Phase I Study of Recombinant Human CD40 Ligand in Cancer Patients

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Purpose: **To determine the toxicity, maximum-tolerated dose (MTD), and pharmacokinetics of recombinant human CD40 ligand (rhuCD40L) (Avrend; Immunex Corp, Seattle, WA), suggested in preclinical studies to mediate cytotoxicity against CD40-expressing tumors and immune stimulation.**

Patients and Methods: **Patients with advanced solid tumors or intermediate- or high-grade non-Hodgkin's lymphoma (NHL) received rhuCD40L subcutaneously daily for 5 days in a phase I dose-escalation study. Subsequent courses were given until disease progression.**

Results: **Thirty-two patients received rhuCD40L at three dose levels. A total of 65 courses were administered. The MTD was 0.1 mg/kg/d based on dose-related but transient elevations of serum liver transaminases. Grade 3 or 4 transaminase elevations occurred in 14%, 28%, and 57% of patients treated at 0.05, 0.10, and 0.15 mg/kg/d, respectively. Other toxicities were mild to moderate. At the MTD, the half-life of rhuCD40L was calculated at 24.8 ± 22.8 hours. Two patients (6%) had a partial response on study (one patient with laryngeal carcinoma and one with NHL). For the patient with laryngeal cancer, a partial response was sustained for 12 months before the patient was taken off therapy and observed on no additional therapy. Three months later, the patient was found to have a complete response and remains biospy-proven free of disease at 24 months. Twelve patients (38%) had stable disease after one course, which was sustained in four patients through four courses.**

Conclusion: **The MTD of rhuCD40L when administered subcutaneously daily for 5 days was defined by transient serum elevations in hepatic transaminases. Encouraging antitumor activity, including a long-term complete remission, was observed. Phase II studies are warranted.**

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THE CELL SURFACE molecule CD40, a member of the tumor necrosis factor receptor superfamily, has been studied as a target for antitumor therapy because of its expression on nearly all B-cell malignancies and the majority of solid tumors.[1-3] This expression includes all types of B-cell tumors, including non-Hodgkin's lymphoma (NHL), acute lymphoblastic leukemia, chronic lymphocytic leukemia, and myeloma. In solid tumors, CD40 expression is found on 35% to 100% of established cell lines, depending on histology, and on more than 60% of tumor biopsy specimens obtained from patients with melanoma and many epithelial tumors (eg, pancreas, lung, ovary, bladder, breast, colon, prostate, and squamous cell cancer of the head and neck).[4,5]

In normal tissues, signaling through CD40 has been implicated in enhanced antigen presentation function and cytokine release.[3,6,7] In B cells, CD40 cross-linking results in immunoglobulin class switching and growth stimulation.[8-10] CD40 has been demonstrated on all normal B-lymphocytes, as well as populations of epithelial cells, $CD34^+$ hematopoietic progenitor cells, monocytes, dendritic cells, endothelial cells, and fibroblasts.[11-15] Although CD40 expression on B-cell malignancies may reflect preexisting expression on nonmalignant precursor cells,[1] the biologic role of CD40 in malignant tissues and, in particular, on solid tumors is unknown.

CD40 ligand (CD40L), also known as CD154, functions as the natural ligand for CD40.[9,16] It is expressed primarily on the surface of activated T lymphocytes[16] but has also been found on activated platelets.[17] The CD40L gene maps to human chromosome Xq26, and its mutation results in X-linked immunodeficiency (hyperimmunoglobulin M) syndrome.[18] CD40L is a major component of T-cell help for B cells in antibody isotype switching and in the formation of memory B cells and germinal centers.[1] Interactions between CD40 and CD40L provide critical costimulatory signals that trigger T-lymphocyte expansion.[19] Antibodies that cross-link CD40 mimic the signal of CD40L[20] and can substitute

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for $CD4^+$ lymphocytes in murine models of T-cell–mediated immunity.[21-23]

