

Safety, Pharmacokinetics, and Biological Pharmacodynamics of the Immunocytokine EMD 273066 (huKS-IL2)

Results of a Phase I Trial in Patients with Prostate Cancer

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Abstract: This phase 1 clinical trial was conducted to evaluate the safety and to determine the maximum tolerated dose (MTD) of the immunocytokine EMD 273066 huKS-IL2 and, secondarily, to assess its pharmacokinetics, immunogenic potential, and immunologic activity in patients with androgen-independent prostate cancer (n = 22). EMD 273066 was administered in 3-day cycles (separated by 4 weeks) of once-daily, 4-hour intravenous infusions at a dose determined by an escalation protocol (0.4, 0.7, 1.4, 2.8, 4.3, 6.4, or 8.5 mg/m²/d). Approximately 2/3 of patients received a second cycle of treatment. The results show that the MTD of EMD 273066 [ie, one dose level below that producing dose-limiting toxicity (DLT) in at least 33% of patients in a dosing group] was 6.4 mg/m²/d. EMD 273066 was generally well tolerated up to a dose of 4.3 mg/m²/d. No DLTs, defined as drug-related toxicities $\geq$ Grade 3 occurring during the first treatment cycle, were observed among patients in the 0.4-, 0.7-, 1.4-, or 4.3-mg/m²/d dosing groups. Four patients treated with 2.8, 6.4, or 8.5 mg/m²/d EMD 273066 experienced DLTs. Titers of both antiimmunocytokine and anti-FcIL-2 antibody responses were observed after the first dose cycle and either decreased or remained stable during a second course of treatment. No hypersensitivity reactions were observed. EMD 273066 exhibited immunologic activity as demonstrated by increases in lymphocyte counts, natural killer cell number and specific activity, and antibody-dependent cellular cyto-

toxicity activity. On average, C_{max}, which was dose-dependent, was achieved within 1 hour after infusion. Mean t_{1/2}, which was independent of dose, ranged from 4.0 to 6.7 hours across doses. A zero-compartment body model with one-order kinetics best described the concentration-time profiles. These data demonstrate that the novel immunocytokine EMD 273066 is well tolerated at doses above a level of observed systemic biologic activity in patients with androgen-independent prostate cancer.

Key Words: immunocytokines; EMD 273066; prostate cancer; pharmacokinetics

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Immunocytokines, recombinant antibody–cytokine fusion proteins that retain both antigen-binding capacity and cytokine activity, are being developed as a novel modality of cancer immunotherapy that combines the tumor-targeting ability of tumor-specific antibodies with the broad-based immunomodulatory activities of cytokines.¹ Immunocytokine development is motivated by the rationale that immunocytokines can improve antitumor immune responses relative to those obtained with current immunotherapies by providing both antibody effector activities and a cytokine cosignal for the generation of cytotoxic cellular immunity. Furthermore, it is postulated that targeted delivery of immunocytokines to the tumor microenvironment, where they may be more effective in mobilizing antitumor immune responses, will improve on the benefit:risk ratio of currently available systemic cytokine therapy.^{1,2} Even at high doses of untargeted IL-2, which are associated with severe, potentially lethal side effects, the expected cytokine concentrations near the tumor are below those required for optimal antitumor responses.^{1–3}

EMD 273066 huKS-IL2 is composed of two molecules of interleukin-2 (IL-2) genetically fused to a humanized monoclonal antibody directed against human adenocarcinoma-associated antigen (KSA, also known as EpCAM, or epithelial cell adhesion molecule), which is highly expressed on

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many epithelial cancers including prostate, colon, breast, and lung.^{4,5} As one of the most potent known antitumor cytokines, IL-2 modulates the proliferation and activity of a broad range of antitumor effector cells and mediators and has demonstrated antitumor activity in patients with renal cell carcinoma or melanoma.^{1,6-8} Results of studies using models of murine carcinoma metastases show that EMD 273066 can induce antitumor effects via T-cell-mediated immune responses,⁴ which are associated with protective, long-lived T-cell memory even in the absence of antigen,⁹ as well as via T-cell-independent immune responses.¹⁰ In animal models, the amount of IL-2 required to stimulate antitumor responses was lower when IL-2 was administered as EMD 273066 than when IL-2 was given alone.⁴ Moreover, overall survival in mice in which metastases of human prostate carcinoma were suppressed with EMD 273066 was 4 times longer than that in mice administered a mixture of unfused anti-KSA antibody and recombinant human IL-2.¹⁰

Both the antibody and the cytokine components of EMD 273066 have been administered individually to humans, but the effects of EMD 273066 in humans have not been previously reported. This phase I clinical trial was conducted to evaluate the safety and to determine the maximum tolerated dose (MTD) of EMD 273066 in patients with advanced prostate cancer. Secondary objectives included assessment of pharmacokinetics, immunogenic potential, and immunologic activity.

