Phase I Study of Recombinant Human Interleukin-7 Administration in Subjects with Refractory Malignancy

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Abstract

Purpose: Interleukin-7 (IL-7) has critical and nonredundant roles in T-cell development, hematopoiesis, and postdevelopmental immune functions as a prototypic homeostatic cytokine. Based on a large body of preclinical evidence, it may have multiple therapeutic applications in immunodeficiency states, either physiologic (immunosenescence), pathologic (HIV), or iatrogenic (postchemotherapy and posthematopoietic stem cell transplant), and may have roles in immune reconstitution or enhancement of immunotherapy. We report here on the toxicity and biological activity of recombinant human IL-7 (rhIL-7) in humans.

Design: Subjects with incurable malignancy received rhIL-7 subcutaneously every other day for 2 weeks in a phase I interpatient dose escalation study (3, 10, 30, and 60 μg/kg/dose). The objectives were safety and dose-limiting toxicity determination, identification of a range of biologically active doses, and characterization of biological and, possibly, antitumor effects.

Results: Mild to moderate constitutional symptoms, reversible spleen and lymph node enlargement, and marked increase in peripheral CD3^{+}, CD4^{+}, and CD8^{+} lymphocytes were seen in a dose-dependent and age-independent manner in all subjects receiving ≥10 μg/kg/dose, resulting in a rejuvenated circulating T-cell profile, resembling that seen earlier in life. In some subjects, rhIL-7 induced in the bone marrow a marked, transient polyclonal proliferation of pre-B cells showing a spectrum of maturation as well as an increase in circulating transitional B cells.

Conclusion: This study shows the potent biological activity of rhIL-7 in humans over a well-tolerated dose range and allows further exploration of its possible therapeutic applications. *Clin Cancer Res; 16(2); 727–35. ©2010 AACR.*

Interleukin-7 (IL-7) has a wide range of biological activities including a critical role in T-cell development as well as roles in hematopoiesis and postdevelopmental immune functions (1–4). Unlike in the mouse, IL-7 is not required for human B-cell development. IL-7 plays a critical and nonredundant role in maintaining peripheral T-cell homeostasis and is considered a prototypic homeostatic cytokine. It is required to maintain naive CD4^{+} and CD8^{+} cells *in vivo* (5–7) and IL-7 levels are inversely correlated to the T-cell depletion status (7, 8).

A multitude of murine and simian preclinical models have shown the wide array of immune-enhancing properties of IL-7, which may find clinical applications. Recombinant human IL-7 (rhIL-7) improves immune reconstitution in nonhuman primate models. In SIV-infected monkeys, it increases the number of circulating naive and memory CD4^{+} and CD8^{+} T cells (9, 10). IL-7 enhances immune reconstitution, improving CD4^{+} reconstitution following autologous CD34^{+} selected stem cell transplantation in monkeys (11) as well as in mice (12–18). It also enhances anti-infectious cytotoxicity in several animal models following adoptive transfer of specific T cells (19) or bone marrow transplant (20).

IL-7 enhances antitumor immune responses in mice (13, 21, 22). It is critical in the proliferation and *in vivo* persistence of tumor-specific T cells (23) and enhances antitumor responses and survival (24). IL-7 administration post-transplant results in both improved T-cell recovery and prolonged tumor-free survival (12). Immune

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Translational Relevance

Interleukin-7 (IL-7) has critical and nonredundant roles in T-cell development, hematopoiesis, and post-developmental immune functions. Based on a large body of preclinical evidence, it is a prototypic homeostatic cytokine that may have multiple therapeutic applications in a wide array of immunodeficiency states, either physiologic (immunosenescence), pathologic (e.g., HIV), or iatrogenic (e.g., postchemotherapy and posthematopoietic stem cell transplant), and, in particular, may have roles in immune reconstitution or enhancement of immunotherapy in multimodality cancer therapy strategies. Indeed, IL-7 has been ranked number 5 on the National Cancer Institute list of "Top 20 Agents with High Potential for Use in Treating Cancer" at the July 12, 2007 National Cancer Institute Immunotherapy Agent Workshop (http://web.ncifcrf.gov/research/brb/workshops/NCI%20Immunotherapy%20Workshop%207-12-07.pdf). This article shows that recombinant human IL-7 has potent biological activity over a dose range that is well tolerated in humans and therefore will allow further exploration of its possible therapeutic applications.

enhancement after severe immune depletion such as following stem cell transplantation may have clinical value because early lymphocyte reconstitution correlates with clinical outcome following autologous transplantation (25–28). Furthermore, standard or high-dose radiochemotherapy often only induces temporary complete or partial disease responses but no cure, leaving candidates for novel immunotherapy strategies immunologically depleted, which may hinder the effectiveness of such strategies.

