

A Phase I Clinical Trial of the hu14.18-IL2 (EMD 273063) as a Treatment for Children with Refractory or Recurrent Neuroblastoma and Melanoma: a Study of the Children's Oncology Group

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Abstract Purpose: Evaluate the clinical safety, toxicity, immune activation/modulation, and maximal tolerated dose of hu14.18-IL2 (EMD 273063) in pediatric patients with recurrent/refractory neuroblastoma and other GD2-positive solid tumors.

Experimental Design: Twenty-seven pediatric patients with recurrent/refractory neuroblastoma and one with melanoma were treated with a humanized anti-GD2 monoclonal antibody linked to human interleukin 2 (IL-2). Cohorts of patients received hu14.18-IL2, administered i.v. over 4 hours for three consecutive days, at varying doses. Patients with stable disease, partial, or complete responses were eligible to receive up to three additional courses of therapy.

Results: Most of the clinical toxicities were anticipated and similar to those reported with IL-2 and anti-GD2 monoclonal antibody therapy and to those noted in the initial phase I study of hu14.18-IL2 in adults with metastatic melanoma. The maximal tolerated dose was determined to be 12 mg/m²/d, with agent-related dose-limiting toxicities of hypotension, allergic reaction, blurred vision, neutropenia, thrombocytopenia, and leukopenia. Three patients developed dose-limiting toxicity during course 1; seven patients in courses 2 to 4. Two patients required dopamine for hypotension. There were no treatment-related deaths, and all toxicity was reversible. Treatment with hu14.18-IL2 led to immune activation/modulation as evidenced by elevated serum levels of soluble IL-2 receptor α (sIL2R α) and lymphocytosis. The median half-life of hu14.18-IL2 was 3.1 hours. There were no measurable complete or partial responses to hu14.18-IL2 in this study; however, three patients did show evidence of antitumor activity.

Conclusion: Hu14.18-IL2 (EMD 273063) can be administered safely with reversible toxicities in pediatric patients at doses that induce immune activation. A phase II clinical trial of hu14.18-IL2, administered at a dose of 12 mg/m²/d \times 3 days repeated every 28 days, will be done in pediatric patients with recurrent/refractory neuroblastoma.

Neuroblastoma is the second most common solid tumor in childhood. It is responsible for 15% of pediatric deaths due to malignancy. Children with advanced stage disease or those with refractory disease, despite currently available therapies, have a

poor prognosis. Therefore, innovative and novel approaches, such as immunotherapy, are sought.

Interleukin-2 (IL-2) has been used alone and in combination with other therapies in the treatment of malignancies with evidence of occasional antitumor effects (1). There are two mechanisms in which IL-2 treatment can mediate antitumor effects, as suggested by murine models (2). IL-2 treatment augments activation of preexisting antigen-specific T cells to enhance their recognition and destruction of neoplastic tissue. More importantly, IL-2 also activates natural killer (NK) cells (3, 4). A more selective induction of tumor-specific T cells, or localization of activated NK cells to sites of tumor, may provide better tumor specificity and minimize side effects of IL-2 (5).

The development of immunocytokines may provide this localized immune attack with acceptable tumor specificity. Immunocytokines are tumor reactive monoclonal antibodies (mAb) genetically linked to cytokines, such as IL-2. Preclinical studies in selected murine models bearing syngeneic tumors have evaluated the antitumor activity of immunocytokines and determined that immunocytokines can induce potent antitumor effects *in vivo*, exceeding the antitumor activity of comparable amounts of mAb and IL-2 given as separate

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molecules (6). It seems that the immunocytokine molecule localizes to the tumor cell surface due to the recognition by the mAb component of the immunocytokine molecule. This interaction allows the IL-2 receptors on T and NK cells to be activated by the IL-2 component of the immunocytokine molecule. Antibody-dependent cell-mediated cytotoxicity of the tumor cells results when NK cells are further activated through their Fc receptors by the Fc component of the tumor-bound immunocytokine molecule.

The immunocytokine evaluated in this clinical trial is the same molecule we investigated in adult melanoma patients (7). It consists of the humanized 14.18 antibody genetically linked to a molecule of human IL-2. The hu14.18 mAb recognizes the GD2 disialoganglioside, expressed on tumors, such as melanoma, neuroblastoma, and certain sarcomas (8, 9). *In vitro*, anti-GD2 antibodies can mediate substantial antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity against GD2⁺ tumor target cells (9). Clinical trials of the murine 3F8 and 14.G2a anti-GD2 antibodies and of the human-mouse chimeric form of the 14.18 mAb (designated ch14.18) have been done in children with neuroblastoma and adults with melanoma, including trials combining them with IL-2 or granulocyte macrophage colony-stimulating factor to induce NK or neutrophil-mediated, antibody-dependent cell-mediated cytotoxicity, respectively (5, 10–13). Toxicities specifically related to the anti-GD2 mAb included fever and chills, felt to result from cytokine activation. Another known toxicity is neuropathic pain controllable with the administration of narcotics. This pain is felt to be due to the recognition of GD2 on nerve fibers by the mAb (5, 14–16).

Preclinical data with immunocytokines, combined with the clinical experience obtained using murine or chimeric anti-GD2 mAb as therapy, provide the rationale for initiation of clinical testing of immunocytokine molecules as potential cancer therapy. Therefore, the primary objectives of this study were to assess the safety, toxicity, immune activation/modulation, and the maximal tolerated dose (MTD) of hu14.18-IL2 when administered as three daily 4-hour i.v. infusions in pediatric patients with refractory or recurrent neuroblastoma or melanoma.

