamic measurements.*** Blood samples were collected before each rhIL-18 infusion on days 1 to 5, at 6 and 12 hours after the start of the infusion on day 1, and on days 6 and 15 for determination of serum concentrations of IFN-γ, neopterin, and soluble Fas ligand and plasma concentrations of granulocyte macrophage colony-stimulating factor, IL-18 BP, and IL-12. Protein concentrations were measured using specific ELISA methods. Blood samples were collected before each dose on days 1 to 5, at 4 and 8 hours after the start of the infusion on day 1, and on days 6, 10, and 15 for analysis of leukocyte subsets by flow cytometry. Aliquots of blood were incubated with FITC-, phycoerythrin-, and peridinin chlorophyll protein–conjugated monoclonal antibodies in erythrocyte lysis buffer, washed, fixed, and analyzed by flow cytometry. During analysis, forward and side scattering properties were used to create a lymphocyte gate. Thresholds for discriminating specific staining above background were established by analysis of samples stained with control antibodies. Histograms were generated and corrected by subtraction of isotype controls using CellQuest software (Becton Dickinson, Franklin Lakes, NJ). Circulating lymphocyte and neutrophil counts were obtained before starting the infusion on days 1 to 5, at the end of the 2-hour infusion on days 1 and 5, and on days 6, 10, 15, 22, and 29.

***Detection of anti-rhIL-18 antibodies.*** Blood samples were collected for detection of antibodies to study drug before enrollment on study and on days 10, 15, 22, and 29 of study. Antibodies to rhIL-18 were assessed using an electrochemiluminescence immunoassay. *In vitro* neutralizing activity was assessed using a bioassay, in which IL-18-mediated induction of IFN-γ in KG-1 cells was assessed in the presence or absence of anti-IL-18 antibodies.

***Criteria for response evaluation.*** Tumor measurements were obtained within 28 days before the first dose of rhIL-18 and on day 29 of study. Tumor measurements beyond day 29 of study were not mandated by the study protocol and were done at the discretion of a patient's physician. Tumor responses were assessed using Response Evaluation Criteria in Solid Tumors criteria.

## Results

***Patient characteristics.*** Twenty-eight patients were enrolled on study (Table 1). The median age was 58 years (range, 20-75 years). All patients had received one or more prior treatments with immunotherapy, chemotherapy, or radiation therapy. The patient with Hodgkin's lymphoma had received multiple prior treatments, including autologous hematopoietic stem cell transplantation. Of the 21 patients with renal cell carcinoma, 19 (90%) had undergone prior nephrectomy and 17 (81%) had received prior treatment with IL-2, 4 (19%) with IFN, 3 (14%) with IL-12, and 2 (9%) with chemotherapy. Of the six patients with melanoma, two (33%) had received prior treatment with IL-2, three (50%) with IFN, and three (50%) with chemotherapy. Common sites of metastatic disease at the time of enrollment on study included lung in 26 (93%), lymph nodes in 20 (71%), bone in 11 (39%), and liver in 8 (29%) patients.

***Administration of rhIL-18.*** rhIL-18 was administered in doses of 3 μg/kg ($n$ = 3 patients), 10 μg/kg ($n$ = 4 patients), 30 μg/kg ($n$ = 3 patients), 100 μg/kg ($n$ = 6 patients), 200 μg/kg ($n$ = 3 patients), 300 μg/kg ($n$ = 3 patients), 600 μg/kg ($n$ = 3

**Table 1.** Patient characteristics

| | *n* | % |
|---|---|---|
| Sex | | |
| Male | 18 | 64 |
| Female | 10 | 36 |
| Tumor type | | |
| Renal cell carcinoma | 21 | 75 |
| Prior nephrectomy | 19 | 68 |
| Melanoma | 6 | 21 |
| Hodgkin's lymphoma | 1 | 4 |
| Prior therapy | | |
| Radiation | 9 | 32 |
| Chemotherapy | 6 | 21 |
| IL-2 | 19 | 68 |
| IFN | 7 | 25 |
| Other | 9 | 32 |

patients), and 1,000 μg/kg ($n$ = 3 patients). All patients received the five planned doses of rhIL-18, except for one patient enrolled in the 100 μg/kg cohort who developed a DLT after receiving a single dose of rhIL-18 (see below). One patient with Hodgkin's lymphoma enrolled in the 10 μg/kg cohort received all five planned doses but exhibited disease progression requiring corticosteroid therapy on day 6 of study. This patient was taken off the study, and an additional patient was enrolled in the 10 μg/kg cohort. Two additional patients in the 200 μg/kg ($n$ = 1) and 1,000 μg/kg ($n$ = 1) dose cohorts completed the planned five doses of rhIL-18 and were assessable for safety through day 15 but developed interval disease progression before day 29 of the study.