MATERIALS AND METHODS

Patients

Patients were men at least 18 years of age with advanced, androgen-independent prostate cancer, an Eastern Cooperative Oncology Group (ECOG) performance status¹¹ of 0 or 1, and an estimated life expectancy of at least 12 weeks. Exclusion criteria included prior treatment with monoclonal antibodies or stem cells processed with monoclonal antibodies (unless the patient was seronegative for KS antibody reactivity); chemotherapeutic agents, radiation therapy, or other immunosuppressive therapy within 4 weeks before the study; and treatment within 6 weeks before with mitomycin C, nitrosoureas, or melphalan (L-PAM). All patients provided written informed consent.

Procedures

The protocol for this open-label, multicenter, dose-escalation study was approved by institutional review boards at each study site. EMD 273066 was administered on an inpatient basis in 2 treatment cycles separated by approximately 4 weeks. Each treatment cycle comprised 3 consecutive days (days 1 through 3 and days 29 through 31) of a once-daily, 4-hour intravenous infusion of EMD 273066. Up to 2 additional cycles of treatment beyond the second could be admin-

istered at 4-week intervals at the investigators' discretion based on the incidence and severity of toxicities and the absence of disease progression.

Adverse events, laboratory values, and vital signs were graded by using the National Cancer Institute's Common Toxicity Criteria (CTC) scale version 2.0,¹¹ which grades adverse events as 0 (no adverse event), 1 (mild), 2 (moderate), 3 (severe and undesirable), 4 (life-threatening or disabling), or 5 (death). Because version 2.0 of the CTC scale does not assess IL-2-specific toxicities, an IL-2-specific scale employing a grading scheme similar to that of the CTC scale was used for potentially IL-2-related events including changes in systolic blood pressure, weight gain, changes in ECOG performance status, and changes in temperature (Table 1). The IL-2-specific scale took precedence over the CTC scale for IL-2-related events.

Dose escalation in this study was guided by the principles of an accelerated titration design, which, compared with traditional designs for phase 1 trials, has been shown to reduce the number of patients who receive subtherapeutic doses of a chemotherapeutic agent, expedites completion of phase 1 clinical trials, and increases the amount of information for planning subsequent studies.¹² Treatment with EMD 273066 was started in the first patient at a dose of 0.4 mg/m²/d (less than 1/10 of the limiting dose in preclinical toxicology studies) and, provided that all toxicities recovered to Grade 1 or lower or to 1 grade above baseline, could be increased in subsequent patients according to a predetermined schedule (0.4, 0.7, 1.4, 2.8, 4.3, 6.4, and 8.5 mg/m²/d). No inpatient dose escalation was permitted. One patient was enrolled per dose level until a toxicity of any grade occurred, after which time subsequent dose levels were to include 3 patients. If the toxicity met the definition of a dose-limiting toxicity (DLT, ie, any drug-related toxicity at least Grade 3 occurring during the first treatment cycle), the 3 patients were to be enrolled at 1 dose level below the level that produced the first DLT. However, if the toxicity did not meet the definition of a DLT, subsequent patients were enrolled at the same dose level as that that produced the toxicity in the single patient. The MTD was defined as the dose that is 1 level below one at which at least 33% of patients experienced a DLT.

TABLE 1. IL-2 Toxicity Grading Scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Increase in systolic blood pressure (mm Hg)	≤20	21 to 39	≥40
ECOG performance status (grade)	2	3	4
Weight gain (% body mass)	5 to 10	11 to 14	≥15
Temperature (°C)	≤38	38.1 to 39.9	≥40

Among individual patients, treatment was stopped for the first occurrence of a Grade 3 toxicity but could be resumed after resolution of the toxicity to Grade 1 or lower or to 1 grade above baseline. For a Grade 4 toxicity or the second occurrence of a Grade 3 toxicity, patients were to be discontinued from the study. Treatment was also to be stopped for an ECOG performance status of 4 (ie, completely disabled) but could be resumed if the status improved to ≤ 2 .