Patients and Methods

Patients. Eligible patients for this study (Children's Oncology Group Study ADVL0018) included those with biopsy-proven neuroblastoma, or other tumors expressing the GD2 antigen as shown by immunohistochemistry. Patient's disease needed to be considered refractory to chemotherapy or recurrent following multiagent chemotherapy. Patients could have either measurable or evaluable metastatic disease, or they could have no evidence of disease if the patient had a complete response of all recurrent disease following treatment with surgery, radiation therapy, and/or chemotherapy. All patients needed to have adequate bone marrow function (defined by absolute neutrophil count > 1,000/ μ L, platelets \geq 75,000/ μ L, and hemoglobin \geq 9.0 g/dL), adequate liver function (defined by alanine aminotransferase or aspartate aminotransferase \leq 2.5 times normal and a total bilirubin < 1.5 mg/dL), adequate renal function (defined as serum creatinine \leq 1.5 mg/dL or a creatinine clearance or radioisotope GFR of \geq 60 mL/min/1.73 m²), adequate cardiac function (defined by a shortening fraction of \geq 27% by echocardiogram or ejection function of >50% by gated radionuclide study), and adequate respiratory function with no evidence of dyspnea at rest, no exercise intolerance, and a pulse oximetry of >94% on room air. All patients had a performance status of \geq 60% by Karnofsky scale if >10 years of age and \geq 60% by Lansky scale

for children \leq 10 years of age, and a life expectancy of at least 12 weeks. Patients with prior central nervous system metastasis were eligible provided the patients' central nervous system disease had been previously treated and clinically stable, off steroids, for 4 weeks before study enrollment. Patients who had received chemotherapy, radiation therapy, or other immunosuppressive/immunostimulatory therapy within 3 weeks of study entry were excluded. Treatment with retinoic acid, growth factor, or other immunomodulatory therapy needed to be completed at least 7 days before study entry. Patients who previously received *in vivo* mAbs for biological therapy or tumor imaging were excluded, unless there was serologic evidence documenting the absence of detectable antibody to hu14.18. Written consent/assent was obtained from all patients and/or their parents or legal guardians.

Hu14.18-IL2 immunocytokine. The hu14.18-IL2 immunocytokine (EMD 273063) was provided by EMD Lexigen Research Center (Billerica, MA). Preclinical evaluation has shown that 1 mg of the fusion protein contains $\sim 3 \times 10^6$ IU of IL-2 (based on a proliferative assay with IL-2 responsive Tf-1 β cells) and ~ 0.8 mg of the hu14.18 mAb (17).⁹

Study design. This phase I clinical trial [clinical trial registry number (NCT00003750) assigned by <http://www.clinicaltrials.gov>] was designed as an open-label, nonrandomized study. There were seven dose levels (2, 4, 6, 8, 10, 12, and 14.4 mg/m²/d) evaluated. Patients were enrolled in cohorts of 3. Hu14.18-IL2 was administered on an inpatient basis as a 4-hour i.v. infusion over three consecutive days, based on preclinical testing. Patients were discharged from the hospital, if clinically stable, 24 hours following completion of the third infusion. Adverse events and toxicities were graded as per National Cancer Institute Common Toxicity Criteria (version 2.0). Dose-limiting toxicity (DLT) was defined as any grade 3 or 4 toxicity using the above stated toxicity criteria with certain exceptions to this definition based on known rapidly reversible side effects of systemic IL-2 and ch14.18 chimeric antibody. Therefore, to accurately grade toxicity and determine the clinical meaningfulness of the MTD, there were several transient toxicities associated with IL-2 or ch14.18 that were not considered dose limiting for the purpose of drug discontinuation or DLT/MTD determination in this study. These exceptions included but were not limited to grade 3 pain requiring i.v. narcotics, fever lasting <6 hours and controllable with antipyretics, hypotension that resolves within 48 hours after completion of immunocytokine, capillary leak, allergic reactions readily controlled with supportive antiallergic (nonsteroidal) treatments, and hematologic, renal, hepatic, or metabolic abnormalities reversing within 48 hours.

Patients who experienced a DLT had their treatment with hu14.18-IL2 stopped and if toxicity resolved were allowed to resume treatment at 50% of the dose that caused the toxicity. Patients with DLT were taken off study if these toxicities did not recover to grade \leq 2 within 2 weeks or grade <2 after 4 weeks. Disease status was assessed following each course of treatment. Patients with stabilization of disease or regression of disease (partial or complete clinical response) and recovery of any toxicity to grade \leq 1 were eligible for additional courses of treatment, starting on day 29 of the previous course. Patients with progressive disease (defined as an increase in measurement of at least 20%, or the appearance of a new lesion) were removed from the study. Patients with no evaluable disease or those with no evidence of measurable disease were eligible to receive four courses of therapy. Eligible patients were given a maximum of four courses of treatment. The MTD was defined as the dose level at which 0 of 6 or 1 of 6 patients experienced a DLT with at least 2 of 3 or 2 of 6 patients encountering DLT at the next higher dose.

Clinical laboratory monitoring. Clinical hematology and chemistry evaluations were done by standard methodology at each participating institution. Blood samples were obtained in the morning of the designated protocol day, before immunocytokine administration.

⁹ J.A. Hank, unpublished data.

Immunologic monitoring. Serum samples were obtained before hu14.18-IL2 treatment, as well as at the end of immunocytokine infusion and 30 minutes, 1, 2, 4, 12, and 24 hours following the completion of immunocytokine infusion on days 1 and 3 of each course. Serum was also collected on days 8, 15, and 22 of each course and day 29 of the last course. Once drawn, the serum was immediately placed on ice and kept refrigerated until it could be frozen at -20°C .

ELISA methods for detection of sIL2R α and immunocytokine levels. SIL-2R α levels were measured by a commercial (Immunotech, Marseilles, France) double-mAb ELISA kit, according to the manufacturer's specifications. Measurement of immunocytokine levels in patients' sera by ELISA was done as previously described (18, 19). ELISA plates were coated with 1A7, an anti-idiotypic antibody to hu14.18. Diluted serum samples were then incubated overnight at 4°C . Plates were then washed and incubated with biotinylated goat anti-human IL-2 antibody (R&D Systems, Minneapolis, MN) followed by ExtrAvidin-AP (Sigma, St. Louis, MO). A standard curve was constructed based on dilutions of lot no. 31,916 of hu14.18-IL2 as previously reported (7).