***Clinical toxicities and laboratory abnormalities.*** Common side effects associated with rhIL-18 administration included grade 1 to 2 chills, fever, nausea, headache, and hypotension (Table 2). Patients in all dose cohorts experienced fever and chills, the latter typically occurring ~1 hour after the start of study drug infusion. Fever and chills were readily ameliorated with standard supportive care measures. Headache and nausea occurred more frequently in patients receiving rhIL-18 doses >100 μg/kg. Common laboratory abnormalities associated with rhIL-18 administration included elevations in liver transaminases and blood urea nitrogen, hypoalbuminemia, hyperglycemia, and hyponatremia (Table 3). These abnormalities were all of grade 1 to 2 severity, except for two cases of grade 3 hyperglycemia; the latter were not associated with clinical symptoms and rapidly resolved after completion of rhIL-18 administration. Grade 1 elevations of serum creatinine were present before the first study drug infusion in six patients with renal cell cancer; all but one of these patients had undergone prior nephrectomy. These patients did not have any significant worsening of the grade 1 serum creatinine elevations during the study. Five additional patients developed transient, asymptomatic grade 1 elevation in serum creatinine after rhIL-18 administration.

Hematologic toxicity was also generally mild and rapidly reversible. Grade 1 thrombocytopenia occurred in seven patients. The only patient who experienced grade 2 thrombocytopenia had been extensively pretreated and had undergone prior autologous stem cell transplantation for refractory Hodgkin's lymphoma. Grade 1 to 2 neutropenia occurred frequently and was not obviously dose dependent (Table 3). However, grade 3 neutropenia was only observed in patients treated with rhIL-18 doses of ≥300 μg/kg. No patient experienced infection attributed to neutropenia. Grade 1 to 2 anemia, presumably due to advanced cancer, was present in 16 patients before receiving the first dose of rhIL-18. Most of the 12 patients with normal baseline hemoglobin levels experienced transient, grade 1 anemia. However, 4 of these 12 patients maintained a normal hemoglobin level throughout the study period, whereas 2 patients still had grade 1 anemia on the last day of the study.

***Serious adverse events and DLT.*** Only one serious adverse event occurred that was attributed to study drug administration. One patient with renal cell cancer enrolled in the 100 μg/kg dose cohort developed grade 3 hypotension and grade 2 sinus bradycardia during his first infusion of study drug. The infusion was stopped and the patient recovered after being given i.v. fluids. As this was deemed to be a DLT according to the protocol, the patient was taken off the study and the 100 μg/kg cohort was expanded to include six patients. No DLT was observed in subsequent patients enrolled in the 100, 200, 300, 600, and 1,000 μg/kg cohorts. Therefore, a maximum tolerated dose of rhIL-18, as given by this schedule, was not determined.

***Pharmacokinetic results.*** Daily i.v. infusion of rhIL-18 for 5 consecutive days resulted in 2.5-fold accumulation with a geometric mean accumulation half-life ($t_{1/2}$) of 35 hours (Table 4). Systemic exposure (area under the plasma concentration versus time curve) generally increased with increasing dose.

***Biological effects of rhIL-18* in vivo.** rhIL-18 induced a rapid, transient increase in both IFN-γ and granulocyte macrophage colony-stimulating factor from below limits of detection at baseline to the highest observed values at the first sampling time point 6 hours after initiation of the first infusion and returning to below detection by 24 hours in most patients. Granulocyte macrophage colony-stimulating factor (Fig. 1A), soluble Fas ligand, and neopterin (data not shown) increased in all patients after dosing. IFN-γ increased in 18 patients and was not measurable in 10 patients (Fig. 1B). Induction of IL-18 BP was evident in all patients (Fig. 2) with increases above baseline detected at the first sampling time point (6 hours) and the highest observed values detected between days 2 and 5. IL-18 BP concentrations were generally maintained during the 5 days of dosing returning to baseline by day 15. IL-12 was not detected in any sample.