The potential therapeutic uses of rhIL-7 are centered on its immunomodulatory effects on T cells and could include generating or optimizing T-cell responses to novel antitumor or anti-infectious immune interventions in a variety of clinical circumstances with severe immune depletion, be they physiologic (e.g., aging), pathologic (e.g., HIV), or iatrogenic (immunodepleting therapies). We conducted a phase I study of the administration of rhIL-7 to determine dose-limiting toxicity (DLT), maximum tolerated dose, and a range of biologically active doses in humans.

Materials and Methods

Eligibility. Adults diagnosed with incurable nonhematologic malignancy with a life expectancy greater than 3 months were eligible. A peripheral $CD3^+$ cells count above 300 cells/mm^3 was required. Also required were normal left ventricular ejection fraction, DLCO/VA and FEV1 >50%, AST and ALT less than three times the upper limit of normal, absolute neutrophil count >1,000/mm^3, platelet count >100,000/mm^3, PT/PTT within 1.5 times the up-

per limit of normal, creatinine clearance of >60 mL/min, and Karnofsky performance status >70%. Exclusions were recent use or need for anticoagulation, systemic corticosteroids, and cytotoxic or immunosuppressive therapy; splenectomy, splenomegaly, and prior solid organ or allogeneic stem cell transplantation; infections with HIV and hepatitis B or C; and history of autoimmune disease and severe uncontrolled asthma. Toxicity was evaluated according to the National Cancer Institute "Common Terminology Criteria for Adverse Events." The study was approved by the National Cancer Institute Institution Review Board and the Food and Drug Administration. Subjects signed written informed consent before enrollment.

Trial design. "CYT 99 007" (*Escherichia coli* rhIL-7; Cytheris) was given subcutaneously every other day for 2 weeks in successive cohorts of three to six patients in a standard phase I design (dose levels: 3, 10, 30, and 60 μg/kg/dose). All subjects, except the first on study, received the same product lot. The primary study objectives were safety, DLT, and maximum tolerated dose determination. Secondary objectives included defining a range of biologically active doses, defining rhIL-7 pharmacokinetics and evaluating possible antitumor effects. Biological activity was defined as 50% increase over baseline in the number of peripheral blood $CD3^+$ cells/mm^3.

Subjects underwent body computed tomography scan at baseline, end of therapy (day 14), and day 28. Subjects with stable disease at day 28 were followed if possible until disease progression. Bone marrow aspirate and biopsy were done at baseline and day 14.

Flow cytometry. Peripheral blood mononuclear cell analysis: We analyzed fresh, EDTA-anticoagulated peripheral blood at the specified time points as described previously (29).

Bone marrow flow cytometry. Bone marrow aspirates were collected into sterile sodium heparin, prelysed with ammonium chloride, and stained for 30 min at room temperature with antibodies directed against CD13, CD33, and CD36 (Beckman Coulter); CD3, CD10, CD14, CD16, CD19, CD22, CD34, CD56, CD45, and CD71 (BD Biosciences); CD64 (Caltag Laboratories); and CD20, κ, λ (DakoCytomation). Cells were fixed in 1.0% paraformaldehyde and stored at 4°C for up to 12 h before acquisition. Four-color cytometry was done with a Becton Dickinson FACSCalibur. Data were analyzed with CellQuest software (Becton Dickinson). Granulocytes, monocytes, mature lymphocytes, nucleated RBC, and immature hematopoietic precursors were determined based on levels of CD45 expression, side scatter, and pattern of antigen expression.

Immunoglobulin gene rearrangement. DNA was extracted from Ficoll-Hypaque separated bone marrow mononuclear cells and PCR-amplified for detection of immunoglobulin heavy-chain gene rearrangements using primers to framework region III and the joining region of the immunoglobulin heavy-chain gene (FRIII-IGH PCR). Additional PCR was done in duplicate using consensus primers to framework region II and the immunoglobulin heavy-chain

Table 1. Summary of subject characteristics, toxicity, and immunogenicity

UPN	Cohort no./ (µg/kg/dose)	Age	Diagnosis	Toxicity (possibly, probably, or definitely related), maximum toxicity grade and type (grade)		Anti-IL7 antibody titer	
						Binding	Neutralizing
01	1/(3)	70	Renal cell carcinoma*	1	IS (1); EO (1)	Positive (1/1,600)	0
02		48	Hemangiopericytoma	2	ALT (2)	Negative (0)	NA
03		67	Melanoma			Negative (0)	NA
04	2/(10)	56	Melanoma	2	IS (2); CS (1)	Negative (1/100)	0
05		71	Renal cell carcinoma	2	IS (2); CS (1); ALT (1)	Negative (0)	NA
06		28	Carcinoma of unknown primary	2	IS (2); CS (2)	Negative (0)	NA
07	3/(30)	21	Osteogenic sarcoma†	3	AST, bili (3); CS (1)	NA	NA
08		20	Rhabdomyosarcoma	1	IS (1); CS (1)	Positive (1/400)	0
09		28	Synovial cell sarcoma	1	IS (1); CS (1); SM (1)	Negative (1/100)	0
10		48	Duodenal carcinoma	2	IS (2); CS (1)	Negative (1/100)	0
11		61	Colon carcinoma			Positive (1/1,200)	0
12		62	Melanoma	2	IS (2); CS, SOB (2); ALT, bili (1)	Positive (1/400)	0
13	4/(60)	19	Melanoma	2	IS (2); CS, bili (2); ALT, AP, Plt (1)	Positive (1/1,200)	0
14		35	Pheochromocytoma‡	3	Cardiac§ (3); AST, ALT (1)	Negative (1/100)	NA
15		43	Ovarian carcinoma	3	ALT (3); AST, IS (2); CS, AP (1)	Positive (1/1,200)	0
16		66	Melanoma	1	IS, CS, bili (1)	Negative (0)	NA