Anti Fc-IL2 antibody detection by ELISA. C8 Maxisorp microtiter plates (Nunc, Neerijse, Belgium) were coated overnight at 4°C with 120 μL of 2 $\mu\text{g}/\text{mL}$ neutralizing rat monoclonal anti-human IL-2 antibody MQ1-17H12 (BD PharMingen, San Diego, CA) in buffer containing 7.5 mmol/L Na_2CO_3 , 17.4 mmol/L NaHCO_3 , and 1.5 mmol/L NaN_3 at pH 9.6. Plates were washed thrice with 0.05% Tween 20/PBS, blocked for 3 hours at room temperature with 5% nonfat dried milk/PBS, and then washed thrice with 0.05% Tween/PBS. Serum samples were diluted 1:5 with a solution of 5 ng/mL biotinylated hu14.18 Fc-IL2 fragment (Fc-IL2, a gift from Stephen Gillies, EMD Lexigen Research Center) in 2% milk/PBS and added to plates at 100 $\mu\text{L}/\text{well}$. Plates were washed five times with Tris/Tween and alkaline phosphatase-conjugated ExtrAvidin (Sigma) diluted 1:70,000 in Tris/Tween was added at 100 $\mu\text{L}/\text{well}$ and incubated for 1 hour on a horizontal shaker at room temperature. Plates were washed five times, and 100 $\mu\text{L}/\text{well}$ of 1 mg/mL p-NPP substrate (Sigma) in a solution containing 0.5 mol/L diethanolamine/PBS was incubated for ~ 45 minutes at room temperature on a horizontal shaker in the dark. A standard curve was constructed with dilutions of biotinylated Fc-IL2 containing 20% normal human serum. Results were determined by measuring the absorbance at 405 nm and calculating the concentration of detectable Fc-IL2. For serum samples from each patient, results are presented as "% inhibition," where the amount of Fc-IL2 detected in pretreatment serum is defined as 0% inhibition for that patient.

Anti-idiotypic antibody detection by ELISA. C8 Maxisorp microtiter plates (Nunc) were coated overnight at 4°C with 120 μL of 2 $\mu\text{g}/\text{mL}$ 1A7 (Titan Pharmaceuticals, Scottsdale, AZ) in buffer containing 7.5 mmol/L Na_2CO_3 , 17.4 mmol/L NaHCO_3 , and 1.5 mmol/L NaN_3 at pH 9.6. Plates were washed thrice with 0.05% Tween 20/PBS, blocked for 3 hours at room temperature with 5% nonfat dried milk/PBS, and then washed thrice with 0.05% Tween/PBS. Serum samples were diluted 1:5 with a solution of 3.1 ng/mL biotinylated hu14.18 (hu14.18 mAb was a gift from Stephen Gillies) in 2% Milk/PBS and added to plates at 100 $\mu\text{L}/\text{well}$. Plates were washed five times, and alkaline phosphatase-conjugated ExtrAvidin (Sigma) diluted 1:70,000 in Tris/Tween was added at 100 $\mu\text{L}/\text{well}$ and incubated for 1 hour on a horizontal shaker at room temperature. Plates were washed five times, and 100 $\mu\text{L}/\text{well}$ of 1 mg/mL p-NPP substrate (Sigma) in a solution containing 0.5 mol/L diethanolamine/PBS was incubated for ~ 45 minutes at room temperature on a horizontal shaker in the dark. A standard curve was constructed with dilutions of biotinylated hu14.18 containing 20% normal human serum. Results were determined by measuring the absorbance at 405 nm and calculating the concentration of detectable hu14.18 and are presented as "% inhibition," as for the antiFc-IL2 assay above.

Statistical methods. Pharmacokinetic variables were summarized using descriptive statistics on hu14.18-IL2 levels in serum samples

obtained before and after the hu14.18-IL2 infusions on days 1 and 3 of each treatment course. The area under the curve from time 0 to 24 hours was calculated with the trapezoidal method. The terminal half-life was determined by linear regression analysis of time versus the log-transformed concentration. Jonckheere-Terpstra trend test was done to evaluate the association between increasing dose level and each of the pharmacokinetic variables such as area under the curve (AUC) and peak concentration. A Spearman's rank correlation analysis was also done to determine the relationship between the actual dose administered and the pharmacokinetic variable. Dose proportionality of AUC and peak concentration was assessed by ANOVA and by the power model (20). Differences of pharmacokinetic variables between days 1 and 3 were evaluated for each course using nonparametric Wilcoxon signed rank tests. The association between immunologic variables and dose was examined by Spearman's rank correlation analysis and linear regression analysis. All statistical analyses were done with SAS software (version 8.2, SAS Institute, Inc., Cary, NC). All *P*s are two sided and were not adjusted for the number of variables evaluated. As such, they should only be interpreted as exploratory.

Results

Patient characteristics. A total of 28 patients were enrolled, of whom 27 were fully evaluable for toxicity, and all 28 were evaluable for response. Pretreatment characteristics are outlined in Table 1. Twenty-seven of the enrolled patients had neuroblastoma, and one patient had melanoma. There were 13 (46.4%) male and 15 (53.5%) female patients. The median age of the 28 patients at the time of study entry was 6.63 years (range, 2.5-17.7 years), and all had performance status scores by Lansky or Karnofsky of ≥ 90 . Prior therapies included surgery (23 patients), biological therapy (4 patients), radiotherapy (24 patients), and chemotherapy (28 patients). Eighteen patients had prior autologous bone marrow transplant. All patients had metastatic, stage IV disease at the time of study entry, and the mean time between diagnosis and study entry was 1.7 years. The dose escalation schedule is shown in Table 2. There were four patients treated at dose level 4 (8 mg/m²/d) because one patient was given dexamethasone for grade 2 allergic symptoms, making this patient ineligible for further toxicity assessment. This patient, during the first day of infusion, developed hives and was treated with 10 mg/m² dexamethasone as opposed to benadryl as directed by the protocol. This required an additional patient be enrolled at that dose level to adequately evaluate toxicity.