rhIL-18 administration was also accompanied by a rapid decline in total lymphocyte counts from normal levels at baseline to a nadir at the end of the first 2-hour infusion followed by gradual recovery to baseline between days 6 and 10 (Fig. 3A). This lymphopenia (grade 1-3), observed in all patients, was considered to be a biological rather than toxic effect (see Discussion). The temporal shifts in total lymphocyte counts reflected underlying changes in NK cells, $CD8^+$ and $CD4^+$ T cells, and NK T cells. The most profoundly and consistently affected lymphocyte subset was NK cells (Fig. 3B), which remained significantly lower than baseline as late as 24 hours after the dose. The temporal shifts in $CD8^+$ and $CD4^+$ T cells, NK T cells, and neutrophils (data not shown) following dosing were similar to NK cells, but the decline in cell number was smaller and more variable with a slower recovery to baseline by day 10. Activation of lymphocyte subsets was also observed, generally coincident with the nadir in circulating counts. The percentage of NK cells expressing Fas ligand increased up to 147-fold from baseline by 4 hours after initiation of the first infusion on day 1 and returned to baseline by 24 hours where it remained despite continued dosing through day 5 (Fig. 3C). Expansion of other activated lymphocyte subsets was also observed. The proportion of $CD8^+$ T cells and NK T cells expressing Fas ligand increased up to 280- and 27-fold from baseline, respectively, by 4 hours after start of infusion on day 1 and returned to baseline by 24 hours. The proportion of $CD8^+$ T cells expressing CD69 increased up to 5-fold from baseline by 48 to 72 hours and returned to baseline by day 10. Figure 4 displays representative individual examples of the change in intensity of expression for several activation antigens measured before dosing and 4 hours after the first dose of rhIL-18. Increased expression intensity (right shift) was observed for Fas ligand on $CD8^+$ T cells and NK cells (despite a decline in counts from 1,710 to 312) but not on $CD4^+$ T cells. Similarly, expression of CD11b was increased on monocytes and NK cells (despite a decline in counts from

**Table 2.** Adverse events experienced by more than one subject in all treatment groups

| Body system event | Grade level* | Dose (μg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 10 | 30 | 100 | 200 | 300 | 600 | 1,000 | Total |
| *n* | | 3 | 4 | 3 | 6 | 3 | 3 | 3 | 3 | 28 |
| No. subjects with any event | | 3 | 3 | 3 | 6 | 3 | 3 | 3 | 3 | 27 |
| Cardiac disorders | | | | | | | | | | |
| Sinus bradycardia | 3 | | | | | | | | | 3 |
| | 2 | | | | 1 | | | | | |
| | 1 | | | 1 | 1 | | | | | |
| Gastrointestinal disorders | | | | | | | | | | |
| Nausea | 3 | | | | | | | | | 11 |
| | 2 | 1 | | | | 1 | 2 | | | |
| | 1 | | | 1 | 3 | 1 | | 1 | 1 | |
| General disorders | | | | | | | | | | |
| Chills | 3 | | | | | | | | | 21 |
| | 2 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | |
| | 1 | 1 | 1 | 1 | 3 | 2 | 3 | 1 | 2 | |
| Pyrexia | 3 | | | | | | | | | 20 |
| | 2 | | 1 | | 2 | 1 | | | | |
| | 1 | 2 | 2 | 3 | 3 | 1 | 3 | 1 | 1 | |
| Musculoskeletal and connective tissue disorders | | | | | | | | | | |
| Arthralgia | 3 | | | | | | | | 1 | 5 |
| | 2 | | | | 1 | | | | 1 | |
| | 1 | | 1 | | | 1 | | | | |
| Nervous system disorders | | | | | | | | | | |
| Headache | 3 | | | | | | | | | 10 |
| | 2 | | | | | 1 | | | | |
| | 1 | 1 | | 2 | 1 | 1 | 1 | 2 | 1 | |
| Skin and s.c. tissue disorders | | | | | | | | | | |
| Rash | 3 | | | | | | | | | 5 |
| | 2 | | | | | 1† | 1 | | | |
| | 1 | | | 1 | | 2 | | | | |
| Vascular disorders | | | | | | | | | | |
| Hypotension | 3 | | | | 1 | | | | 1 | 8 |
| | 2 | | 1 | | | | | | | |
| | 1 | | 1 | 1 | | | 1 | 1 | 1 | |
| Hypertension | 3 | | | | | | | | | 2 |
| | 2 | | | | | | | | | |
| | 1 | | | | 2 | | | | | |

*There were no grade 4 adverse events reported.
†Includes preferred terms of rash and rash papular.

2,299 to 642), and CD69 was increased on $CD8^+$ T cells. The induction of plasma cytokines and shifts in circulating lymphocyte subsets described above were observed at all doses. However, interpatient variability in all of these measures was high, and a simple relationship between response and dose was not evident.

***Production of antibodies to rhIL-18.*** Twenty-six patients were evaluable for development of treatment-related anti-IL-18 antibodies. Antibodies to study drug were detected in the serum of 10 of 26 (38%) evaluable patients. *In vitro* neutralizing activity was detectable in 1 of 10 patients with anti-IL-18 antibodies. Anti-IL-18 antibodies were initially detected at a median of 14 days (range, 8-15 days) after the first injection of study drug. After first being detected, anti-IL-18 antibodies persisted in all subsequent serum samples tested in five patients, were intermittently detected in two patients, and became undetectable in three patients. There were no obvious differences in toxicity, basic pharmacokinetic variables, or biological effects between patients who developed anti-IL-18 antibodies and those who did not.