To enhance the signaling activity of CD40L via cell surface CD40, a trimeric recombinant form of human CD40 ligand (rhuCD40L) (Avrend; Immunex Corp, Seattle, WA) was produced that incorporates an isoleucine zipper motif.[24] Two main observations have suggested that rhuCD40L could be used therapeutically to target CD40-expressing solid tumors and B-cell malignancies.[3] First, rhuCD40L is cytotoxic against CD40-expressing tumors.[25-32] In preclinical studies, rhuCD40L induced in vitro growth inhibition and cell killing of $CD40^+$ human carcinoma and B-cell lymphoma cell lines. Furthermore, rhCD40L improved survival in vivo of severe-combined immunodeficient mice implanted with MDA231 breast cancer cells.[30] Similar protective effects of rhuCD40L were observed in a severe-combined immunodeficient mouse–human lymphoma model.[25]

A second potential antitumor effect of rhuCD40L is related to immune stimulation observed after CD40 cross-linking, including the enhancement of antigen presentation by dendritic cells, monocytes, and B cells, and the triggering of antigen-specific T-cell responses.[3] In B-cell malignancies, treatment with CD40L can even upregulate antigen presentation by tumor cells themselves and can stimulate autologous antitumor T-cell responses,[6,33-35] a notion currently being tested in vivo in patients with B-cell lymphoma or leukemias.[36,37]

In preclinical toxicology studies, rhuCD40L was well tolerated by nonhuman primates treated daily for 28 days and by mice given trimeric recombinant murine CD40L in a total of 10 injections every other day. The most prominent clinical findings included injection site reactions (ISRs) and enlargement of lymphoid organs after subcutaneous administration. At substantially higher doses (four- to 10-fold increase) than planned for phase I investigation in humans, rhuCD40L resulted in moderate to severe myelosuppression and mild hepatotoxicity in monkeys.

Here, we report the results of the first clinical trial to treat cancer patients with rhuCD40L. The main objective of this phase I study was to determine the safety and maximum-tolerated dose (MTD) of rhuCD40L when administered subcutaneously daily for 5 days. Other objectives included the evaluation of the pharmacokinetics of rhuCD40L administered in this fashion and the assessment of antitumor effects in cancer patients.

PATIENTS AND METHODS

To be eligible, patients had to have recurrent intermediate or high-grade NHL (Working Formulation), chemotherapy-resistant solid tumors, or solid tumors for which conventional cytotoxic therapy was considered unlikely to achieve a meaningful antitumor response. Patients had to be older than 18 and younger than 75 years of age, with a Karnofsky performance status greater than 70%. They had to have adequate hematologic function (hemoglobin $>$ 9 g/dL, WBC count $>$ 3,000 cells/mm^3, absolute neutrophil count $>$ 1,500 cells/mm^3, and platelet count $>$ 100,000 cells/mm^3), renal function (serum creatinine $<$ 2.0 mg/dL), and hepatic function (total bilirubin $<$ 2.0 mg/dL and AST and ALT$<$ 2.5 times the upper limit of normal values). For female patients of childbearing potential, a negative pregnancy test was required, taken within 24 hours before the first dose of rhuCD40L. Patients were not eligible if they had a known personal or immediate family history of autoimmune disease; significant chronic neurologic, hepatic, renal, respiratory, dermatologic, endocrinologic, or cardiovascular disease; alcohol abuse or illicit drug use within 12 months of enrollment; or second malignancy within the previous 5 years, except for carcinoma in situ of the uterus and superficial epithelial skin cancers. Use of systemic chemotherapy, radiotherapy, antitumor biologic therapy, or investigational drugs was not allowed within 30 days before the first dose, nor was the use of hematopoietic growth factors allowed within 10 days. Signed, written, and informed consent was obtained, as required, from each patient. The study was approved by the investigational review boards of the three participating institutions, as well as the United States Food and Drug Administration.