Toxicity. Clinical toxicities grade ≥ 2 are detailed in Table 3. Twenty-six (93%) patients had grade 2 or 3 fevers. Eighteen (64%) patients had grade 2 or 3 pain (rectal, pelvic, myalgia, neuropathic, abdominal, arthralgia, chest, bone, and headache). Seventeen patients (61%) experienced grade ≥ 2 hypotension. Of the 17 patients with hypotension, three were grade 3 and one was grade 4 (Table 4). The latter patient (8 mg/m²/d) had two separate episodes of hypotension in courses 2 (grade 3) and 4 (grade 4). Two patients required dopamine to support systemic blood pressure, and the remaining two responded to i.v. fluids.

Toxicities determined by clinical lab evaluation as grade ≥ 2 are listed in Table 5. The majority of patients (68%) had grade 2 or 3 anemia. Eight patients (29%) had grade 2 or 3 neutropenia, and three patients had grade 4. Ten patients (36%) had thrombocytopenia. The majority of these were grade 2 or 3 with one grade 4 toxicity. Six patients (21%) experienced grade 3 and 4 lymphopenia, a known immune effect of IL-2.

Table 1. Patient demographics

Total no. subjects	28
Median age (y), (range)	6.63 (2.5-17.7)
Sex	
Male	13
Female	15
Prior therapy*	
Surgery	23
Biologic	4
Radiation	24
Chemotherapy	28
Performance status	
Mean Lansky score	92.4
Mean Karnofsky score	90
Time from diagnosis to immunocytokine therapy	
Mean (y)	1.7
Stage of disease for all patients	IV

*No prior therapy data for two patients.

Although the MTD was determined by DLT in course one only, DLT was evaluated in each course of therapy for each patient. If a patient experienced a DLT in any course, if eligible for further therapy, there was a dose reduction for future courses. During course 1, one patient required 50% dose reduction on day 2 of infusion and omission of day 3 due to an anaphylactic reaction. This patient was assigned to the 12 mg/m²/d dose level at study entry and only received one course of therapy. Six patients (30% of patients completing two courses of therapy) required dose modifications during course 2. One patient at 2 mg/m²/d received 50% dose reduction for course 2 and the subsequent two courses due to neutropenia. One patient assigned to 8 mg/m²/d (patient 13 in Table 4) required 50% dose reduction on day 3 of infusion and subsequent courses due to hypotension. One patient experienced blurred vision during course 2 at a dose level of 12 mg/m²/d requiring a 50% dose reduction for course 3. Another patient at 12 mg/m²/d required omission of the infusion on day 3, course 2 and subsequent dose reduction of 50% for course 3 due to hypotension. One patient who entered the study at 12 mg/m²/d required a 50% dose reduction for course 3 due to a hand and foot rash reaction that occurred during course 2. One patient who entered the study at 14.4 mg/m²/d required a 50% dose reduction for course 2 due to neutropenia and did not complete a third course of therapy. Two patients (13% of patients completing three courses of immunocytokine) required dose modifications during course 3. One patient experienced hemorrhagic cystitis on day 2 of course 3 (likely related to prior cyclophosphamide treatment) at 2 mg/m²/d necessitating a 50% dose reduction for the fourth course. The same patient who had the hand and foot rash, also became hypotensive during course 3 at a dose of 6 mg/m²/d, requiring further dose reduction by 50% on day 3 of course 3. All of these toxicities were reversible upon completion of protocol therapy.

MTD determination in the first course of treatment. The MTD for this study was defined in the protocol as the highest dose

level at which no more than two of six treated patients had DLT in the first course of treatment. All episodes of DLT are shown in Table 6. Three additional patients were treated at 2 mg/m²/d because one patient had neutropenia. Dose escalation proceeded for each of the next five dose levels; three evaluable patients were treated in each without DLT during course 1. This included the first three patients in course 1 at the dose of 12 mg/m²/d. One of three had DLT (neutropenia and leukopenia) at 14.4 mg/m²/d. Traditionally, three more patients would have been enrolled at 14.4 mg/m²/d as course 1 was tolerated well by two of three patients at that dose level. However, some patients were showing IL-2-related hypotension in courses 2 to 4 at lower doses (Table 6). Of the four patients with grade 3 or 4 hypotension, three of these developed hypotension only in the latter courses (Table 4). This hypotension in courses 2 to 4, while significant, did not meet DLT criteria for the MTD determination since it occurred after course 1. Thus, the decision was made to enroll the next three patients at 12 mg/m²/d to further evaluate late toxicities with hu14.18-IL2 at this lower dose. One of these three patients had a DLT during the first course. This was an anaphylactic reaction requiring epinephrine, benadryl, dexamethasone, nebulized albuterol, and supplemental oxygen. This patient had no history of prior biologic/mAb therapy before enrollment onto this study. Thus, five of six patients tolerated the first course of therapy at this dose. Even so, one of these five patients did have significant DLT of hypotension during course 2. Therefore, 12 mg/m²/d was determined to be the appropriate phase II dose, and it was designated as the MTD for this study acknowledging that the protocol defined MTD of this agent may not have been reached in the phase I study. No additional patients were entered.

Clinical outcome. A total of 28 patients were treated on this study. Fifty-four percent (15 of 28) had stable disease for two or more courses of hu14.18-IL2 therapy. Those patients with stable disease following four courses of therapy with hu14.18-IL2 went on to receive a variety of therapeutic interventions following completion of immunocytokine treatment. With a median follow-up of 20 months, 57% (16 of 28) of all patients enrolled on this study were deceased. All deaths were related to his/her disease. There were no deaths reported while on protocol therapy or within one month following completion of protocol therapy.