Abbreviations: ALT and AST, transaminases; AP, alkaline phosphatase; bili, total bilirubin; CS, constitutional symptoms; EO, eosinophilia; IS, injection site; erythema, induration, pruritus; Plt, decreased platelets; SM, splenomegaly; SOB, shortness of breath.
*Received initial lot of "CYT 99 007."
†Received only one dose.
‡Received only three doses.
§See text.

joining region (FRII-IGH PCR) according to the method of Ramasamy et al. (30).

Serum anti–IL-7 antibody determination. Antibody detection used a two-step ELISA and a bioassay for neutralization of IL-7 bioactivity, both developed for and used during rhIL-7 preclinical development by Cytheris. Pretreatment and day 28 serum samples were assayed. Assays were repeated at days 35 and 42 if equivocal at day 28. Titer ≥1/200 was considered positive for the ELISA, but a neutralization bioassay was done for any titer ≥1/100. A neutralizing antibody titer ≥1/400 at either day 28 or 42 was to be considered positive and a DLT.

Serum rhIL-7 level determination and pharmacokinetics. Serum IL-7 levels were determined serially for pharmacokinetics following the first and last doses. The Diaclone Research (Cell Systems) Sandwich IL-7 ELISA kit was used for validation and sample analysis. The two-site sandwich ELISA used anti–rhIL-7 monoclonal antibodies, one as coating antibody for capture and the other, biotinylated, for detection. The linear relationship between rhIL-7 concentrations and absorbance extended up to 200 pg/mL; concentrations <12.5 pg/mL were treated as zero for pharmacokinetic calculations (Kinetica Software version 4.2) with the noncompartmental extravascular model. The area under the curves (AUC) were computed using the log linear method, trapezoidal when $C_n > C_{n-1}$. Half-lives were calculated between $t = 2$ h (T_{max}) and $T = 24$ h.

Statistics. Using the data presented in Table 2, differences between pretreatment and post-treatment values and the relative difference (post-pre)/pre were formed for the four markers of interest of mature B cells (CD19+/CD45bright and CD19+/CD10−) and immature B cells (CD19+/CD45dim and CD19+/CD10+). In the one case in which the value was noted to be <1.0, this was changed to be 0.5 for simplicity. It was determined that the association between either the absolute or the relative difference was approximately equally well associated with the pretreatment value; thus, for simplicity, the absolute difference was evaluated to determine if the change was equal to zero. A Wilcoxon signed rank test was used to determine if the change was equal to zero after determining that the differences were not normally distributed. All P values are two-sided and presented without formal adjustment for multiple comparisons.

Results

Twelve men and 4 women, ages 19 to 71 years (median, 49 years), with incurable malignancies, were treated. Table 1 summarizes subject characteristics, toxicity, and immunogenicity data.

Toxicity. "CYT 99 007" was well tolerated. Following most injections, subjects receiving ≥10 μg/kg developed grade 1 to 2 constitutional symptoms 6 to 8 h following administration and mild local reactions with erythema, pruritus, and induration. Two subjects did not complete therapy because of toxicity. One developed rapidly reversible grade 3 liver enzyme and bilirubin elevation following the first dose at the 30 μg/kg and treatment was terminated. The other developed grade 3 chest pain and hypertension with mild troponin elevation several hours after the third dose of 60 μg/kg; treatment was stopped and all abnormalities abated within 2 days (see Discussion). Subsequently, no significant electrocardiogram changes were seen when systematically performed at 3 and 24 h following every injection on all remaining subjects. No symptoms or signs of autoimmunity were seen.