Treatment

Patients were stratified by diagnosis, with NHL patients in one group and solid tumor patients in a second. Each dose cohort in the NHL group included three patients, whereas each dose cohort in the solid tumor group had four patients. Determination of CD40 tumor expression was not required for enrollment. CD40 expression in NHL is nearly 100% but is roughly 75% in solid tumors; consequently, solid tumor patient groups were larger than NHL groups because of this lower expected CD40 expression rate. There was no expected difference with regard to the primary end point of toxicity between patients with solid tumors versus those with NHL. Patients received rhuCD40L (Avrend) by daily subcutaneous injection for 5 consecutive days, a treatment schedule based on pharmacologic data from preclinical studies in nonhuman primates. Five doses were scheduled for evaluation: 0.05, 0.10, 0.20, 0.40, and 0.80 mg/kg/d. Escalation between doses was based on tolerance of lower doses. In the absence of progressive disease or grade 3 or 4 major organ toxicity, patients could receive a second 5-day course of rhuCD40L beginning 6 weeks after the first dose in course one. Third and subsequent courses were administered at 4-week intervals. Dose-limiting toxicity (DLT) was defined based on events during the first course of therapy as any grade 3 or 4 major organ toxicity or any grade 3 or 4 nonmajor organ toxicity requiring discontinuation from the study. An isolated, 1-day grade 3 elevation of ALT in one patient was not considered a DLT. Before enrollment at the next dose level was permitted, at least three patients in the stratification group had to complete 5 days of dosing and 28 days of follow-up without DLT. If one patient experienced DLT at a given dose level, three additional patients were enrolled at the same dose level. If two or more patients experienced DLT at a given dose level, the dose level preceding the one at which DLT was observed was considered the MTD. If two or more patients at a given dose level within a stratification group experienced grade 2 major organ toxicity, the dose was only increased 50% in the next dose cohort. If the intermediate dose level was well tolerated, patients were enrolled at the next scheduled dose level; otherwise, the dose immediately preceding the intermediate dose was considered the MTD. Eight additional patients were then enrolled at the MTD.

At the time of enrollment, medical histories were obtained and patients underwent a physical examination, including measurement of weight, vital signs, and performance status. Pretreatment evaluation also included complete blood count, reticulocyte count, serum chemistry profile, urinalysis, prothrombin time, partial thromboplastin time, quantitative serum immunoglobulins, an ECG, a chest radiograph, and assessment of known tumor sites using appropriate imaging techniques. Patients were evaluated for toxicity (common toxicity criteria) on days 1 through 7, 9, 14, 21, 28, and 35 during courses 1 and 2 and slightly less frequently during subsequent courses. Quantitative serum immunoglobulins, an ECG, a chest radiograph, and tumor response evaluation were repeated at least at the end of courses 1 and 2 and every two cycles thereafter.

Pharmacokinetics

For courses 1 and 2, serum samples for pharmacokinetic studies were obtained at baseline; at 1, 2, 4, 6, and 8 hours postdose on day 1; and at 4, 6, 8, 24, 48, 96, and 216 hours after the day 5 dose. Parameters included half-life, maximum concentration, and area under the curve. Enzyme-linked immunosorbent assay (ELISA) plates were coated with a solution of isoleucine zipper-specific monoclonal antibody. RhuCD40L standard (Immunex Corp) and human sera were diluted in a sample buffer, and the rhuCD40L standard curve ranged from 1,600 to 25 pg/mL in two-fold increments. Sera were tested at a minimal dilution of 1:5 and were titrated two-fold in duplicate through four wells. Incubation and washing were followed by a 1-hour incubation with rabbit polyclonal anti-rhuCD40L antiserum. Detection used peroxidase-conjugated goat antirabbit immunoglobulin (Ig) G (H+L), and color was developed with 3,3',5,5'-tetramethylbenzidine. The reaction was stopped with acid, and optical densities (OD) were determined at a wavelength of 450 nm. The standard curve was generated with a four-parameter logistic model, and sample concentrations were estimated by interpolation from the fitted curve.

Immunoassessment

During course 1, blood samples for flow cytometry were collected in heparinized tubes at baseline and on day 5 and shipped immediately overnight at room temperature to a central laboratory. Peripheral-blood mononuclear cells were obtained after Ficoll (Amersham Pharmacia Biotech, Uppsala, Sweden) centrifugation and preincubated at 4°C with 1% normal rabbit serum and 2% normal goat serum in phosphate-buffered saline/NaN_3 to block nonspecific binding of test antibodies. Cells were then incubated for 30 minutes at 4°C with the relevant phycoerythrin– or fluorescein isothiocyanate–conjugated monoclonal antibodies, washed three times in phosphate-buffered saline/NaN_3 and analyzed by flow cytometry using a FACScan (Becton Dickinson, Mountain View, CA). Monoclonal antibodies used in this study were CD3, CD4, CD8, CD14, CD16, CD19, and CD56 (BD Pharmingen, Torrey Pines, CA).

