Anti-tumor activity of hu14.18-IL2 (EMD 273063) in relapsed/refractory neuroblastoma patients: a Children's Oncology Group (COG) phase II study

Suzanne Shusterman[1], Wendy B. London[1,2], Stephen D. Gillies[3], Jacquelyn A. Hank[4], Stephan D. Voss[1], Robert C. Seeger[5], C. Patrick Reynolds[6], Jenny Kimball[4], Mark A. Albertini[4], Barrett Wagner[4], Jacek Gan[4], Jens Eickhoff[4], Kenneth B. DeSantes[4], Susan L. Cohn[7], Toby Hecht[8], Brian Gadbaw[4], Ralph A. Reisfeld[9], John M. Maris[10] and Paul M. Sondel[4]

[1]Dana Farber Cancer Institute and Children's Hospital Boston, Boston MA,
[2]Children's Oncology Group Statistics and Data Center (COG SDC)
[3] Provenance Biopharmaceuticals Corp. Waltham MA
[4]University of Wisconsin Carbone Cancer Center, Madison WI
[5]Childrens Hospital of Los Angeles, Los Angeles CA
[6]University of Texas, Lubbock TX
[7]University of Chicago, Chicago IL
[8]National Cancer Institute, Biologic Resources Branch, Frederick MD
[9]The Scripps Research Institute, La Jolla CA
[10]The Children's Hospital of Philadelphia, Philadelphia PA

Corresponding Author:
Paul M. Sondel MD PhD
Department of Pediatrics
4159 MACC Fund Childhood Cancer Research Wing
Wisconsin Institute of Medical Research, UW-Madison
1111 Highland Ave.
Madison WI, 53792
608-263-9069 (ph)
608-263-4226 (FAX)
pmsondel@humonc.wisc.edu

Acknowledgement of Research Support:

This work was supported by NIH/NCI U10CA98543 COG Group Chair Grant, NIH/NCI U10 CA98413 COG Statistics and Data Center, and NIH/NCI R01-CA-32685-25, CA87025, CA81403, RR03186, and grants from the Midwest Athletes for Childhood Cancer Fund, the Crawdaddy Foundation and The Evan Dunbar Foundation. Hu14.18-IL2 (EMD 273063) was produced through an MCRADA between the NCI and Merck KGaA).

Running Head: Phase II clinical activity of hu14.18-IL2 in neuroblastoma

Prior Presentation: This study has not been previously published but was presented at the 2008 ASCO meeting (abstract 3002, J. Clin. Oncol 26: May 20 suppl; p 132s, 2008)

Disclaimers: none

ABSTRACT

Purpose: The hu14.18-IL2 fusion protein consists of IL2 molecularly linked to a humanized monoclonal antibody (mAb) that recognizes the GD2 disialoganglioside expressed on neuroblastoma cells. This Phase II study assessed the anti-tumor activity of hu14.18-IL2 in two strata of patients with recurrent or refractory neuroblastoma.

Patients and Methods: Hu14.18-IL2 was given intravenously (12 mg/m^2/daily) for three days every 4 weeks for patients with disease measurable by standard radiographic criteria (stratum-1) and for patients with disease evaluable only by MIBG scintigraphy and/or bone marrow (BM) histology (stratum-2). Response was established by independent radiology review as well as BM histology and immunocytology, and durability assessed by repeat evaluation after > 3 weeks.

Results: 39 patients were enrolled (36 evaluable). No responses were seen in stratum-1 (n=13). Of 23 evaluable patients in stratum-2, 5 patients (21.7%) responded; all had a complete response (CR) of 9, 13, 20, 30 and 35$^+$ months duration, respectively. Grade 3 and 4 non-hematologic toxicities included capillary leak, hypoxia, pain, rash, allergic reaction, elevated transaminases and hyperbilirubinemia. Two patients required dopamine for hypotension and 1 required ventillatory support for hypoxia. Most toxicities were reversible within a few days of completing a treatment course and were expected based on phase I results.

Conclusions: Patients with disease evaluable only by MIBG and/or BM histology had a 21.7% CR rate to hu14.8-IL2 while patients with bulky disease did not respond. Hu14.18-IL2 warrants further testing in children with non-bulky high-risk neuroblastoma.