Table 2. Dose escalation schedule

Dose level	mg/m ² /d	No. patients in each course			
		Course 1	Course 2	Course 3	Course 4
1	2	6	4	4	4*
2	4	3	2	2	2
3	6	3	2	1	1
4	8	4	3	3	2
5	10	3	3	2	2
6	12	6	3	3	0
7	14.4	3	3	0	0

*One patient at this dose received six courses.

Table 3. Clinical toxicities observed with hu14.18-IL2

Dose (mg/m ² /d)	n*	No. courses [†]	Number of patients with the highest toxicity observed in all courses of therapy [‡]																							
			Fever grade [‡]			Rigors grade			Hypotension grade			Hypoxia grade			Pruritus grade			Pain grade			Fatigue grade					
			2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4			
2	6	20	2	4		1			1											2	1					
4	3	9	1	2		2			2											2						
6	3	7	3						2											1						
8	4	12	2	2		3			2	1										1	2					
10	3	10	1	2		1			3											2						
12	6	12	3	1		1			1	2									1	2	4		1			
14.4	3	6	1	2		1			2	1			1							1			1			

NOTE: Clinical toxicities graded 1 to 4 as per National Cancer Institute Common Toxicity Criteria, version 2.0.

*Total number of patients treated at each dose level.

†Total number of courses administered for all patients at the indicated dose level.

‡For each category of toxicity, each patient was scored based on their highest grade of toxicity for each course of treatment. For each dose level of hu14.18-IL2, the number of patients showing each grade of toxicity as their highest grade is shown.

Although this phase I study was not designed to evaluate overall tumor response to therapy, all patients were monitored for antitumor activity of hu14.18-IL2. There were no measurable complete or partial responses; however, one patient did show evidence of antitumor activity while receiving immunocytokine.

This patient was an 8-year-old female who at the time of study entry had been heavily pretreated with systemic chemotherapy, radiation, and surgery and had undergone prior stem cell transplant with residual disease shown at multiple sites, seen only by MIBG scan. At the conclusion of 5 months of 13-*cis*-retinoic acid treatment, this patient continued to have multiple sites of disease detectable only by MIBG. Because of persistent MIBG positive disease, she then received six cycles of Fenretinide (800 mg/m² daily × 7 days, followed by 14 days of rest) with minimal change in disease status, as detected by MIBG. She then enrolled in this study and received the allowed 4 courses of hu14.18-IL2 at the lowest dose level (2 mg/m²/d) with persistent MIBG detectable disease noted at the same multiple sites following the fourth course of treatment. One month after completing immunocytokine therapy, this patient was restarted on fenretinide at 800 mg/m² daily × 7 days. One week after restarting the fenretinide, an MIBG scan was initially read as normal and reevaluated to show a minimal single parietal skull lesion, consistent with improvement. Based on the possibility that this may have been a delayed clinical response related to the recently completed hu14.18-IL2, approval was granted for two more cycles of hu14.18-IL2

at the same 2 mg/m²/d dose. At the conclusion of the sixth course of hu14.18-IL2, radiographic evidence of disease remained essentially unchanged. This patient was then restarted on fenretinide 1 week following the aforementioned MIBG scan. Repeat imaging 3 months later showed no evidence of disease. This patient has had several MIBG studies done with no radiographic evidence of disease at this time, now 2.5 years after completing hu14.18-IL2 therapy. It should be noted, however, that the disappearance of MIBG is possible in patients with neuroblastoma even without intervention. Late disappearance of MIBG can be observed due to loss of MIBG avidity by the tumor, as well as technical factors that can modify MIBG uptake (21). At the time of study entry, this patient's bone marrow showed one neuroblastoma cell per 100,000 cells by immunocytochemistry. At the conclusion of four courses of hu14.18-IL2, this patient's bone marrow had three neuroblastoma cells per 100,000 cells with occasional ganglioneuroma cells noted. This would suggest stability of bone marrow disease based on immunocytochemistry after four courses of immunocytokine therapy. Repeat bone marrow since then have shown occasional ganglioneuroma cells, of uncertain significance, and a repeat immunocytochemistry assay detected no neuroblastoma.

In addition, two patients with bulky disease as well as metastatic sites, including the bone marrow, showed evidence of decreasing bone marrow disease. One was detected by repeated bone marrow histology, and one was determined by a

Table 4. Characteristics of patients with grade 3 or 4 hypotension

Patient ID no.	Dose (mg/m ² /d)	Grade of toxicity	Course/day (details)	Treatment required
13	8	3	2/3 (before infusion)	i.v. fluids
13	8	4	4/3 (30 min into infusion)	i.v. fluids and dopamine × 24 h
22	12	3	2/2 (1 h after infusion complete)	i.v. fluids and dopamine × 48 h
28	12	3	3/2	i.v. fluids and albumin infusion
23	14.4	3	1/2 (30 min after infusion)	i.v. fluids

Table 7. Pharmacokinetic variables for course 1

Dose (mg/m ² /d)	n	Peak concentration (µg/mL)*†		Clearance (L/h)		Half-life (h)		AUC (µg/mL·h)*	
		Median	Range	Median	Range	Median	Range	Median	Range
Day 1									
2	6	0.83	0.37-1.30	0.45	0.25-1.07	2.43	1.13-5.15	4.66	1.37-11.0
4	3	1.84	1.56-2.17	0.26	0.25-0.35	4.05	3.41-4.47	11.3	10.9-12.1
6	3	1.91	1.08-3.24	0.41	0.40-3.42	2.88	1.87-4.29	10.4	4.42-25.6
8	4	3.33	2.09-4.62	0.38	0.24-0.61	3.44	3.07-3.57	22.3	15.2-28.8
10	3	3.20	3.06-3.39	0.35	0.32-0.44	2.88	2.86-4.28	20.3	17.2-24.1
12	6	6.22	3.67-11.6	0.38	0.16-0.49	3.19	2.45-3.46	41.6	16.1-67.0
14.4	3	6.06	4.86-8.30	0.47	0.31-0.51	2.99	2.38-3.03	29.0	26.8-44.3
Day 3									
2	6	0.80	0.38-1.17	0.69	0.37-0.95	1.84	0.89-3.38	3.46	1.54-7.49
4	3	1.88	0.71-2.27	0.33	0.25-0.83	2.21	2.10-2.25	11.3	3.40-12.7
6	3	1.33	1.31-2.51	0.55	0.47-2.26	3.09	1.59-3.43	7.61	6.68-22.5
8	4	2.05	1.28-3.26	0.71	0.45-1.28	2.17	1.95-2.23	10.2	8.12-16.4
10	3	3.50	2.32-7.66	0.44	0.20-0.58	1.98	1.87-2.48	20.1	10.6-39.7
12	5	3.79	2.63-4.88	0.72	0.46-1.33	1.96	1.74-2.08	20.1	8.19-23.8
14.4	3	6.40	3.99-9.66	0.45	0.34-0.66	2.18	1.98-2.36	30.7	22.6-36.9