Serum samples obtained at baseline and day 5 were kept frozen at −80°C. Levels of polyclonal IgM, IgG, IgA, and IgE were then determined by ELISA, as described previously.[38] Sera from normal donors (n = 6) was used to determine normal ranges. Antigen-specific titers of IgG were determined by ELISA by coating plates with the following partially purified antigens: tetanus toxoid (Connaught Laboratories Inc, Swiftwater, PA), measles, influenza, or varicella (Viral Antigens Inc, Memphis, TN), followed by biotinylated-antihuman IgG (Cappel, Malvern, PA). Sera from normal donors was used to generate a standard curve for each antigen.

Table 1. Patient Characteristics

Characteristic	Solid Tumor (n = 23) No.	Solid Tumor (n = 23) %	NHL (n = 9) No.	NHL (n = 9) %
Sex				
Women	14	61	4	44
Men	9	39	5	56
Race				
White	17	74	6	67
Hispanic	2	9	3	33
Asian	2	9	0	0
African-American	2	9	0	0
Age, years				
Median	59		53	
Range	23-75		21-71	
Stage IV disease		100		78
Prior cancer therapy				
Chemotherapy		65		100
Radiation		65		67
Biologic		39		67

Serum levels of interleukin 12 (IL-12), macrophage inflammatory protein (MIP1) α, and MIP1β, and regulated upon activation, normal T cell expressed and secreted (RANTES) were determined by ELISA using paired monoclonal and polyclonal antibodies specific for each cytokine (R&D Systems, Minneapolis, MN). The sensitivities of the ELISAs were as follows: IL-12, 6 pg/mL; MIP1α, 23 pg/mL; MIP1β, 6 pg/mL; and RANTES, 15 pg/mL.

rhuCD40L Antibodies

Serum samples obtained on the first, 14th, and last day of each course were tested for the presence of antibodies to rhuCD40L. ELISA plates were coated overnight with rhuCD40L, and after washing, controls and serum samples were added to the wells and incubated. Bound antibody was detected, after washing, with a peroxidase-labeled antihuman Ig conjugate. A sample was scored positive if the posttreatment sample exhibited a statistically significant OD increase when compared with the corresponding pretreatment sample OD.

Confirmation of positive antibody results was tested by assessing the ability of sample to influence the binding of CD40L to CD40. Immobilized CD40 was exposed to sample (preincubated with a fixed quantity of rhuCD40L), washed, and exposed to biotinylated rhuCD40L. Bound biotinylated rhuCD40L was detected with streptavidin-horseradish peroxidase conjugate. A sample was scored positive (confirmed) if it exhibited a statistically significant difference from the control.

RESULTS

Patient Characteristics

Thirty-two patients including 23 with solid tumors and nine with NHL were evaluated in this phase I study from July 1998 to August 1999 at three institutions. Patient characteristics are given in Table 1. Histology of the solid tumors included renal cell carcinoma (n = 10), head and neck cancer (n = 4), cervical cancer (n = 2), sarcoma (n = 2), and one patient each

Table 2. Incidence and Grade of Serum Transaminase Elevations (all courses)

	Solid Tumor (n = 23)						NHL (n = 9)							
	0.05 mg/kg/d (n = 4)		0.10 mg/kg/d (n = 15)		0.15 mg/kg/d (n = 4)		0.05 mg/kg/d (n = 3)		0.10 mg/kg/d (n = 3)		0.15 mg/kg/d (n = 3)		Total (N = 32)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
AST high														
Grade 1	1	25	3	20	0	0	0	0	0	0	2	67	6	19
Grade 2	0	0	2	13	1	25	0	0	2	67	0	0	5	16
Grade 3	1	25	5	33	2	50	0	0	0	0	1	33	9	28
Grade 4	0	0	0	0	1	25	0	0	0	0	0	0	1	3
ALT high														
Grade 1	0	0	4	27	0	0	0	0	0	0	1	33	5	16
Grade 2	0	0	1	7	1	25	0	0	2	67	0	0	4	13
Grade 3	1	25	3	20	2	50	0	0	0	0	1	33	7	22
Grade 4	0	0	0	0	1	25	0	0	0	0	0	0	1	3

with rectal carcinoma, esophageal carcinoma, breast carcinoma, peritoneal carcinomatosis, and adenocarcinoma of unknown primary tumor. Histology in the NHL patients included B-cell NHL (n = 8) and T-cell NHL (n = 1). B-cell NHL diagnoses were diffuse large cell (n = 4); diffuse, mixed small and large cell (n = 1); diffuse, small cleaved cell (n = 1); and transformed follicular (n = 2).