Vital signs were measured before and during infusions on each day of treatment as well as during clinic visits throughout the treatment period when no infusions were scheduled. Adverse events, defined as any untoward medical occurrence regardless of its suspected etiology, and ECOG performance status were assessed according to a similar schedule. Blood samples for standard chemistry, hematology, and urinalysis tests were obtained at screening and on days 1, 2, 4, 7, 14, 21, 28, 30, 32, 35, and 43.

Premedications that were permitted (but not necessarily used at all study sites) included diphenhydramine 50 mg IV/PO, nizatidine 150 mg PO, ranitidine 150 mg PO, acetaminophen 650 mg PO, and morphine sulfate 2 to 4 mg IV. Known IL-2 side effects were managed according to predetermined protocols with medications including acetaminophen, diphenhydramine, and indomethacin. Any anaphylactic reaction was to be treated with dexamethasone and epinephrine. Chemotherapeutic agents, radiation therapy (other than palliative therapy for bone pain), or other immunosuppressive therapy; mitomycin C; nitrosoureas; L-PAM; monoclonal antibodies; and stem cells processed with monoclonal antibodies were not permitted.

Pharmacokinetic Analyses

Blood samples for pharmacokinetic analysis of EMD 273066 were taken on the first and third days of the first and second treatment cycles. On each sampling day, venous blood was obtained before the start of infusion and 0.5, 4, 4.5, 5, 6, 8, 16, and 24 hours after the start of infusion. Samples were stored frozen during the study and then analyzed using an enzyme-linked immunosorbent assay (ELISA) with a lower limit of quantification of 25 ng/mL. Standard pharmacokinetic parameters were calculated based on the serum concentration data. Serum concentrations below the lower limit of quantification were set to 0 for calculation of descriptive statistics. Noncompartmental and compartmental analyses were performed using KINETICA™ software, version 3.0.

Immunogenicity

The immunogenicity of EMD 273066 was assessed by using a sandwich ELISA. Antiimmunocytokine antibodies as well as antibodies against the Fc and IL-2 components (FcIL-2) of EMD 273066 were measured. Serum samples were obtained preinfusion and on days 1, 2, 5, 8, 15, and 22 of the first and second treatment cycles. In the antiimmunocytokine anti-

body assay, EMD 273066 was used as capture. Following incubation with test sera, biotinylated EMD 273066 was used for detection. Anti-human IgG₁, κ light chain-specific, was used as an internal control antibody. Reactivity was based on the ability of antiimmunocytokine antibodies to bridge plate-bound and soluble, biotinylated EMD 273066. In the anti-FcIL-2 antibody assay, hu-FcIL-2 was used as capture. This molecule constitutes the heavy chain constant region of IgG₁, the linker region generated by the fusion of IL-2 to the carboxyl terminus, and human IL-2. Goat anti-human Ig κ chain and λ chain-specific antibodies were used for detection of any bound immunoglobulin including IgM and IgG. Goat anti-human Ig κ chain and goat anti-human λ chain-specific antibodies were used as internal controls. An antiimmunocytokine or anti-IL-2 response was defined as an optical density value of all dilutions (up to 1:270 for antiimmunocytokine and 1:810 for anti-IL-2) at least 3 times higher than the preinfusion value.

Immunologic Activity

Immunologic activity was assessed by determining the numbers of lymphocytes and natural killer (NK) cells as well as with NK cell activity and antibody-dependent cellular cytotoxicity (ADCC) activity. Blood samples for lymphocyte counts were obtained at screening and on days 1, 2, 4, 7, 14, 21, 28, 30, 32, 35, and 43. Blood samples for the NK-cell assays were obtained before infusion on days 1 (preinfusion), 8, and 29 of the first treatment cycle and, if possible, at similar times during the second treatment cycle.

NK-cell numbers were determined by Fluorescence-Activated Cell Sorter (FACS) analysis by using antibodies targeting the NK-cell-specific surface markers CD56 and FcRIII (CD16). Values were recorded for cells displaying either of these markers as well as for those displaying both markers simultaneously (double positives). For analysis of the time profile of changes in NK-cell number, data from all patients were pooled at each time point regardless of the dose level. Numbers were normalized to the predose value for each patient individually, and then the mean was calculated for all normalized data at each time point. Data were reported as total CD56⁺ cells, and "activated" NK number was taken as the CD16/CD56 double-positive fraction.