INTRODUCTION

Most children with neuroblastoma present with metastatic disease and/or high-risk features.[1-2] Despite multimodal intensive induction and consolidation therapy that provides responses for approximately 80% of patients, <40% of patients with high-risk disease are cured.[2-3] The majority of responding patients eventually die from recurrent disease, indicating that they still harbor viable neuroblastoma following frontline therapy.

The GD2 disialoganglioside is expressed on most neuroblastomas and melanomas, and weakly on peripheral nerves.[4-6] Clinical trials using murine (3F8 and 14.G2a) and chimeric (ch14.18) anti-GD2 mAbs have shown controllable toxicity (including pain and fever), but rare anti-tumor effects against measurable disease.[7-11] Preclinical data suggest *in vivo* activity is mediated by antibody dependent cell-mediated cytotoxicity (ADCC) and is most effective in the minimal residual disease (MRD) setting.[12-15] ADCC may be enhanced by interleukin-2 (IL2), which activates NK cells,[16-17] and by GM-CSF, which activates neutrophils and macrophages.[18] Clinical trials have administered anti-GD2 mAbs together with IL2 and/or GM-CSF. [19-26] Recently a COG phase III trial in high risk neuroblastoma patients, showed a 66% vs. 46% (p=0.01) advantage in event free survival (EFS) and a 86% vs. 75% (p=0.02) advantage in overall survival (OS) using a regimen of ch14.18 + GM-CSF + IL2 and isotretinoin versus isotretinoin alone.[27]

The hu14.18-IL2 fusion protein consists of the humanized 14.18 anti-GD2 mAb linked to IL2.[28] Hu14.18-IL2 localizes to $GD2^+$-tumor cell surfaces via the mAb

component. The IL2 component binds to and activates both NK and T cells, via their IL2 receptors; while the Fc end triggers ADCC and complement-dependent cytotoxicity.[28-31] Hu14.18-IL2 has preclinical activity in neuroblastoma-bearing mice via NK-mediated effects, especially when there is a smaller tumor burden.[14,32] In mice hu14.18-IL2 has superior anti-tumor activity compared to ch14.18 mAb combined with IL2.[13,33]

Phase I testing of hu14.18-IL2 demonstrated biologic activity, clinical tolerability, and a maximal tolerated dose (MTD) of 12 mg/m^2/day for 3 days.[34-35] Dose limiting toxicities (DLT) included hypotension and allergic reactions.

The primary objective of this study was to determine the anti-tumor activity of hu14.18-IL2 in subjects with measurable disease and subjects with disease evaluable only by MIBG scintigraphy and/or BM histology.

PATIENTS AND METHODS

Eligibility

Patients with recurrent or refractory neuroblastoma (from 12 months – 22 years) were eligible. Primary refractory disease (persistent tumor after frontline therapy) required a biopsy demonstrating viable tumor. There were no prior therapy limitations. Eligibility required organ function, performance status, recovery from prior therapy and life expectancy standard for COG Phase II trials. Patients with central nervous system disease were excluded as were patients requiring immunosuppression. IRB-approved informed consent (and assent when applicable) was obtained for all patients.

Study Design

This phase II, single arm, trial evaluated the activity of hu14.18-IL2 separately for two patient strata. Stratum-1 included patients with disease measurable by CT and/or MRI using standard radiographic criteria. Stratum-2 included patients with disease evaluable only by ^{123}I-MIBG scintigraphy and/or BM histology.

Hu14.18-IL2 (EMD 273063) was supplied collaboratively by the NCI (Bethesda, MD) as well as EMD pharmaceuticals (Durham, NC) and Merck KGaA (Darmstadt, Germany). Hu14.18-IL2 (12 mg/m^2/dose) was administered on an inpatient basis as a 4 hour intravenous infusion over three consecutive days. Patients received Indomethacin (0.5 mg/kg/dose, every 6 hours). Treatment cycles were 28 days. Toxicities were graded by the NCI Common Toxicity Criteria (v3.0). DLT was defined as any $\geq$ grade 3 toxicity, with certain reversible exceptions identified in the phase I studies.[34-35] Treatment was held for DLT and restarted at 50% the previous dose once toxicity resolved. Disease evaluations were done every two courses.[36] Treatment was continued for four courses in the absence of progressive disease or drug intolerance. Subsequent treatment could continue for two courses after reaching a CR.