Patients were treated at three different dose levels. The total number of courses administered was 65, and the mean number of courses per patient was two (range, one to 12). Six solid tumor patients and none of the nine NHL patients received three or more courses. Every patient except one completed at least one full 5-day course of therapy. One patient with breast carcinoma developed grade 4 elevations in AST and ALT after four injections of rhuCD40L, so the final day of therapy was not given. This patient was included in the analyses of toxicity and tumor response but not pharmacokinetics.

Hepatic Toxicity and MTD

Dose-related, transient serum elevations in liver transaminases were observed as detailed in Table 2. In the solid tumor cohort of patients, this toxicity defined the MTD of rhuCD40L administered on this schedule to be 0.10 mg/kg/d. No MTD was defined based on NHL patients. Among all patients, grade 3 or 4 elevations of either AST or ALT were observed in 14%, 28%, and 57% of patients treated at 0.05, 0.1, and 0.15 mg/kg/d, respectively. Grade 4 elevation in AST or ALT occurred only at the highest dose. Elevations were transient, usually peaking on day 6, and generally returned to within normal limits by day 14. Two patients with liver metastases had sustained elevations of AST and ALT. In the six patients treated with three or more courses of rhuCD40L, there was no increase in the incidence or severity of liver enzyme elevations with repeated courses. When observed in these six patients, grade 3 or 4 elevations in liver transaminases always initially occurred during the first course, except for one patient who developed grade 3 abnormalities only during the second course.

Hematopoietic Toxicity

Six patients developed grade 3 (n = 5) or grade 4 (n = 1) reductions in hemoglobin, although this toxicity was not clearly dose-dependent. Two patients had grade 3 or 4 leukopenia, and three had grade 3 or 4 neutropenia. Seven (78%) of the nine NHL patients and 15 (68%) of the 23 solid tumor patients had grade 3 lymphopenia, although three of these NHL patients and four of these solid tumor patients had grade 3 lymphopenia at baseline. In the six patients treated with three or more courses of rhuCD40L, there was no increase in the incidence or severity of these hematopoietic toxicities, which when observed always initially occurred during the first course. One patient with renal cell carcinoma, bone metastases, and no evidence of bleeding developed asymptomatic grade 4 reduction in hemoglobin after two courses and was transfused two units of packed RBCs.

Other Toxicities

Other toxicities were generally mild to moderate (Table 3). Grade 3 toxicities included five patients with pain and one patient each with dyspnea, asthenia, dyspepsia, and pneumonia. A grade 3 seizure occurred in one patient who had completed radiation therapy for brain metastases eight months before treatment with rhuCD40L. One patient had two episodes of gastrointestinal bleeding (one grade 3 and one grade 4) relating to an invasive small bowel tumor. One patient developed a renal stone. Two deaths occurred on study, 26 and 30 days after the last dose of rhuCD40L, and each was due to disease progression.

Table 3. Adverse Events Other Than Hepatic and Hematologic Toxicities Occurring in ≥ 10% of All Patients (all grades, all courses)