Biological activity. Starting at 10 μg/kg/dose, all subjects showed evidence of biological activity (a trend toward activity was seen at 3 μg/kg/dose). A 50% to 60% drop in absolute lymphocyte counts following the first dose was seen in all subjects on day 1 as in most preclinical models. All patients showed a dose-dependent marked increase in absolute lymphocyte count, which persisted for several weeks after treatment (Fig. 1A). Although several hematologic parameters, serum albumin, total protein, and C-reactive protein showed mild fluctuations during the

study, none, except for absolute lymphocyte count, appeared clinically significant; transient platelet decrease during treatment; monocyte decrease after treatment; and eosinophils peak on day 21, all (except the C-reactive protein values) remaining within the normal institutional range. Lymphoid organ enlargement (spleen and lymph nodes but not thymus) was seen in a dose-dependent manner on computed tomography scan, maximum on day 14, returning to baseline over several weeks (Fig. 1B).

T-cell populations. For all T-cell populations, the absolute cell numbers peaked on day 21 and remained elevated for weeks, whereas lymphopenia following treatment was never observed. In most subjects, CD3$^+$ αβ and γδ T cells increased equally and the increase was similar in CD4$^+$ and CD8$^+$ cells as well as CD3$^+$ (but not CD3$^-$) CD16$^+$ or CD56$^+$ cells. An in-depth analysis of T-cell subset kinetics and their consequences on the circulating T-cell makeup and T-cell repertoire diversity following rhIL-7 therapy has been reported (29) and is summarized in the discussion. Briefly, up to 60% of circulating CD3$^+$ cells were in cycle (Ki-67$^+$) on day 7 of treatment. In spite of treatment continuation, the fraction of circulating cells in cycle had decreased by the end of treatment and returned to baseline 1 week after the end of treatment. Both IL-7Rα surface expression and mRNA were decreased during the treatment and returned

Table 2. Lymphoid bone marrow expansion with rhIL-7 therapy

UPN (age, y)	Mature B cells				Immature B cells			
	CD19$^+$/CD45^{bright}		CD19$^+$/CD10$^-$		CD19$^+$/CD45^{dim}		CD19$^+$/CD10$^+$	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
01 (70)	0.4	1	0.4	0	0.2	8	0.2	0
02 (48)	4	2	2	1	0.4	10	1	11
03 (67)	5	3	4	3	1	<1	2	1
04 (56)	6.9	2	5.5	1	7	59	10	55
05 (71)	3.5	27	2	1.9	11	23	12	47
06 (28)	4.2	2	4.3	15	1.2	18	0.8	5
08 (20)	26	35	17	6	17	51	22	80
09 (28)	11	4	9.4	4	12	8	13	8
10 (48)	7.3	23	6.6	4	6.5	21	7	39
11 (61)	10	2	10	2	6	6	4	10
12 (62)	10	0.8	7	1	2	7	2	6
13 (19)	6.9	1.5	6.3	0.7	4.6	33	4.6	30
15 (43)	14.8	1	10.7	1	19.2	80	11.9	80
16 (66)	3	2	3	4	3	11	3	9
Mean	8.07	7.59	6.30	3.19	6.51	23.96	6.68	27.21
Median	6.90	2.0	5.90	1.95	5.30	14.50	4.30	10.50
Post-Pre		−0.48		−3.11		17.46		20.54
P		0.38		0.024		0.0012		0.0029

NOTE: Summary of bone marrow flow cytometry data. Percentage of bone marrow mononuclear cells for the marker combinations: mature B cells: CD19$^+$/CD45^{bright} and CD19$^+$/CD10$^-$; immature B cells: CD19$^+$/CD45^{dim} and CD19$^+$/CD10$^+$. UPN: unique patient number. *P* value given (Wilcoxon signed rank test) on the absolute difference between "pre" and "post" values for each marker combination.

to baseline after the end of treatment. Interestingly, the subject who received only the first three doses of rhIL-7 showed the same cell cycling and IL-7Rα downregulation patterns (29) at day 7 and had a day 28 increase in absolute lymphocyte count similar to that of the other subjects, suggesting that maximum therapeutic effects may be reached early possibly because of IL-7Rα downregulation (see Discussion).

IL-2Rα (CD25) surface expression was studied in a selected group of patients receiving the higher doses of rhIL-7. There was evidence of increased CD25 surface expression in the CD4+ non-Tregs and CD8+ T-cell populations. The mean percent increase over baseline of CD25+ cells were 55% (95% confidence interval, 40-70) at day 7 and 57% (95% confidence interval, 45-69) at day 14 for CD4+ cells and 74% (95% confidence interval, 63-85) at day 7 and 85% (95% confidence interval, 72-98) at day 14 for CD8+ cells with return to baseline at day 21.

Circulating B cells. Mature B cells (CD20+) consistently showed a modest and not demonstrably dose-dependent decrease in absolute number during therapy and returned to baseline by day 28 (Fig. 1C). Several subjects showed a mild expansion of circulating CD19+/CD10+ B cells [immature/transitional B cells (31, 32)], peaking on day 21 (Fig. 1C) with no correlation between cell number and subjects' age or magnitude of their bone marrow pre-B-cell proliferation (see below). There was no increase in circulating activated germinal center B cells (CD19+/CD27+/CD10+).