NK cell activity and ADCC were determined using 4-hour chromium release assays that employed ⁵¹Cr-labeled natural killer target cell K562 (an EpCAM-negative human erythroleukemia line) and ADCC target cell DU145 (an EpCAM-positive human prostate carcinoma line), respectively. Target cells were collected for labeling with ⁵¹Cr by first centrifuging at 1500 rpm for 10 minutes to form pellets. Media were decanted to leave approximately 200 μ L (3×10^6 target cells) to which 300 μ L RPMI/10% FCS was added. Cells were washed in PBS and resuspended in RPMI/10% FCS. Labeled target cells were stored at room temperature until use.

Effector cells were prepared from patients' peripheral blood leukocytes. Blood was diluted with RPMI and layered over Ficoll in 50-mL tubes. Tubes were centrifuged at 1800 to 2000 rpm for 20 minutes, and peripheral blood leukocytes were washed with RPMI. Cells were cultured overnight in 10% FCS/RPMI at $2.5\text{--}5 \times 10^6$ cells/mL, washed the next day, and resuspended in RPMI/10% FCS.

For the ADCC assay, diluted antibody (50 μ L) was added to wells containing peripheral blood leukocytes to yield antibody concentrations of 0, 20, and 200 ng/mL. ^{51}Cr -labeled target wells were added to each well. After centrifugation and harvesting, samples were counted in a γ counter. Results were compared with target cells totally lysed with 100 μ L 2% Triton X 100.

For analysis of NK activity and Fc-mediated ADCC activity, results with K562 and DU145, respectively, from all patients were pooled at each time point regardless of the dose level. Numbers were normalized to the predose value for each patient individually, and then the mean was calculated for all normalized data at each time point.

RESULTS

Patients

Twenty-two patients (ECOG 0, 7 patients; ECOG 1, 15 patients) were enrolled and received at least 1 infusion of EMD 273066. Patients' mean age was 67.2 years (range 41 to 80). Most patients (16/22) were white; the remainder were Latino/Hispanic (3/22), black (2/22), or East Indian (1/22).

All patients received at least 1 infusion of EMD 273066. More than half of the patients (15/22) completed at least 2 treatment cycles as specified by the protocol (Table 2). Three patients received more than 2 cycles of EMD 273066. One of these patients received 4 treatment cycles at 1.4 mg/m²/d followed by 2 cycles at 2.8 mg/m²/d under an exemption allowing for additional courses of therapy on the basis of positive response in PSA and clinical tumor measurements.

Dose-Limiting Toxicities and Maximum Tolerated Dose

The MTD of EMD 273066 (ie, one dose below that producing DLT among at least 33% of patients in a dosing group) was 6.4 mg/m²/d. No DLTs occurred among patients in the 0.4-, 0.7-, 1.4-, or 4.3-mg/m²/d dosing groups. One patient each in the 2.8- and 6.4-mg/m²/d dosing group and 2 patients in the 8.5-mg/m²/d dosing group experienced DLTs (Table 3). The patient receiving 2.8 mg/m²/d experienced dyspnea meeting the definition of a DLT as well as other symptoms (eg, edema, pleural effusion) consistent with vascular leak syndrome on day 3 of the first treatment cycle. Symptoms resolved during treatment with furosemide. The patient was later discontinued from the study because of disease progression. The patient receiving 6.4 mg/m²/d experienced hypotension meeting the definition of a DLT on day 2 of the first treatment cycle. The hypotension resolved spontaneously. This patient had preexisting abnormal kidney function unrelated to study medication (but met study inclusion criteria) and was discontinued from the study because of bilateral hydronephrosis. The first patient receiving 8.5 mg/m²/d experienced chills, rigors, and crackling at the base of the lung on day 2 of the first treatment cycle. These symptoms met the definition of a DLT and were considered to be consistent with vascular leak syndrome. This patient was discontinued from the study. The second patient receiving 8.5 mg/m²/d experienced hypertension, anemia, and asthenia during the first treatment cycle. The patient was discontinued from the study when the ECOG performance score decreased (initially to 3 and then lower) such that it met the criterion for study withdrawal.