*Peak concentration, clearance, half-life, and AUC were obtained at course 1, day 1; Values shown are median and range for all patients at each dose level.

† The peak concentration and AUC were both dose dependent ($P < 0.001$).

reported similar patterns of blood lymphocyte counts when immunocytokine was used to treat adults with metastatic melanoma (7). The noted lymphopenia followed by lymphocytosis was shown in all courses of immunocytokine treatment; however, in courses 2 to 4, the differences were not statistically significant. This may be due to fewer numbers of patients in those courses resulting in less statistical power. There was a relationship between the dose of hu14.18-IL2 administered and the increase in lymphocyte count from baseline (day 0) to day 8 in course 1 ($P = 0.002$, Fig. 2). There was also a dose effect noted in course 2 ($P = 0.006$). When comparing lymphocyte counts with peak concentration of hu14.18-IL2, there is a positive correlation between the change in lymphocyte counts from days 0 to 8 compared with peak immunocytokine concentrations on days 1 and 3 ($P = 0.001$ and $P = 0.0006$, respectively; Fig. 3).

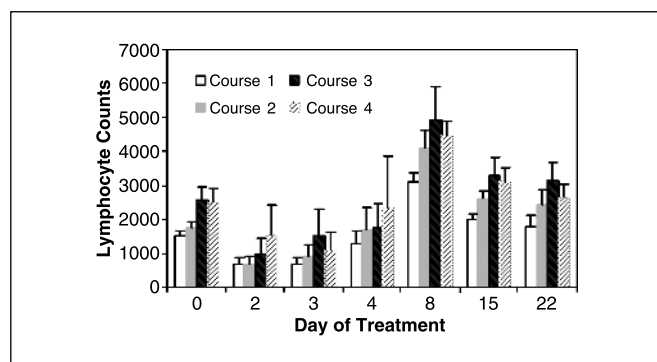


Fig. 1. Lymphocyte counts. Columns, mean lymphocyte counts of all 28 patients in all four courses of immunocytokine therapy based on blood samples before treatment on the indicated days; bars, SE.

Immune activation/modulation was also determined based on soluble IL-2 receptor (sIL2R) levels in patient serum (Fig. 4). There is evidence to suggest immune activation after immunocytokine infusion as the level of sIL2R was significantly lower on day 1, course 1, compared with days 2 to 8 ($P < 0.001$). Although the baseline level of sIL2R of courses 1 and 2 seem to be similar, the level on day 1, course 2 is higher than day 1, course 1 ($P = 0.02$), indicating a statistically significant higher baseline level of sIL2R for course 2 compared with course 1, due to the immune activation induced in course 1. Similarly, the sIL2R AUC values for courses 2, 3, and 4 were statistically greater than that of course 1 ($P = 0.002$, 0.04, and 0.06, respectively) with an overall course effect showing higher sIL2R AUC values with subsequent courses ($P = 0.008$). For courses 1

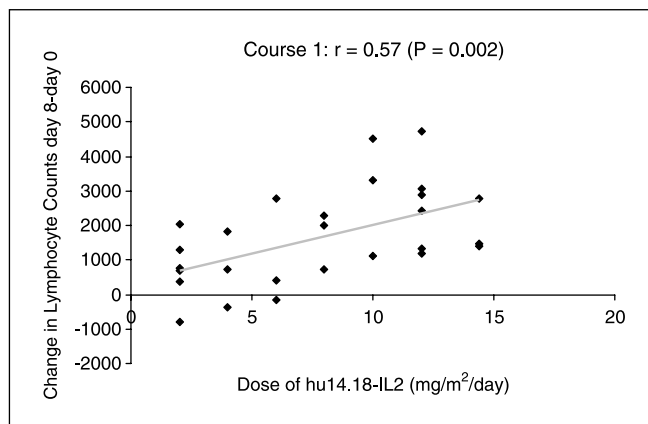


Fig. 2. Correlation of boost in lymphocyte counts on day 8 (lymphocyte count on day 8 lymphocyte count on day 0) of course 1 and dose level. The boost in lymphocyte count on day 8 shows a positive correlation with the dose of immunocytokine given ($P = 0.002$).

and 2, the sIL2R values also increased with increasing dose of hu14.18-IL2 administered ($P = 0.002$). The sIL2R AUC values in course 1 correlated directly with the peak level of hu14.18-IL2 in the serum on day 1 of course 1 ($P = 0.009$; Fig. 5). Therefore, a higher sIL2R AUC was associated with higher maximum concentrations of immunocytokine.