	Solid Tumor (n = 23)						NHL (n = 9)							
	0.05 mg/kg/d (n = 4)		0.10 mg/kg/d (n = 15)*		0.15 mg/kg/d (n = 4)		0.05 mg/kg/d (n = 3)		0.10 mg/kg/d (n = 3)		0.15 mg/kg/d (n = 3)		Total (N = 32)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Injection site reaction	2	50	9	60	3	75	0	0	2	67	3	100	23	72
Asthenia	3	75	6	38	0	0	2	67	0	0	1	33	12	38
Fever	2	50	3	19	1	25	2	67	1	33	1	33	10	31
Abdominal pain	3	75	4	25	0	0	1	33	1	33	0	0	9	28
Anorexia	2	50	3	19	0	0	2	67	1	33	0	0	8	25
Constipation	1	25	3	19	0	0	2	67	2	67	0	0	8	25
Dyspnea	2	50	3	19	1	25	2	67	0	0	0	0	8	25
Pain	2	50	3	19	1	25	1	33	1	33	0	0	8	25
Chills	1	25	1	6	1	25	0	0	1	33	1	33	5	16
Diarrhea	1	25	2	13	1	25	1	33	0	0	0	0	5	16
Nausea	2	50	2	13	1	25	0	0	0	0	0	0	5	16
Vasodilation	1	25	2	13	0	0	0	0	1	33	1	33	5	16
Vomiting	2	50	3	19	0	0	0	0	0	0	0	0	5	16
Back pain	2	50	1	6	0	0	0	0	1	33	0	0	4	13
Cough increase	1	25	2	13	0	0	0	0	1	33	0	0	4	13
Pruritus	0	0	1	6	1	25	2	67	0	0	0	0	4	13
Sinusitis	1	25	1	6	0	0	1	33	0	0	1	33	4	13
Tachycardia	1	25	1	6	0	0	0	0	2	67	0	0	4	13
Weight decrease	0	0	2	13	0	0	2	67	0	0	0	0	4	13

*One patient in the solid tumor group who received 0.15 mg/kg/d of rhuCD40L in courses 1 and 2 and 0.10 mg/kg/d in courses 3 and 4 is double-counted such that the denominator is 16 for calculating percentages.

ISRs were the only clinical adverse event that seemed to be dose-related, but were all grade 1 or 2. Among solid tumor patients, ISRs were observed in 50%, 60%, and 75% of patients treated with 0.05, 0.10, and 0.15 mg/kg/d, respectively. Among NHL patients, ISRs were observed in 100% of patients in the 0.15 mg/kg/d group compared with 0% at 0.05 mg/kg/d and 67% at 0.10 mg/kg/d.

One patient with renal cell carcinoma developed subclinical hypothyroidism after one course of rhuCD40L. This patient had received a 5-month course of interleukin-2 therapy 4 months before entering onto the study and was retrospectively found to have had elevated antithyroid peroxidase antibody levels (but normal thyroid function) before treatment with rhuCD40L. No other autoimmune sequelae were observed in patients on this study.

Antitumor Activity

Two patients (6%) had a partial tumor response while on study. One of these patients, a 60-year-old man with stage IV squamous cell carcinoma of the larynx that was progressive despite prior conventional and experimental therapy, had a more than 50% reduction in his laryngeal tumor mass after one course of 0.1 mg/kg/d of rhuCD40L. Prior therapy for recurrent disease included external-beam radiation, four single-agent chemotherapeutic agents, and a phase I agent targeted at the epidermal growth factor receptor. The partial response to rhuCD40L was sustained for 11 additional courses, and he was then taken off treatment after receiving 1 year of rhuCD40L without progression. He was subsequently observed while not receiving any anticancer therapy. Three months after discontinuing rhuCD40L, follow-up endoscopic examination revealed a complete tumor response, which was confirmed pathologically by biopsy 5 months later. The patient remains free of disease 1 year after completing rhuCD40L and 2 years after enrollment.

The second patient with a partial response had a more than 50% reduction in his NHL after one course of 0.05 mg/kg/d of rhuCD40L but was found to have progressive disease after two courses. Prior therapy included two regimens of chemotherapy and six treatments with external-beam radiation. Twelve patients (38%) had stable disease after one course of rhuCD40L, which was sustained in five patients after two courses. Four patients continued with stable disease through the fourth course.