Adverse Events and Other Safety Measures

The most common adverse events considered by the investigators to be at least possibly related to study medication and that were Grade 2 or above were fever, asthenia, and chills (Table 4). The majority of adverse events were Grade 1 or 0; 7 patients had Grade 3 adverse events; and no patient had a

TABLE 2. EMD 273066 Exposure

	Dose (mg/m ² /day)						
	0.4 (n = 1)	0.7 (n = 1)	1.4 (n = 3)	2.8 (n = 6)	4.3 (n = 3)	6.4 (n = 6)	8.5 (n = 2)
1 cycle	0	0	1	2	0	2	2
2 cycles	1	1	1	4*	2	4	0
3 cycles	0	0	0	1	0	0	0
4 cycles	0	0	1*	0	1	0	0

*One patient received 4 treatment cycles of huKS-IL-2, 1.4 mg/m² day, followed by 2 cycles of 2.8 mg/m²/day.

TABLE 3. Dose-Limiting Toxicities (DLTs)*

Dose (mg/m ² /day)	n	Number with Dose-Limiting Toxicities
0.4	1	0
0.7	1	0
1.4	3	0
2.8	6	1
4.3	3	0
6.4	6	1
8.5	2	2

*DLTs were defined as any drug-related toxicity $\geq$ Grade 3 occurring during the first treatment cycle.

Grade 4 adverse event. No unexpected changes in vital signs or the results of clinical laboratory tests were observed.

Pharmacokinetics

Concentration–time profiles for doses of 0.7 mg/m²/d through 8.5 mg/m²/d are shown in Fig. 1. At the lowest dose level, concentrations of EMD 273066 were below the limit of quantification. High intra- and interindividual variability was detected for all pharmacokinetic parameters. On average, C_{max} , which was dose-dependent, was achieved within 1 hour after infusion. Mean $t_{1/2}$, which was independent of dose, ranged from 4.0 to 6.7 hours. Mean volume of distribution was between 3.9 and 7.1 L. Serum concentrations of EMD 273066 increased with dose but dose-proportionality could not be de-

tected because of the high variability of C_{max} and AUC. A one-compartment body model with zero-order input best described the concentration–time profiles.

Immunogenicity

Both antiimmunocytokine and anti-FcIL-2 antibody responses, as defined by optical density values of all dilutions at least 3 times higher than preinfusion values, were observed. The antiimmunocytokine response during the first treatment cycle was first observed on day 8, when 14 of 20 patients tested positive for titers of antiimmunocytokine antibodies to EMD 273066. Titers did not increase during the second cycle. An anti-FcIL-2 response was observed during both the first and second treatment cycles, during which 20 of 21 patients tested positive for titers of antibodies against FcIL-2. Titers did not increase during the second cycle. Furthermore, the same samples were negative for binding to recombinant IL-2 but not to FcIL-2. No hypersensitivity reactions were observed after dosing with EMD 273066.

Immunologic Activity

Lymphocyte counts reflected lymphopenia during each treatment cycle followed by rebound lymphocytosis (Fig. 2). NK-cell number and specific activity as well as ADCC activity increased after administration of EMD 273066. NK-cell number relative to pretreatment value was elevated by factors of 2.9, 3.3, and 3.2 on days 8, 29, and 36, respectively (Fig. 3). At an effector cell ratio of 25:1 with 10 ng immunocytokine added, NK-cell activity relative to pretreatment value increased by 13%, 47%, and 67% on days 8, 29, and 36, respec-

TABLE 4. Number of Patients with Drug-Related Adverse Events Grade 2 or Above*

	Dosing Group (mg/m ² /day)						
	0.4 (n = 1)	0.7 (n = 1)	1.4 (n = 3)	2.8 (n = 6)	4.3 (n = 3)	6.4 (n = 6)	8.5 (n = 2)
Fever	0	1	3	2	3	6	2
Asthenia	0	0	1	2	1	1	2
Chills	0	0	1	1	2	1	2
Nausea	0	0	0	0	1	1	1
Rash	0	0	1	3	0	1	1
Vomiting	0	0	1	0	1	2	1
Dyspnea	0	0	0	3	0	1	0
Anemia	0	0	0	0	1	0	2
Hypotension	0	0	2	0	1	2	1
Edema	0	0	0	1	0	1	0
Movement disorder	0	0	0	0	0	0	2
Pain	0	0	0	1	1	0	0
Pruritis	0	0	0	2	0	0	0

*Adverse events reported in $\geq 10\%$ of patients across dosing groups and of a severity of Grade 2 or above are listed.