Bone marrow. All subjects underwent bone marrow aspiration and biopsy evaluation before and at the end of therapy by detailed analysis of H&E, immunostains, and flow cytometry. Although 17 different markers or marker combinations spanning the spectrum of bone marrow lineages and of differentiation stages were analyzed by flow cytometry, most showed variations between pretreatment and post-treatment samples that remained within the normally occurring wide normal range. In general, rhIL-7 treatment resulted, by day 14, in an increased cellularity, usually within normal limits. There were no significant abnormalities in morphology or abundance of the myeloid, erythroid, or megakaryocytic lineages. The proportion of CD4+ and CD8+ T cells remained largely unchanged and most subjects showed a statistically significant increase in CD19+/CD10+ and/or CD19+/CD45dim early pre-B cells (Table 2). In 6 subjects, however, the CD10 and Tdt abnormalities on biopsy immunostains would have triggered further flow cytometry investigation in other clinical settings (Fig. 2), and in these cases, post-treatment early pre-B cells represented 30% to 86% of all bone marrow mononuclear cells by flow cytometry, showing no commensurate increase in mature B cells (Table 2) and no correlation with treatment dose or subjects' age (Fig. 3A). As shown in Fig. 3B, CD19+ B-cell
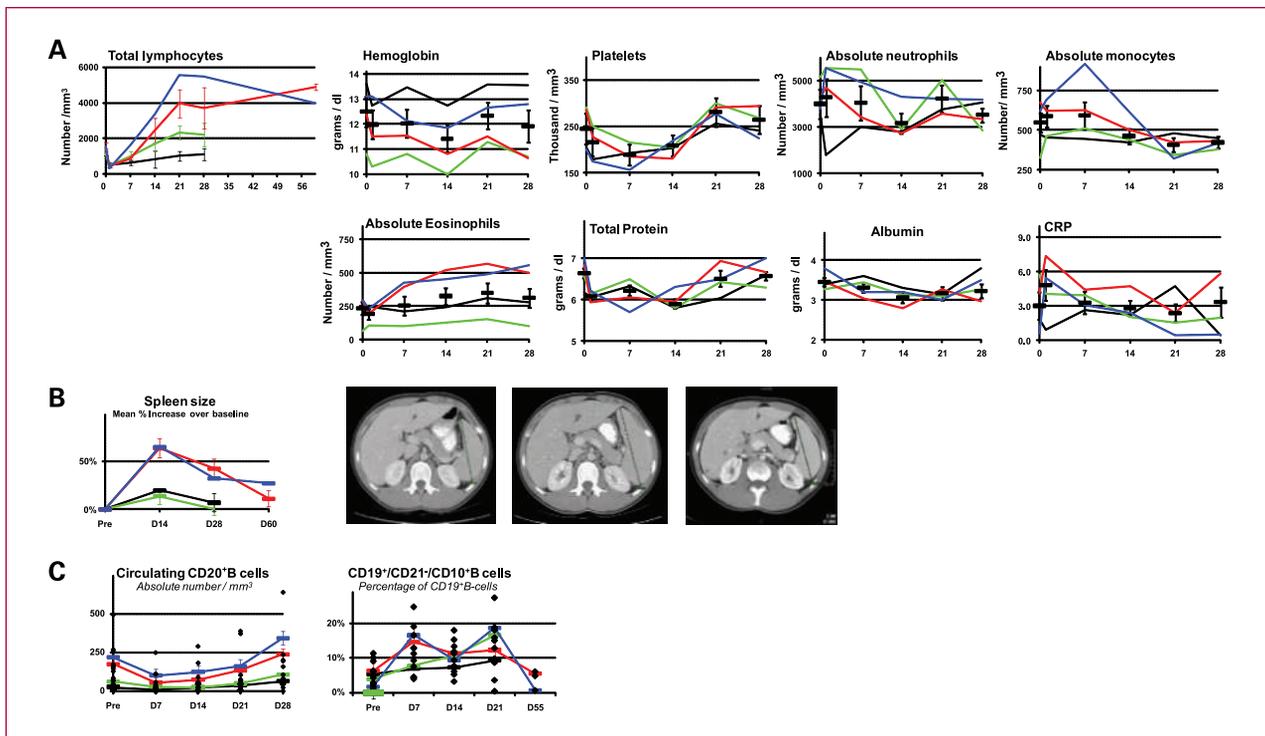
Fig. 1. Clinical parameter kinetics with rhIL-7 therapy. Cohorts: 1, black; 2, green: 3, red: 4, blue. *A,* top left, absolute lymphocyte count cohort mean ± SE. Others: mean ± SE for all subjects. *B,* left, mean spleen size; spleen on computed tomography: baseline, day 14, 2 months. *C,* left, mature B cells, mean absolute numbers; right, immature B cells, mean percentage of CD19+ population.
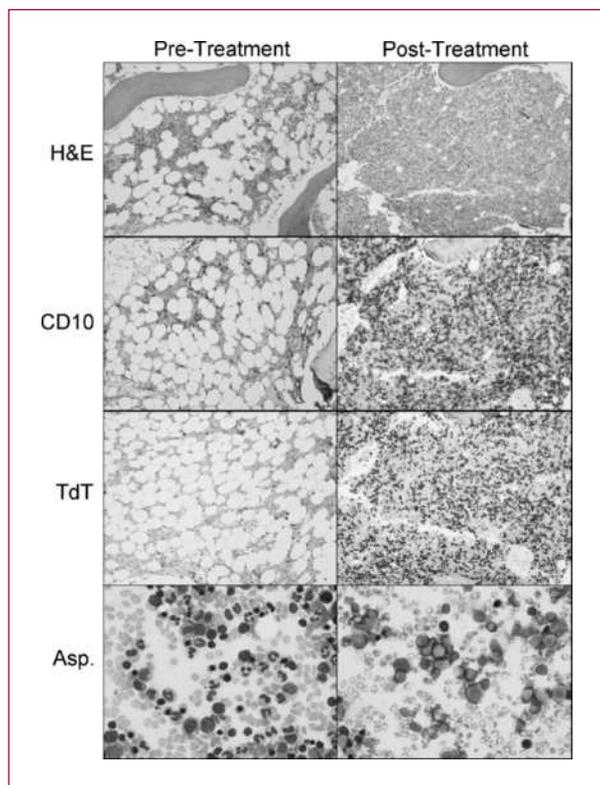
Fig. 2. H&E, immunostaining (CD10 and Tdt; ×200), and Giemsa stain (×400), before and after rhIL-7 therapy, in subject with marked marrow expansion (UPN08).