Pharmacokinetics

Serum concentrations of rhuCD40L were barely detectable in the 0.05 mg/kg/d group. For 18 patients treated at 0.10 mg/kg/d and six patients treated at 0.15 mg/kg/d, absorption was rapid after subcutaneous administration, with peak serum concentrations generally observed within 4 hours after dosing. At the MTD of 0.10 mg/kg/d, the terminal half-life was 24.8

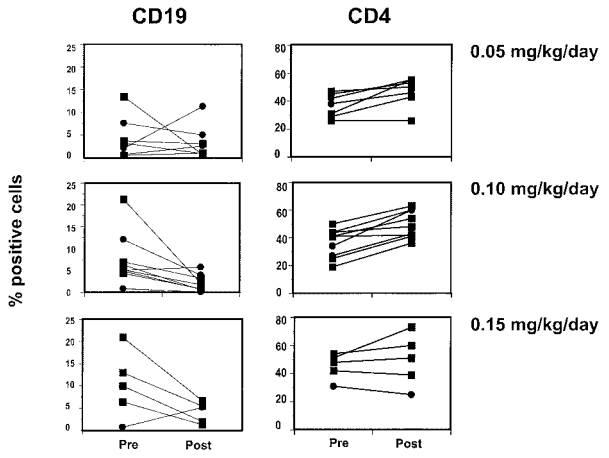

Fig 1. Analysis of CD19$^+$ and CD4$^+$ peripheral lymphocyte subsets from patients before and after the 5-day administration of rhuCD40L at 0.05, 0.10, or 0.15 mg/kg/d. ■, solid tumor patients; ●, NHL patients.

hours (SD, 22.8 hours; range, 12 to 73 hours). The maximum concentration at this dose was 2.9 ± 1.7 ng/mL, and the area under the curve from 0 to 24 hours was 41.6 ± 24.6 ng-hr/mL. There were no significant changes in pharmacokinetic parameters between dosing days.

Immunoassessment

Blood was obtained at baseline and on day 5 from 23 patients (15 with solid tumors and eight with NHL) to measure lymphocyte subsets by flow cytometry. Peripheral-blood mononuclear cells were obtained in sufficient numbers for analysis from 21 patients. For 16 of 21 patients, there was a decrease of at least 5% in the percentage of CD19$^+$ cells on day 5 compared with baseline ($P = .027$ by Fisher's exact test (StatXact 4, Cytel Software, Cambridge, MA), particularly evident at doses of 0.1 mg/kg/d and 0.15 mg/kg/d (Fig 1). In contrast, 17 of 21 patients had increases in the percentage of CD4$^+$ cells of at least 5% on day 5 relative to baseline ($P = .007$). There was no overall trend in the change of CD3$^+$, CD8$^+$, CD14$^+$, or CD16/CD56$^+$ cells before and after treatment (data not shown).

Serum obtained at baseline and on day 5 was used to measure polyclonal and antigen-specific antibody levels and levels of pro-inflammatory cytokines, including IL-12, MIP1α, MIP1β, and RANTES. No significant changes were observed before or after treatment in serum antibody or cytokine levels.

rhuCD40L Antibody

Samples were obtained from 31 of 32 patients for the measurement of antibodies to rhuCD40L, and two patients were confirmed to be positive. In one patient, antibodies developed after one of three courses and were still detectable in the serum 4 months later. This patient did not develop immune complex-related phenomenon nor any other adverse event related to the development of antibodies. In a second patient, anti-rhuCD40L antibodies were detected at day 14 of course 1, but the patient died of progressive disease on day 35 before further follow-up was possible.

DISCUSSION

rhuCD40L is a novel recombinant biologic agent whose receptor, CD40, is expressed on the surface of nearly all B-cell malignancies and the majority of solid tumors. Treatment of tumor cell lines in vitro or tumor-bearing animals in vivo with rhuCD40L leads to tumor death or tumor growth inhibition. Furthermore, CD40L (naturally expressed by activated T lymphocytes) is an important component of T-cell help for B-cell differentiation and provides costimulatory signals for T-cell clonal expansion.[11,19] CD40 ligation has been effectively exploited for the induction of antitumor immunity in several animal models.[39-41]

Here, we report the results of the first phase I clinical trial of rhuCD40L in the treatment of patients with advanced solid tumors and NHL. Thirty-two patients were given rhuCD40L subcutaneously daily for 5 days, which could be repeated every 4 to 6 weeks in the absence of progressive disease or toxicity. Overall, rhuCD40L was well tolerated. Based on transient elevations of liver transaminases, the MTD of this approach was 0.1 mg/kg/d every 4 weeks. The exact cause of the liver function test abnormalities is not known, but a cytotoxic effect of rhuCD40L on CD40$^+$ hepatic cells has been reported in one in vitro experimental model.[42] Anemia, leukopenia, and neutropenia were observed in less than 15% of patients, but only one patient required a blood transfusion, and none simultaneously developed fever. Injection site reactions were dose dependent in frequency but were never worse than grade 2. No autoimmune sequelae were observed except possibly for one patient with preexisting antithyroid peroxidase antibodies who developed subclinical hypothyroidism on study.