***Tumor responses.*** Evidence of antitumor activity after administration of rhIL-18 was observed in two patients enrolled in the 100 μg/kg cohort. A patient with metastatic melanoma with s.c. nodules, lung nodules, and lymphadenopathy had stable disease (~26% reduction in target lesions by Response Evaluation Criteria in Solid Tumors criteria) on day 29 physical examination and computed tomography scans. In the absence of any further therapy, the target lesions regressed by 69% on

**Table 3.** Laboratory abnormalities observed during study drug administration

| Abnormality | Grade | Dose (μg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 10 | 30 | 100 | 200 | 300 | 600 | 1,000 |
| | | *n* = 3 | *n* = 4 | *n* = 3 | *n* = 6 | *n* = 3 | *n* = 3 | *n* = 3 | *n* = 3 |
| Clinical chemistry | | | | | | | | | |
| Hypokalemia | 1-2 | | 1 | | | 1 | | 1 | 1 |
| | 3-4 | | | | | | 1 | | |
| Hyponatremia | 1-2 | 2 | 2 | | | 2 | 1 | 1 | 1 |
| | 3-4 | | | | | | | | |
| Hypoalbuminemia | 1-2 | 2 | 1 | 2 | 4 | 2 | 3 | 2 | 3 |
| | 3-4 | | | | | | | | |
| Hyperglycemia | 1-2 | 1 | | 1 | 2 | 1 | | 1 | 2 |
| | 3-4 | | 1 | 1 | | | | | |
| Elevated aspartate aminotransferase | 1-2 | 3 | 2 | 1 | 1 | 2 | 1 | 2 | 1 |
| | 3-4 | | | | | | | | |
| Elevated alanine aminotransferase | 1-2 | 2 | 1 | 1 | 2 | 1 | 2 | | 2 |
| | 3-4 | | | | | | | | |
| Elevated blood urea nitrogen | 1-2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | |
| | 3-4 | | | | | | | | |
| Hematology | | | | | | | | | |
| Anemia | 1-2 | 2 | 1 | 1 | 3 | 1 | | 2 | 2 |
| | 3-4 | | 1 | | | | | | |
| Neutropenia | 1-2 | 2 | 2 | 1 | 4 | 2 | 2 | | 1 |
| | 3-4 | | | | | | 1 | 1 | |
| Leukopenia | 1-2 | 3 | | 2 | 4 | 2 | 2 | 2 | 2 |
| | 3-4 | | 2 | | 1 | | 1 | | |
| Lymphopenia | 1-2 | 2 | 3 | 1 | | 2 | 2 | 1 | 2 |
| | 3-4 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 1 |

evaluation done 3 months after the last dose of study drug. A patient with renal cell carcinoma with bilateral pulmonary nodules had stable disease (7% increase in target lesions by Response Evaluation Criteria in Solid Tumors criteria) on day 29 computed tomography scans. In the absence of further therapy, the target lesions decreased by 56% on computed tomography scans done 5 months after the last dose of study drug.

## Discussion

IL-18 was first described as a novel cytokine that stimulated IFN-γ production by T cells and enhanced the cytolytic activity of NK cells *in vitro* (5). The importance of IL-18 *in vivo* was confirmed by studies of IL-18-deficient mice, which exhibit defective Th1 immune responses and NK cell cytotoxicity (13). IL-18 participates in protective immune responses to intracellular bacteria, fungi, and viruses (1, 2). Moreover, IL-18 has antitumor activity in preclinical models of lung cancer, breast cancer, sarcoma, and melanoma (8–12).

We report the first description of the effects of rhIL-18 in human subjects. rhIL-18 administration was well tolerated by daily i.v. infusion for 5 consecutive days in doses as high as 1,000 μg/kg/d. Common toxicities included grade 1 to 2 chills, fever, nausea, headache, and hypotension. These toxicities were

**Table 4.** Median (range) plasma rhIL-18 pharmacokinetic variables

| rhIL-18 dose, μg/kg (*n*) | Day 1 AUC (h × ng/mL) | Day 5 AUC (h × ng/mL) | Accumulation ratio | Accumulation $t_{1/2}$ (h) |
|---|---|---|---|---|
| 3 (3) | 214 (122-247) | 461 (273-479) | 2.24 (1.87-2.24) | 30 (24-42) |
| 10 (4) | 353 (228-465) | 881 (704-1,045) | 2.27 (1.85-4.57) | 31 (19-94) |
| 30 (3) | 771 (490-858) | 2,099 (2,076-2,250) | 2.69 (2.62-4.29) | 37 (36-38) |
| 100 (5) | 627 (574-852) | 1,993 (1,774-2,790) | 3.27 (2.49-3.70) | 41 (27-61) |
| 200 (3) | 869 (884-904) | 2,252 (1,627-3,108) | 2.55 (1.87-3.44) | 36 (31-37) |
| 300 (3) | 1,983 (1,255-2,392) | 5,527 (2,715-6,357) | 2.31 (2.22-3.21) | 35 (30-44) |
| 600 (3) | 3,443 (3,379-3,869) | 6,192 (5,393-6,456) | 1.60 (1.57-1.91) | 26 (25-30) |
| 1,000 (3) | 5,941 (5,941-8,523) | 9,945 (9,308-17,362) | 1.67 (1.57-2.04) | 36 (34-36) |

Abbreviation: AUC, area under the plasma concentration versus time curve.