immunostaining in the flow cytometry analysis was consistent with ongoing maturation: increasing levels of CD45 and CD19 expression (polygonal gate in Fig. 3A); in gated CD19+ B cells: increasing CD20 with decreasing CD10 expression (Fig. 3B) and increasing CD45 with decreasing CD34 expression (Fig. 3C). PCR studies of both bone marrow and peripheral blood mononuclear cells for immunoglobulin gene rearrangement failed to reveal any clonal population (data not shown). Follow-up bone marrow or peripheral blood studies, performed several weeks after the end of treatment in subjects with the highest pre-B-cell increases, consistently showed either declining or resolved pre-B-cell marrow proliferation by flow cytometry, confirmed to have remained polyclonal by PCR.

Immunogenicity. Sera were tested for anti–IL-7 antibodies on day 28 and, if required, on day 42 (Table 1). The only subject treated with the first lot of rhIL-7 (01) developed a 1/1,600 antibody titer. Excluding this subject who received a different formulation, 5 of the 14 subjects evaluable for immunogenicity developed anti–IL-7 antibodies. None of the 5 receiving 3 or 10 μg/kg and 5 of 9 receiving 30 or 60 μg/kg developed antibodies on day 28, suggesting a dose response. No subject developed anti–IL-7 neutralizing antibodies. None of the subjects with longer follow-up developed late lymphopenia.

Antitumor activity. Although the study was not designed to evaluate antitumor activity, disease follow-up was carried out as feasible. Subject 02 with central nervous system hemangiopericytoma and abdominal metastases (no central nervous system disease at entry on study) had a substantial improvement of abdominal pain and a 20% reduction in mass size at 3 months. The abdominal disease remained stable at the time of a central nervous system recurrence, 9 months following rhIL-7 therapy. All other patients had progressive disease by day 42.

Pharmacokinetics. Pharmacokinetics studies (performed after the first and last rhIL-7 doses) are provided in Table 3. The rhIL-7 half-life ranged from 6.5 to 9.8 h. Whereas the C_{max} increased from the 10 to 30 μg/kg dose, the day 1 total AUC increased throughout the dose range, and clearance decreased, whereas apparent half-life increased with the dose. High-affinity clearance mechanisms, mediated by IL-7R, probably explain a better clearance at low dose. The consistently higher clearance and lower AUC at day 14 compared with day 1 at a given dose probably reflect the larger lymphoid mass at day 14. rhIL-7 pharmacodynamics seem to correlate better with AUC and half-life than with C_{max}, consistent with the characteristic pharmacodynamic/pharmacokinetic profile of growth factors and cytokines: prolonged AUCs and half-lives tend to improve efficacy by sustaining blood levels above activity threshold over a prolong period, whereas peak concentrations well above activity threshold (high C_{max}) contribute less to the biological effects.