Antibody responses against the hu14.18-IL2 immunocytokine. Serum samples from multiple times for each course were evaluated at a low dilution factor of 1:5 for the development of antibody directed at hu14.18-IL2. Based on the structure of the immunocytokine, patients could generate antibody against the idiotype of the immunocytokine, designated anti-idiotype antibody, or against the Fc-IL2 portion of the immunocytokine, designated as anti-Fc-IL2 antibodies. The anti-idiotype antibody and anti-Fc-IL2 antibody were detected in inhibition ELISAs, where the anti-idiotype in the patient serum inhibited the hu14.18 antibody from binding to an ELISA plate coated with a murine monoclonal anti-idiotypic antibody with specificity for the 14.18 idiotype; the anti-Fc-IL2 in patient serum inhibited the Fc-IL2 fragment of the immunocytokine from binding to a plate coated with rat anti-human IL-2 antibody (see Patients and Methods). Inhibition of >29% was considered to be positive (+; based on variability of control specimens) for both the anti-idiotypic and anti-Fc-IL2 antibody responses. A (++) response was defined as >50%

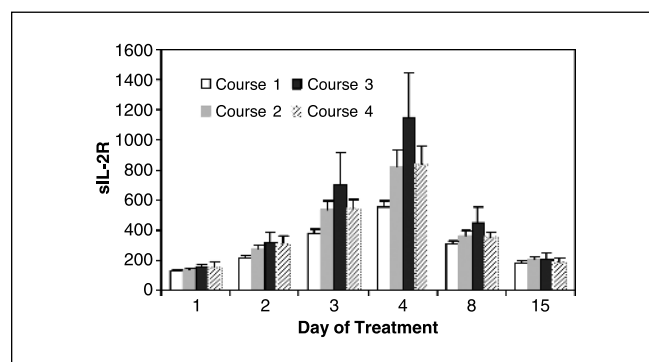


Fig. 4. sIL2R levels on various days of each course of treatment. Columns, average serum sIL2R levels obtained for serum samples from all patients for each of the designated courses; bars, SE.

and 40% inhibition in the anti-idiotypic and anti-Fc-IL2 assays, respectively. In this study, >60% of all patients showed evidence of the development of a (++) anti-idiotype antibody in response to immunocytokine therapy. Similarly, ~50% of these patients developed a (++) anti-Fc-IL2 antibody response (Table 8), as defined by inhibition of binding to a rat anti-human IL-2 antibody. Furthermore, the incidence of (++) anti-idiotype and anti-Fc-IL2 antibody responses seemed to increase with subsequent courses of treatment. The functional and clinical significance of these anti-immunocytokine antibodies is beyond the scope of this article and will be the subject of a separate report.¹⁰

Discussion

The hu14.18-IL2 immunocytokine molecule binds well to human melanoma and neuroblastoma cells *in vitro* and mediates potent antibody-dependent cell-mediated cytotoxicity against these cells *in vitro* using human NK cells as effectors (17, 24). It causes superior antitumor effects than comparable amounts of 14.18 mAb combined with IL-2 when administered daily for 3 to 5 days in immunocompetent mice bearing localized or metastatic B78 murine melanoma or NXS2 murine neuroblastoma (25, 26) and mediates potent NK-dependent antitumor effects in T cell-deficient mice bearing NXS2 (25).

We report the initial clinical trial of hu14.18-IL2 in pediatric patients with recurrent or refractory neuroblastoma ($n = 27$) or melanoma ($n = 1$). As all but one of the patients in this study had neuroblastoma, the reported data may not be representative of children with melanoma. The MTD definition for this trial of hu14.18-IL2 was written into the protocol using standard MTD definition for the first treatment course. However, in determining the MTD for the completed study, a more cautious approach was used to ensure safety because of some toxicities developing in later courses. Thus, the MTD (27) and appropriate phase II dose was determined to be 12 mg/m²/d, acknowledging that the protocol defined MTD of this agent may not have been reached in this phase I study. The MTD for this pediatric study is higher than the MTD determined for this same agent in adults with melanoma (7.5 mg/m²/d; ref. 7). This has also been the case for the MTD of

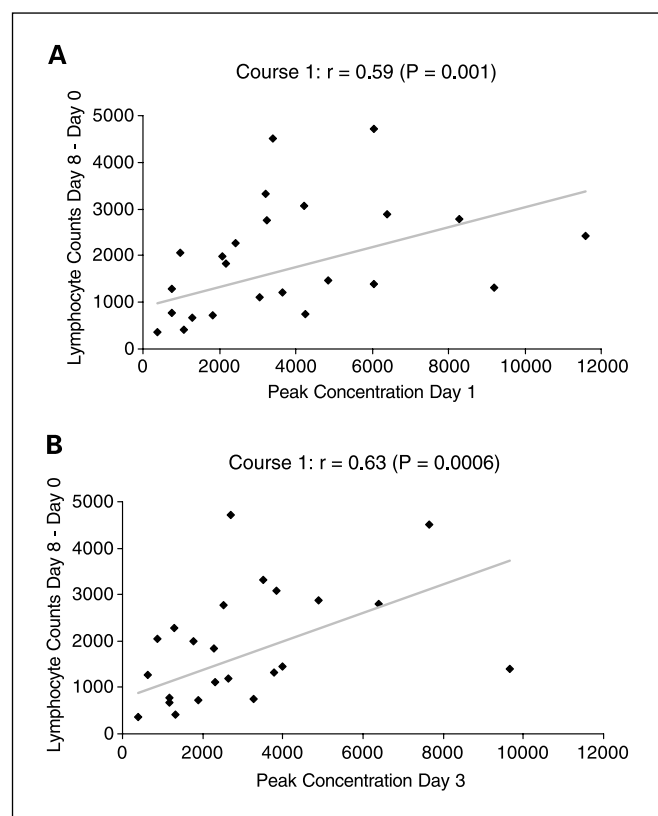


Fig. 3. Correlation of peak concentration of hu14.18-IL2 detected on day 1 and on day 3 with the boost in lymphocyte count detected on day 8 (value for day 8 value for day 0) for course 1. Spearman's rank correlation analysis was done to correlate a change from baseline to day 8 lymphocyte counts versus peak concentration of hu14.18-IL2 on day 1 or 3. There is a significant positive correlation between these variables with $P = 0.001$ for hu14.18-IL2 levels on day 1 (A) and $P = 0.0006$ for the hu14.18-IL2 level on day 3 (B).