Evaluation of Response

All patients who completed $\geq$2 courses of hu14.18-IL2 or who had an event (relapse or progressive disease) were evaluable for response. All responses were confirmed by independent radiology review and marrow immunocytology.

The International Neuroblastoma Response Criteria was used to define response.[37] For measurable disease, response was determined using the RECIST criteria. Response for stratum-2 patients was determined as follows:

MIBG Response. Patients graded locally with CR or PR for MIBG were scored by central review using the Curie scale.[38] CR was defined by complete resolution of all MIBG avid lesions.

BM Response: For patients who entered with BM disease (neuroblastoma identified in the BM aspirate and/or biopsy by the local pathologist using standard histology), CR was defined as no tumor cells detectable by morphology and immunocytologic analysis on two subsequent bilateral BM aspirates/biopsies done ≥3 weeks apart. PD was defined as ≥25% tumor in the marrow and a doubling in the percentage of tumor. Stable disease (SD) was defined as persistence of disease that does not meet criteria for CR or PR. Patients who cleared morphologic tumor but still had immunocytochemistry-detectible tumor (sensitive to 1 tumor cell in $1x10^5$ nucleated cells)[36] were called SD.

Immunologic Monitoring

Absolute lymphocyte counts were determined at each institution pre-treatment and on days 1, 3, 4, 8, 15 of each course. Serum samples were obtained pre-treatment, immediately after treatment on days 1, and 3, and on days 4 and 8 of each course. These were analyzed for hu14.18-IL2 levels, anti-hu14.18-IL2 antibody and soluble IL2 receptor (sIL2R).[39-40]

Statistical considerations

The primary endpoint of this study was response. Responders were defined as evaluable patients who demonstrated a best overall response of CR, very good partial

response (VGPR), or PR. Using a one-stage rule, if 4 or more patients responded out of the first 20 evaluable in a given stratum, the regimen was considered effective.

A two-stage rule was used to monitor for an excessive number of unacceptable DLTs, where 'unacceptable' was defined as a requirement for pressor and/or ventilator support due to acute vascular leak syndrome. Secondary analyses of EFS and OS were performed as intent-to-treat. For EFS, time-to event was from enrollment until first occurrence of relapse, progression, death or secondary malignancy, or until last contact if no event was observed. For OS, the event was death. Survival estimates (Kaplan-Meier) were calculated[41] and reported with standard errors.[42]

Estimates of the mean value of biological correlates are presented ± the standard error. A paired t-test was used to test the change from baseline to a subsequent timepoint. A two-sample t-test was used to compare the level of a particular biological correlate for responders versus non-responders. A non-parametric Spearman's rank correlation analysis was performed to test for association between hu14.18-IL2 levels and anti-hu14.18-IL2 antibody response (both the bridging and the binding assays). All analyses were performed using SAS software version 9.2 (SAS Institute, Cary, NC). P-values less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

A total of 39 patients (all eligible) were enrolled, 15 in stratum-1 and 24 in stratum-2 (**Table 1)**. The 15 patients in stratum-1 received a total of 35 treatment

courses (median, two courses) and the 24 patients in stratum-2 received a total of 76 courses (median 2.5 courses).

Response and outcome

Two patients in stratum-1 were not evaluable for response. One received no treatment due to parental choice, and the other received only one dose of drug secondary to vascular leak and hypotension. Of the 13 evaluable patients in stratum-1, there were no responders: 3 had SD and 10 had PD. One patient in stratum-2 was taken off study secondary to anaphylaxis during cycle 1 and was not evaluable for response, leaving 23 evaluable stratum-2 patients. In the first 20 evaluable stratum-2 patients, there were five responders, all with CR (**Table 2**). The statistical criterion for activity required at least 4 responders in stratum-2, and this boundary was exceeded. Of the 23 evaluable stratum-2 patients, five patients had a CR, four patients had SD and 14 had PD, for an overall response and CR rate of 21.7% (95% confidence interval: 5%, 37%).