Discussion

We present here clinical findings of a phase I study of rhIL-7, defining a toxicity profile and a range of biologically active doses. Enrollment was limited to individuals with a stable CD3+ peripheral count >300 cells/mm³. Studies in other populations (HIV and allogeneic transplant recipients) are under way. Although the first dose level was designed as a "no effect dose" based on nonhuman primate studies (9), early signs of biological activity were apparent at this dose, suggesting a 10- to 30-fold greater sensitivity in humans.

rhIL-7 was well tolerated with no maximum tolerated dose being reached in the treated subjects. The observation of only mild adverse reactions along with the biological activity in the tested dose range suggests a safe therapeutic window. One subject had liver DLT, concomitant with the onset of a catheter infection. The cardiac DLT occurred in the subject with pheochromocytoma, with typical symptoms for this disease (causing the DLT) occurring concurrently with the expected constitutional symptoms. These two potentially rhIL-7–related toxicities will require further scrutiny in subsequent trials. At the two highest doses, spleen and lymph node enlargements were noticeable but not clinically worrisome, although caution during the period of rapid spleen enlargement remains warranted. rhIL-7 immunogenicity was not limiting and no neutralizing antibodies or lymphopenia developed. A glycosylated

rhIL-7 is presently under clinical development, which may prove less immunogenic and allow the evaluation of repeated courses of treatment. There was no suggestion of the development of autoimmunity.

In a limited number of subjects, there was a marked, self-limited expansion of polyclonal bone marrow pre-B cells (hematogones), consistent with *in vitro* observations that human pro-B cells display a functional IL-7R and expand following IL-7 exposure but subsequently die without reaching mature B-cell stage (33–35). Several subjects showed a mild expansion of circulating $CD19^+/CD10^+$ transitional B cells, a population found in HIV patients to correlate with serum IL-7 levels (31). There was no obvious correlation between bone marrow and peripheral blood expansions of immature B cells and the number of circulating mature B cells was largely unaffected by rhIL-7 treatment. IL-7 effects on B-cell development and physiology accumulated in the murine system are mostly irrelevant to humans and the knowledge that rhIL-7 may induce a self-limited, but often marked, polyclonal pre-B-cell expansion in humans is valuable safety information for the future rhIL-7 clinical trials.

rhIL-7 induced a dose-dependent, but age-independent, increase in circulating $CD3^+$ (both $\alpha\beta$ and $\gamma\delta$) lymphocytes, $CD3^+$ (but not $CD3^-$) $CD16^+$ or $CD56^+$ cells, and $CD3^+/CD4^-/CD8^-$ T cells (29). This net increase in circulating lymphocytes was most likely due to proliferation/

expansion taking place in the peripheral blood and/or lymphoid organs with varying contribution by alteration in cell trafficking. The massive increase in the number of cycling circulating lymphocytes is unlikely due solely to trafficking out of lymphoid organs, considering the concomitant spleen and lymph node enlargement as well as the continuing increase in circulating lymphocyte numbers after therapy. Other evidence supported T-cell expansion as resulting from a combination of increased cell cycling and decreased apoptosis (29). The sharp decrease in cell cycling while pharmacologic serum IL-7 levels were sustained during the second week of treatment suggests that downregulation of IL-7Rα plays a role in the self-limited effects of rIL-7, a consideration for the design of future trials.