Two patients (6%) had a partial tumor response while on study. One of these patients, having achieved a sustained partial response of a laryngeal mass, discontinued rhuCD40L after 12 courses (approximately 1 year) and was observed while not receiving any anticancer therapy. After 3 months without therapy, he was found to have a complete response with the disappearance of the laryngeal mass, a finding that was confirmed pathologically on biopsy of the site. It is tempting to speculate that his transition from partial to complete response was immune-mediated, given

the delay in his tumor response, which is typical of T-cell–mediated antitumor effects.[43]

Determination of CD40 tumor expression was not required for study entry, although five of six pretreatment biopsies that were evaluated showed CD40 expression. Nevertheless, evaluation of a correlation between CD40 tumor expression, tumor response, and toxicity was not possible in this study. However, the potential stimulation of host professional antigen-presenting cells, such as dendritic cells, by treatment with rhuCD40L would be independent of tumor CD40 expression. In tumor-bearing mice, for example, treatment with trimeric CD40L has been shown to be effective against CD40-negative tumors.[40,41,44]

In this study, effects of rhuCD40L on the immune system were monitored in part by flow cytometry of peripheral blood. In 76% of the patients tested, there was a decrease in the percentage of circulating $CD19^+$ B lymphocytes on day 5 compared with baseline, possibly related to the peripheral clearance of these $CD40^+$ cells by binding to rhuCD40L. Such clearance was also observed in mice and nonhuman primates treated with CD40L (Armitage, unpublished observations). In contrast, the percentage of $CD4^+$ T lymphocytes increased during this time in 81% of patients. Levels of serum immunoglobulins and cytokines, such as IL-12, were unchanged, although in vitro CD40L treatment stimulates immunoglobulin class switching in B cells and cytokine production from monocytes, dendritic cells, and B cells. In contrast to preclinical animal testing, rhuCD40L did not lead to lymph node enlargement or splenomegaly in any of the patients.

Monomeric forms of CD40L signal inefficiently via CD40, whereas cross-linking of CD40 by trimeric CD40L or by certain antibodies against CD40 markedly enhances signaling.[24,45] RhuCD40L leads to a cascade of intracellular signals in B-cell lymphomas and $CD40^+$ solid tumor cells (Vonderheide and Battle, unpublished observations)[11]; however, the isoleucine-zipper motif used for trimerization of rhuCD40L presents a potential for the development of anti-rhuCD40L antibodies in patients. Although 94% of patients evaluated did not develop anti-rhuCD40L antibodies, two patients were confirmed to have developed such antibodies. There were no clinical adverse events related to the anti-rhuCD40L antibodies.

Pharmacokinetic monitoring of rhuCD40L serum levels indicated that absorption was rapid after subcutaneous administration. The terminal half-life was approximately 24 hours, but there was considerable variability at the MTD. Intravenous dosing of rhuCD40L is being compared with subcutaneous dosing in subsequent trials.

In summary, encouraging antitumor activity, including the induction of a long-term complete remission in one heavily pretreated patient, was observed in this phase I trial of rhuCD40L. The MTD of this dosing schedule was defined by transient elevations in liver transaminases, but other side effects were quite tolerable. Phase II studies are underway to further refine the dosing, toxicity, and efficacy of this novel agent. Given the observed hepatic toxicity in this study, phase II studies include interim evaluations and stopping rules for toxicity. Important additional end points for these subsequent studies will be correlation of efficacy with tumor CD40 expression and the assessment of the effect of rhuCD40L on patients' immune system. Ultimately, the combination of rhuCD40L with chemotherapy, radiotherapy, serotherapy, and other biologic therapy, as suggested by preclinical studies (unpublished observations),[44] will become an important goal.

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