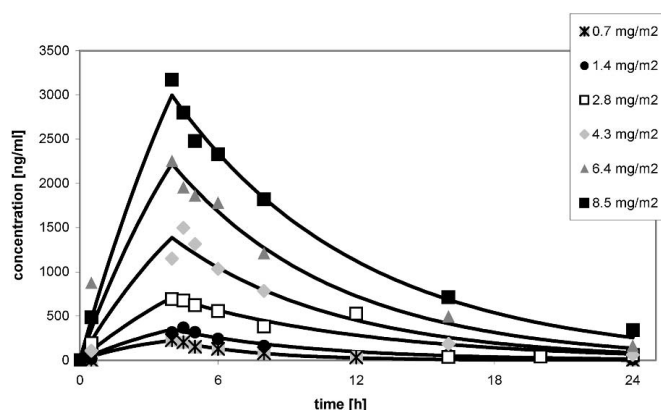


FIGURE 1. Mean concentration–time profiles (symbols) and resulting compartmental fit (lines) for each dosing group on the first day of dosing.

tively. At an effector-cell ratio of 25:1 with 100 ng immunocytokine added, NK-cell activity relative to pretreatment value increased by 2%, 53%, and 152% on days 8, 29, and 36, respectively. Similarly, at an effector-cell ratio of 25:1 with 10 ng immunocytokine added, ADCC activity relative to pretreatment values increased by 23% and 67% on days 8 and 29, respectively. At an effector cell ratio of 25:1 with 100 ng immunocytokine added, ADCC activity relative to pretreatment values increased by 28% and 63%, respectively. No obvious dose-related trends were observed.

DISCUSSION

In this phase I study, the immunocytokine EMD 273066 administered in a 4-hour infusion daily for 3 consecutive days was generally well tolerated up to a dose of 4.3 mg/m²/d. Importantly, systemic biologic activity was observed at doses be-

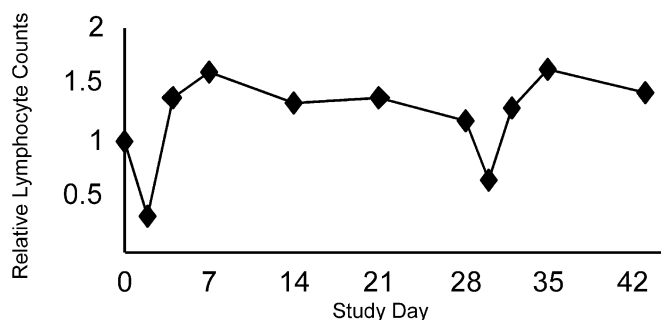


FIGURE 2. Lymphocyte counts relative to baseline. Lymphocyte counts reflected lymphopenia during each treatment cycle, followed by rebound lymphocytosis. Lymphocyte counts for all patients were pooled at each time point regardless of the dose level. Numbers were normalized to the predose value for each patient individually, and then the mean was calculated for all normalized data at each time point.

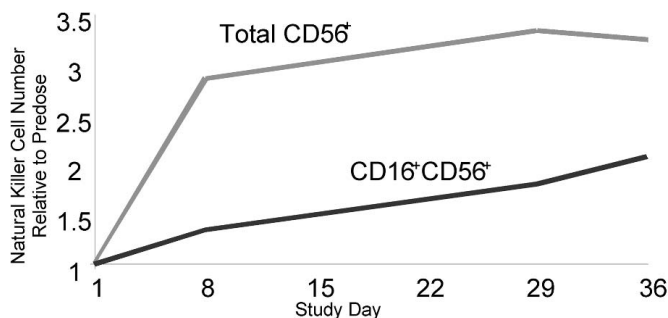


FIGURE 3. NK-cell number relative to predose number was elevated by factors of 2.9, 3.3, and 3.2 on days 8, 29, and 36, respectively. Data are reported as total CD56⁺ cells, and “activated” NK number is taken as the CD16/CD56 double-positive fraction. Numbers were normalized to the predose value for each patient individually, and then the mean was calculated for all normalized data at each time point. The figure includes data from all patients.