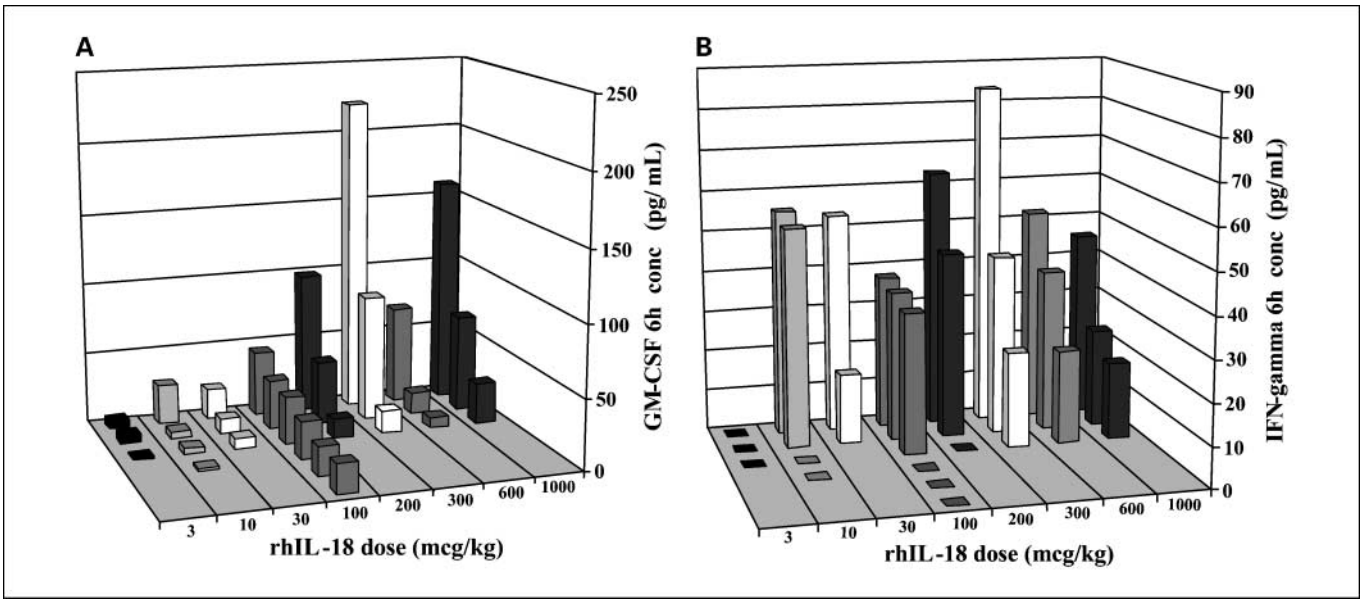


**Figure 1.** Peak plasma granulocyte macrophage colony-stimulating factor (*GM-CSF*; *A*) and serum IFN-γ (*B*) levels in patients receiving rhIL-18. Columns, maximum cytokine concentration observed in an individual patient within each dose cohort. Peak cytokine levels were detected 6 hours after the first rhIL-18 infusion on day 1 in all patients.

expected based on previous experience with immunostimulatory cytokines (14). Common laboratory abnormalities observed during rhIL-18 administration included elevations in liver transaminases and blood urea nitrogen, hypoalbuminemia, hyperglycemia, hyponatremia, and cytopenias. These abnormalities were generally of modest severity (grade 1-2), not associated with any symptoms, and rapidly reversible after cessation of rhIL-18 administration. Unacceptable toxicity occurred in only 1 of 28 patients receiving rhIL-18, and a maximum tolerated dose of rhIL-18 given by this route and schedule has not been defined.

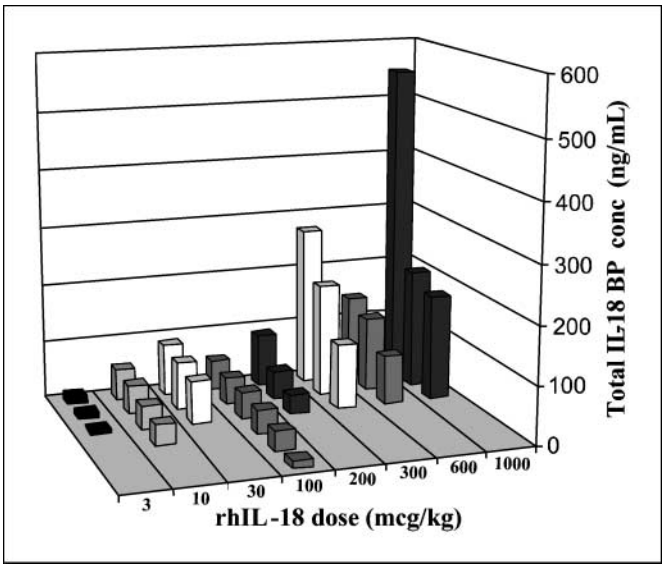


**Figure 2.** Peak plasma IL-18 BP levels in patients receiving rhIL-18. Columns, maximum plasma IL-18 BP concentration observed in an individual patient within each dose cohort. Maximum IL-18 BP concentrations were detected between days 2 and 5 in all patients.