¹⁰ Hank et al., in preparation.

anti-GD2 mAb combined with IL-2 when tested in neuroblastoma versus adult melanoma patients (10, 28) and might reflect the greater level of prior treatment with immunosuppressive chemotherapy received by the children with advanced neuroblastoma. In this phase I study, DLTs included transient hypotension, allergic reactions, and hematologic toxicity. The allergic reactions occurred in patients who had not received prior treatment with mAb. Two other patients had previously received treatment with mAb, and neither showed allergic reactions while receiving hu14.18-IL2 in this study. This agent can be given safely as a 4-hour continuous infusion for three consecutive days and can induce immune activation with acceptable toxicity. Most of the clinical toxicities noted in this study were anticipated and similar to those reported previously with IL-2 and anti-GD2 mAb therapy (1, 7, 11, 12). The majority of patients reported mild to moderate pain with hu14.18-IL2 treatment. This pain was similar to that of patients treated with ch14.18 mAb (14–16). Although the majority of patients experienced pain, it was adequately controlled with i.v. morphine and was not dose limiting. There was no evidence of motor neuropathy associated with hu14.18-IL2. All reported toxicities were temporary and resolved completely.

Immune activation/modulation was monitored by quantifying sIL2R levels and lymphocyte responses to immunocytokine. The level of serum sIL2R increases following hu14.18-IL2 therapy. There is a noted dose effect with sIL2 levels, and the AUC correlates with peak concentration of immunocytokine. Similarly, there was evidence of immune activation/modulation as evidenced by changes in lymphocyte counts. There was initially a significant fall in lymphocyte count followed by lymphocytosis in nearly every patient treated with immunocytokine.

Although there were no complete or partial responses, one patient seemed to show evidence of antitumor activity with immunocytokine treatment and remains stable and free of neuroblastoma after 2.5 years. It is unclear whether the normalization of MIBG in this patient is due to hu14.18-IL2, fenretinide, or the combination of these drugs as this patient was receiving both drugs very close together.

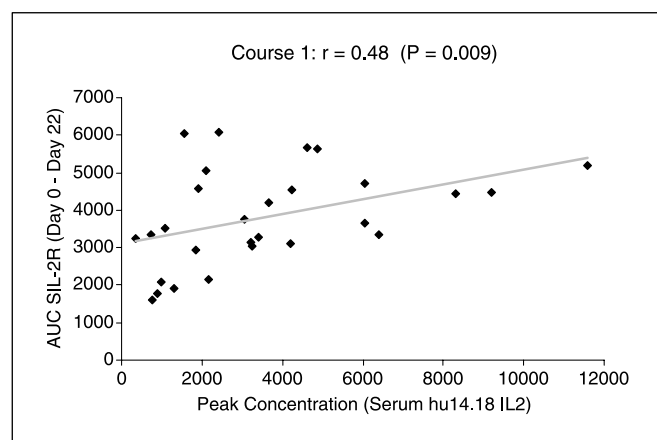


Fig. 5. Correlation between sIL2 receptor levels and peak concentration of immunocytokine. AUC of serum sIL2R levels measured from days 0 to 22, during course 1 was computed using the trapezoid rule for each subject. Spearman's rank correlation coefficient was calculated between sIL2r AUC and peak concentration of immunocytokine for day 1 of course 1. There is a positive correlation between these two variables ($P = 0.009$). Therefore, a higher sIL2r AUC is associated with higher peak concentrations of hu14.18-IL2.

Table 8. Percentage of patients demonstrating antibody response to hu14.18-IL2 following each course

Course*	n	Percentage of patients showing anti-immunocytokine antibody					
		Anti-idiotype			Anti-Fc		
		–	+	++	–	+	++
1	28	39	36	25	50	18	32
2	20	30	20	50	50	15	35
3	15	40	7	53	47	7	47
4	11	27	9	64	36	9	55

NOTE: –, <29% inhibition for both assays; +, 29% to 50% inhibition for anti-idiotype and 29% to 40% inhibition for anti-Fc-IL2; ++, >50% inhibition for anti-idiotype and >40% inhibition for anti-Fc-IL2.

*Serum specimen obtained after each designated course.

As a phase I clinical trial, the main objective of this study was the evaluation of safety, toxicity, immune activation/modulation, and MTD determination. Murine models strongly show that the most potent effects of hu14.18-IL2 are seen in the setting of minimal residual disease (29, 30). The phase II trial to be done by the Children's Oncology Group will address this in one of the three cohorts of eligible patients with neuroblastoma.

ELISA screening systems were used to evaluate the immune response patients made against the hu14.18-IL2 antibody itself. The majority of patients did make a detectable antibody response against the idiotype and the Fc-IL2 components of hu14.18-IL2 when serum was tested at a low (1:5 dilution). A similar anti-immunocytokine response was seen in our prior trial of hu14.18-IL2 in adults with melanoma (7). Similar anti-Fc-IL2 responses were also seen, using a separate screening assay, in the initial phase I trial of a distinct immunocytokine (KS-IL2), that has linked IL-2 to a humanized mAb that recognizes the epithelial cell adhesion molecule (EpCAM) on human epithelial tumors (31). Preliminary data indicate that the anti-Fc-IL2 antibody response in these patients is directed against a neoantigen formed by the linkage of IL-2 to the hu14.18 mAb and not to either the hu14.18- mAb or to IL-2 itself.¹¹ The functional and clinical significance of these anti-immunocytokine antibodies, especially at this low dilution of the patients' sera, is beyond the scope of this article and will be the subject of a separate report.¹⁰

As a result of this trial, the MTD of 12 mg/m²/d is suggested for phase II testing and will require clear guidelines for cessation of therapy and dose adjustment for episodes of DLT. Two separate phase II trials are now under way to determine the antitumor effect of hu14.18-IL2 in pediatric patients with recurrent or refractory neuroblastoma and in patients with advanced melanoma.

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¹¹ Hank et al., unpublished data.

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