Detailed studies of T-cell subsets, reported elsewhere (29), showed that rhIL-7 preferentially expanded the $CD4^+$ naive and central memory T cells as well as $CD8^+$ naive T cells resulting in a statistically significant broadening of T-cell repertoire diversity in both $CD4^+$ and $CD8^+$ T cells. Therefore, within 3 weeks of treatment initiation, rhIL-7 resulted in a marked expansion of T cells, which remained functional (with conserved or increased *in vitro* responsiveness to anti-CD3 stimulation) and exhibited a rejuvenated profile resembling that seen early in life (29). Additionally, $CD4^+/FoxP3^+$ regulatory T cells showed markedly less proliferation, consistent with their low IL-7Rα-chain expression (36, 37), expanded less than
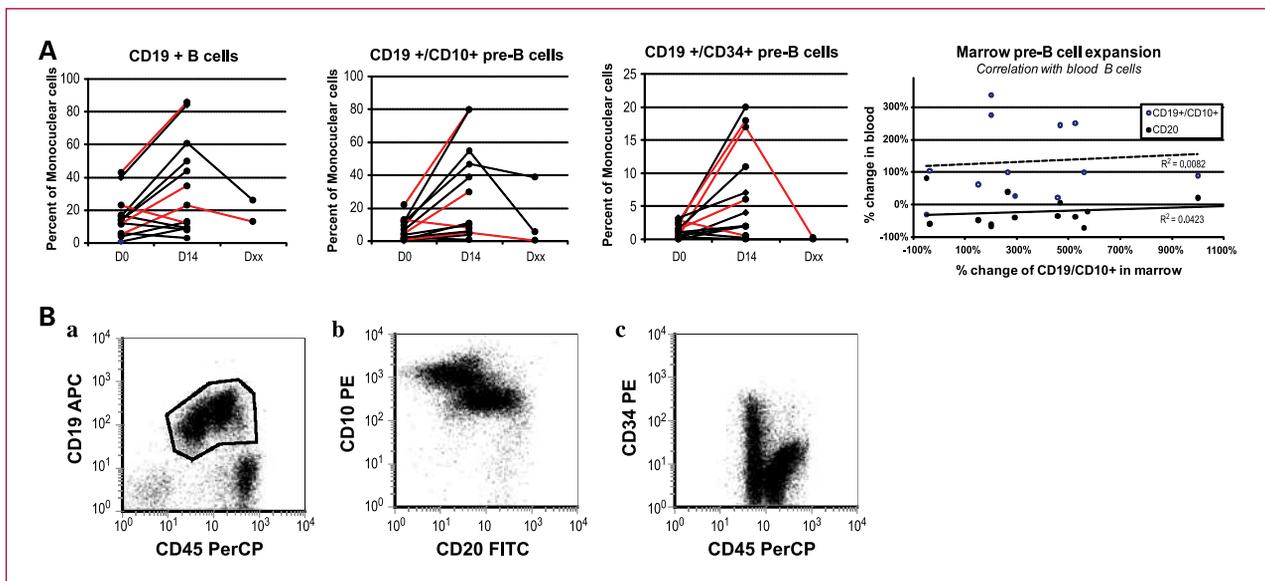
Fig. 3. Bone marrow B cells. $CD19^+$ cells and subsets: % total bone marrow mononuclear cells (subjects ages <30 years in red). Dxx; 4 to 10 weeks; correlation between bone marrow $CD19^+/CD10^+$ pre-B cells and peripheral blood increases in $CD20^+$ (●) and $CD19^+/D10^+$ (○). Flow cytometry scatter plots of marrow early B-cell precursors in subject with marked expansion (UPN08) illustrating progressive maturation. *A,* bone marrow cells (ungated data), X axis CD45 PerCP and Y axis CD19 APC and $CD19^+$ B cells (in polygonal analysis gate), have increasing levels of CD45 and CD19 expression consistent with ongoing maturation. *B,* $CD19^+$ B cells (gate-polygonal gate in A), X axis CD20 FITC and Y axis CD10 PE and $CD19^+$ B cells, have increasing levels of CD20 expression and decreasing levels of CD10 expression consistent with ongoing maturation. *C,* $CD19^+$ B cells (gate-polygonal gate in A), X axis CD45 PerCP and Y axis CD34 PE and $CD19^+$ B cells, have increasing levels of CD45 expression and decreasing levels of CD34 expression consistent with ongoing maturation.

Table 3. rhIL-7 pharmacokinetics

Dose	10 µg/kg		30 µg/kg		60 µg/kg	
rhIL-7 serum levels (pg/mL)						
Time (h)	d 1	d 14	d1	d 14	d 1	d 14
0	6	0	0	10	0	0
0.5	51	35	506	372	534	929
1	93	48	837	399	908	1,019
1.5	121	57	1,335	616	1,279	1,324
2	148	66	1,895	688	1,246	1,354
2.5	147	69	1,766	426	1,223	1,344
4	354	83	1,531	717	1,176	1,211
8	120	53	917	459	821	177
12	87	58	936	438	832	161
24	28	6	296	122	478	139
Pharmacokinetics parameters						
Mean total daily dose (µg)	686	686	2,322	2,322	4,763	5,280
C_{max} (pg/mL)	354	83	1,895	717	1,846	1,697
T_{max} (h)	4.0	4.0	2.0	4.0	4.0	4.0
AUC total (pg/mL min)	168,357	69,697	1,416,470	591,776	1,812,870	1,483,180
$t_{1/2}$ (h)	8.54	9.80	9.03	6.46	8.92	7.23
Clearance (mL/min)	4,077	9,847	1,639	3,890	2,627	3,560

NOTE: Top: mean serum concentrations for cohorts. Bottom: pharmacokinetics parameters (mean values for a cohort). Results are shown after the first dose (day 1) and after the last dose (day 14) for each cohort.

other CD4$^+$ T cells resulting in a decreased proportion of circulating regulatory T cells (29, 38).

In summary, rhIL-7 is well tolerated in humans and has significant biological activity over dose range, indicating a potential safe therapeutic window. It induces an age-independent marked expansion of the T-cell mass resulting in a rejuvenated T-cell profile with an increased T-cell repertoire diversity and a decreased proportion of regulatory T cells. This study paves the way for the systematic investigation of specific clinical applications for enhancement of immune responses or immune reconstitution in a multitude of clinical circumstances of immune depletion, be they iatrogenic (chemotherapy/radiation/transplantation), pathologic (HIV), or physiologic (immunosenescence), and may be considered to optimize strategies of passive or active immunotherapy.

Disclosure of Potential Conflicts of Interest

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