IL-18 and IL-12 share many biological activities *in vitro* and *in vivo* (1, 4). Nonetheless, it seems that rhIL-18 can be given safely to cancer patients in doses that are 3 orders of magnitude higher than doses of rhIL-12 that are prohibitively toxic (15). The reasons for this marked disparity in the toxicity profiles of the two cytokines are currently unclear, although it may in part be related to the observed differences in IFN-γ induction *in vivo* (discussed below). It is possible that IL-12 receptors are expressed *in vivo* by cell types that lack IL-18 receptors. Alternatively, optimal effects of rhIL-18 may require additional costimulatory signals that are not necessary for the effects of rhIL-12. Biological activity was observed at all doses in this study. Therefore, an optimal therapeutic dose or regimen was not immediately apparent. Subsequent studies will be required to address these questions.

Peak concentrations of rhIL-18 ranging from ~30 to 3,000 ng/mL (~17-170 nmol/L) were measured in the plasma of patients receiving rhIL-18 in this study. Such concentrations would be expected to engage both high-affinity ($K_d$, ~300-500 pmol/L) and low-affinity ($K_d$, ~30-50 nmol/L) IL-18 receptors expressed on the cell surface (7, 16, 17). *In vitro*, IL-18 concentrations of 1 to 1,000 ng/mL have been found to activate lymphocytes (18–20). These data are consistent with observed clinical effects, including fever and lymphopenia, which were seen even in patients receiving the lowest dose of rhIL-18 (3 μg/kg). Transient lymphopenia is commonly observed after the administration of immunostimulatory cytokines and is most likely due to *in vivo* activation of lymphocytes with their subsequent extravasation into tissue spaces (21–23). Like the lymphopenia seen after administration of rhIL-12 (22), but unlike that seen after rhIL-2 (21), rhIL-18-induced lymphopenia was followed by recovery of the absolute lymphocyte count to pretreatment baseline levels without a substantial rebound lymphocytosis in most patients.

As expected, administration of rhIL-18 led to *in vivo* production of IFN-γ (24). Nevertheless, the serum IFN-γ

concentrations (~17-90 pg/mL) in cancer patients receiving rhIL-18 in doses of 3 to 1,000 μg/kg were substantially lower than the serum IFN-γ concentrations (~300-9,000 pg/mL) in cancer patients receiving a single i.v. injection of rhIL-12 in doses of only 0.03 to 1 μg/kg (22). This may explain in part the remarkably mild toxicity of rhIL-18 administration, as high serum IFN-γ concentrations *in vivo* are believed to contribute to the toxicities of rhIL-12 therapy (15). Modest *in vivo* production