low 4.3 mg/m²/d. The MTD was 6.4 mg/m²/d. EMD 273066-associated adverse events and toxicities, such as rigors, chills, anemia, and signs of vascular leak syndrome, occurred predominantly at doses exceeding 4.3 mg/m²/d and are consistent with the established side-effect profile of IL-2 and with the effects of antibodies administered alone.^{13,14}

IL-2 (administered alone, not fused with an antibody) has demonstrable antitumor activity in approximately 15% of patients with renal cell carcinoma and melanoma,^{15,16} but its use is limited by potentially lethal side effects including vascular leak syndrome, nephrotoxicity, and hypotension.^{1,17–21} With EMD 273066, the potential delivery of IL-2 directly to the tumor microenvironment, where it may be most effective at stimulating antitumor immune responses, may provide greater antitumor effect than systemic administration of equipotent doses of IL-2 administered alone. Correspondingly, the optimum dose of IL-2 for antitumor effect when IL-2 is administered as EMD 273066 should be lower than that required when IL-2 is administered alone, a hypothesis that has been supported in murine models.⁴ The tumor-targeting property of EMD 273066 thus could theoretically allow administration of a lower dose of IL-2 with a corresponding reduction in the incidence and severity of IL-2-associated adverse events. Results of animal studies are consistent with the theoretical benefits of the ability of EMD 273066 to deliver IL-2 to the tumor microenvironment.⁴ The implications of this property of the immunocytokine await further evaluation in studies conducted in patients with cancer.

Immunocytokine EMD 273066 demonstrated biologic activity as measured by increases in total lymphocyte counts, NK-cell counts, NK-cell activity, and ADCC activity in the peripheral circulation. The increases generally persisted for 3 to 4 weeks. Lymphocyte counts followed known IL-2 patterns of lymphopenia during treatment followed by rebound lymphocytosis.

phocytosis with each cycle of administration. The magnitude of these effects was consistent with what would be expected with this dose of IL-2. These data showing broad-based immunologic activity of EMD 273066 in patients with prostate cancer are consistent with results of animal studies demonstrating that the antitumor activity of EMD 273066 can be effected via either T-cell-dependent or T-cell-independent mechanisms.^{4,10} The broad-based immunologic activity of EMD 273066 differentiates it from cytokine gene therapy, a T-cell-dependent approach that involves patient-specific, ex vivo genetic modification of autologous tumor cells to express cytokines that are used to generate systemic antitumor immune responses.^{1,2} Cytokine gene therapy is limited in its utility because it must be tailored to the individual patient; additional research may demonstrate the relatively broader utility of immunocytokine therapy.

Antibody responses to the immunocytokine and the FcIL-2 components were detected but did not increase after a second course of treatment in any of the patients. The observation that the same samples were negative for binding to recombinant IL-2 but not for Fc-IL-2 suggests that the antibody may recognize the neoepitope at the antibody-IL-2 junction. No hypersensitivity reactions (including anaphylaxis) were observed after initial or subsequent dosing with EMD 273066. Additional studies with greater numbers of patients are needed to further examine the immunogenicity profile, which could distinguish EMD 273066 from other antibody-based immunotherapies associated with hypersensitivity reactions, including anaphylaxis in 1% to 11% of patients.²²⁻²⁶

The pharmacokinetic findings should be interpreted cautiously because they are based on data from few patients. The preliminary data from this study suggest that dose-dependent C_{max} was achieved, on average, within an hour of the completed infusion and that $t_{1/2}$, which was approximately 5 hours, was not dose dependent. Further study is warranted to extend these pharmacokinetic observations. The $t_{1/2}$ of EMD 273066 is notably longer than that of IL-2 administered alone.²⁷ Although efforts are currently under way to enhance the half-life and therefore, presumably, the bioactivity of IL-2,²⁸ the need for such efforts appears to be obviated with EMD 273066 in view of its relatively long half-life.

In conclusion, this phase I study of the novel immunocytokine EMD 273066 administered to patients with advanced prostate cancer demonstrates that it is well tolerated at doses above a level demonstrating systemic biologic activity. These data support additional clinical exploration of EMD 273066. Clinical studies of EMD 273066 administered alone and in combination with chemotherapeutic agents are being planned.

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