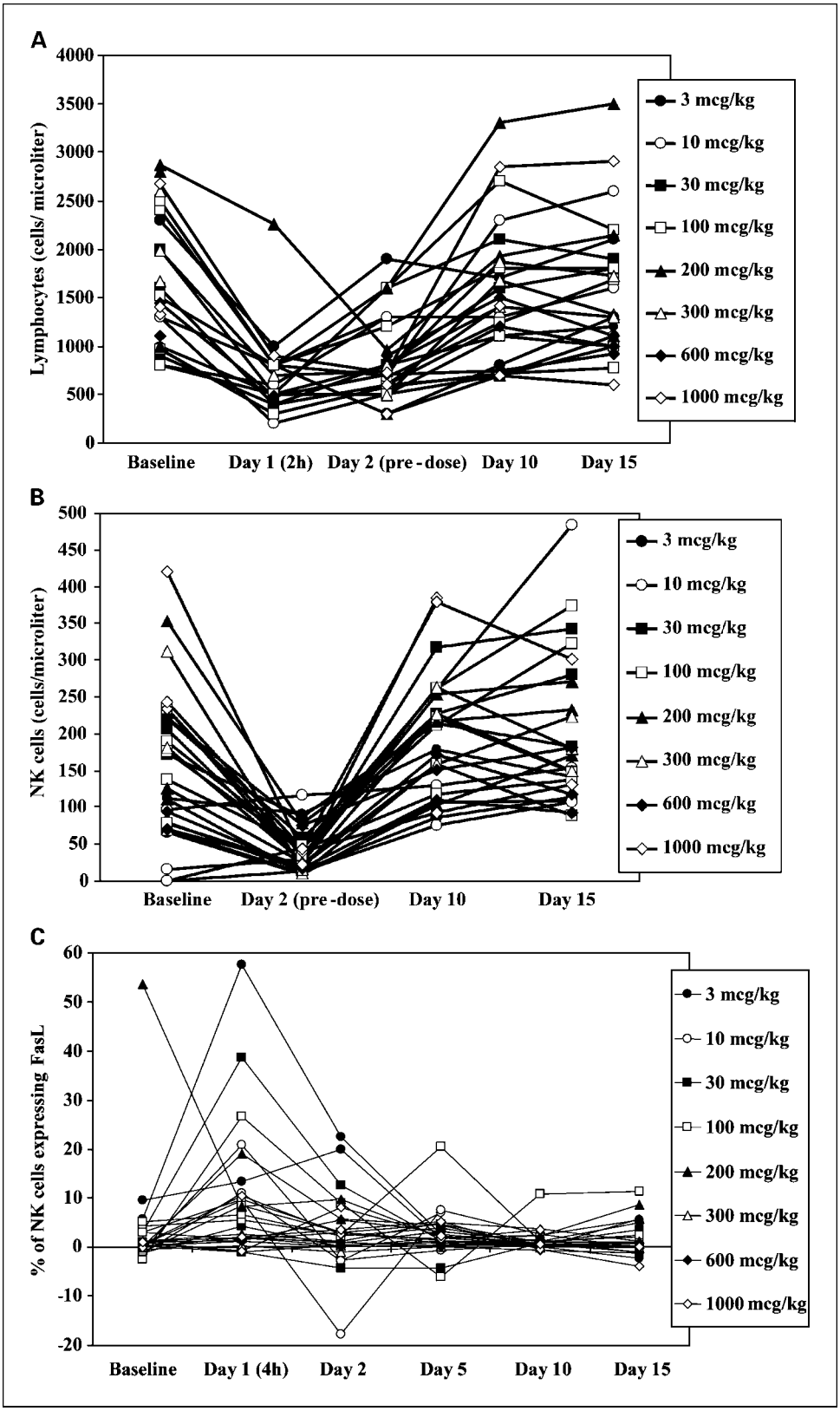


**Figure 3.** Peripheral blood leukocyte subsets in patients receiving rhIL-18. Profiles of individual patients in each cohort showing circulating counts (cells per μL) of total lymphocytes (*A*) and NK cells (*B*) and the percentage of NK cells expressing Fas ligand (*C*) at baseline and subsequent times selected to reflect the general pattern of change over time.

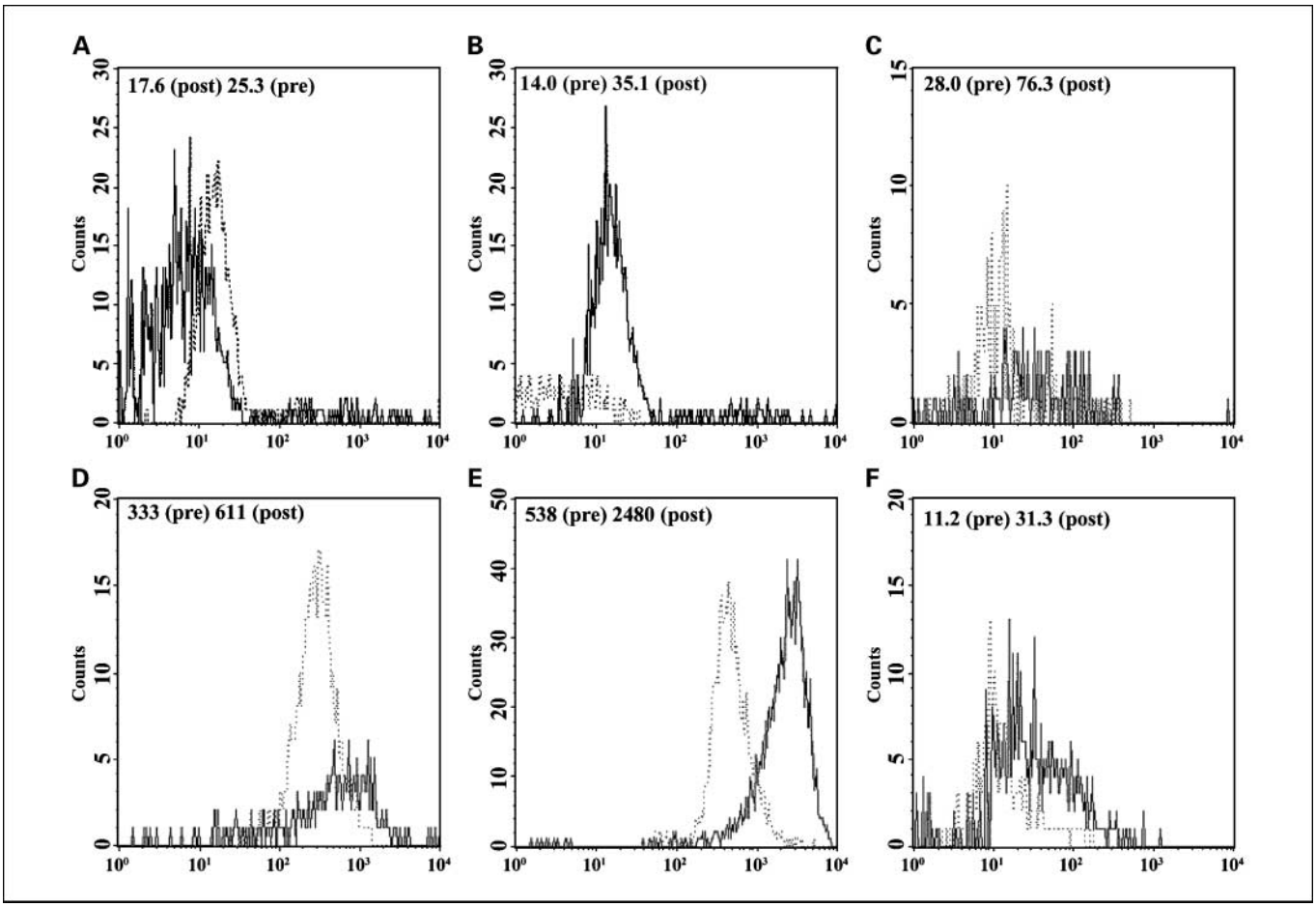


**Figure 4.** Up-regulation of cell surface activation antigens on peripheral blood leukocytes in response to rhIL-18 therapy. Flow cytometric analysis of Fas ligand on $CD4^+$ T cells (*A*), $CD8^+$ T cells (*B*), and NK cells (*C*), CD11b on NK cells (*D*) and monocytes (*E*), and CD69 on $CD8^+$ T cells (*F*) before dosing (*light dashed line*) and 4 hours after the first dose of rhIL-18 (*heavy solid line*) in representative individual patients. Peak mean fluorescence intensity values, corrected by subtraction of isotype control values, for each plot before (*pre*) and 4 hours after (*post*) the first rhIL-18 dose.

of IFN-γ in response to rhIL-18 is consistent with preclinical studies showing that IL-18 (compared with IL-2, IL-12, or IL-15) is a weak inducer of IFN-γ production *in vitro* (7, 20, 25). This does not preclude potential efficacy of rhIL-18 in cancer immunotherapy, as preclinical tumor models have shown that the efficacy of IL-18 (unlike that of IL-12) is not dependent on IFN-γ (9).

The 35-hour $t_{1/2}$ of rhIL-18 is much longer than those of other immunostimulatory cytokines evaluated clinically, such as rhIL-2 ($t_{1/2}$, <30 minutes; ref. 21) or rhIL-12 ($t_{1/2}$, 5-10 hours; ref. 26). This long $t_{1/2}$, together with daily dosing, resulted in 2.5-fold accumulation over the 5-day period. Plasma concentrations of rhIL-18 generally increased with increasing dose. IL-18 BP concentrations also generally increased with increasing doses of rhIL-18 through induction that may be mediated in part by IFN-γ (27–30). Increased plasma IL-18 BP concentrations in those patients with no detectable serum IFN-γ concentrations could be due to IFN-γ that was induced, although it did not achieve concentrations that were measurable in serum at the first sampling time. Alternatively, IFN-γ-independent mechanisms of IL-18 BP induction may occur in humans receiving rhIL-18.

Unconfirmed partial responses were observed in two patients, one each with melanoma and renal cell carcinoma, who received 100 μg/kg rhIL-18. It is interesting to note that tumor regression in both of these patients was delayed, being first detected 3 and 5 months, respectively, after rhIL-18 administration. Although it is impossible to exclude that these responses were due to spontaneous tumor regression, they are also compatible with rhIL-18-induced, immune-mediated antitumor activity. Presumably, expansion of rare, tumor-targeted immune effector cells *in vivo* is required for the delayed tumor regression seen in these settings. Given the very favorable toxicity profile of rhIL-18 observed in this single cycle study, further investigation of rhIL-18-based immunotherapy is warranted. Preliminary data indicate that rhIL-18 can be given safely in repeated 5-day treatment cycles (31). Additional studies are required to determine whether repetitive cycles or more prolonged exposure to the cytokine can augment the antitumor activity of rhIL-18. Moreover, preclinical data suggest that administration of rhIL-18 with other immunostimulatory cytokines might be particularly efficacious for cancer immunotherapy (11, 20).

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