Three of the patients with CR **(Table 3)** enrolled with disease in the BM only. One patient had a single MIBG-avid lesion in the right tibia and the final responder had BM disease as well as multiple MIBG-avid sites. This was the first relapse for four of the five patients who had previously been in a complete remission following myeloablative chemotherapy and autologous stem cell transplant (ASCT). Patient-29 had primary refractory neuroblastoma and enrolled with persistent disease two months following treatment with ^{131}I-MIBG and myeloablative therapy with autologous stem cell rescue. Four of these 5 patients received 6 cycles of therapy and one (patient-10) stopped

therapy after four cycles due to DLT. Two of the responders received isotretinoin following the completion of protocol-determined therapy. Four of the patients achieved CR following 2 cycles of hu14.18-IL2 treatment. Patient 29 had a negative MIBG scan and negative BM morphology following 2 cycles of treatment but remained positive by immunocytology. Both the BM morphology and immunocytology were clear following 4 treatment cycles. All five patients had a prolonged CR and patient 29 remains in CR at 35^{+} months (additional clinical details for these patients are provided in supplemental Table S-1).

In addition to the five CRs, two additional patients in stratum-2 that were scored as SD for protocol-defined agent activity, showed suggestion of improvement and are presented here descriptively (patients 3 and 21 in table S-1). One patient went on study with multiple MIBG-avid sites and biopsy proven bone and marrow disease following ASCT. This patient showed clearing of marrow disease and had a decrease in MIBG avidity that was close to, but did not meet the definition of PR by central review. The other patient went on study with MIBG avid disease and BM biopsies showing 10 – 15% replacement with neuroblastoma. Following 4 courses of treatment, despite a CR by MIBG scintigraphy, the overall response was SD because of substantial improvement, but incomplete clearing in the BM.

The overall (n=39) 1-year EFS and OS were 26%±10% and 63%±11%, respectively, with the curves going much lower after 1 year (**Figure 1a**). For stratum-1 (n=15) and stratum-2 (n=24), both the EFS (**Figure 1b**) and OS (**Figure 1c**) curves trend to similar low values after 1 year.

Toxicity

Of the 38 patients evaluable for toxicity, 8 received only one course of therapy: 6 due to progressive disease, and 2 due to DLT. The grade 3 and 4 toxicities observed over all treatment courses are listed in **Table 4**. Most toxicities were self limited and resolved within a few days of the last dose of hu14.18-IL2 for that treatment course.

Two patients had unacceptable DLTs. One developed grade 3 hypotension after the first dose of hu14.18-IL2 in course-1 and required treatment with dopamine for 24 hours. The other developed capillary leak and hypoxia that required pressors and ventilator support for two weeks. This toxicity developed after the final dose of hu14.18-IL2 during course-2. In retrospect, this patient had 2 prior episodes requiring ventilator support due to capillary leak following ASCT one year prior. Following this event, the protocol was amended to exclude patients with a prior history of ventilator support related to lung injury. All DLTs are listed in Table 5.

Correlative studies

Stratum-1 and stratum-2 patients were combined for these correlative analyses.

Hu14.18-IL2 levels. The mean change in the serum hu14.18-IL2 level from baseline (Course-1, Day-1, prior to first dose) to: a) the Day-1 peak value was 2.4 $\pm$ 0.9 μg/ml (n=36); and, b) the Day-3 peak value was 2.1 $\pm$ 0.8 μg/ml (n=31). During course-1, the change from baseline to Day-3 was less than the change from baseline to Day-1 ($p<0.001$); this was true for all courses (1-6). Within the 36 patients evaluable for response, for each timepoint (Day-1 peak, Day-3 peak) and course (1-6), the hu14.18-

IL2 peak levels for responders (n=5) were similar to those of non-responders (p>0.15 at each time).

Absolute Lymphocyte Count (ALC): As noted previously,[35] subjects showed a significant (p<0.001) drop in their ALC with hu14.18-IL2 treatment [Course-1: baseline to day-3 decrease of 830 ± 940 cells/mcL (n=29); baseline to day-4 decrease of 710 ± 770 cells /mcL (n=25)]. While this drop in ALC is scored as hematologic toxicity, it actually represents immune activation and margination of lymphocytes, a known effect of IL2 .[43] This transient lymphopenia (Supplemental Figure S-1) is followed by lymphocytosis consistent with immune activation [Course-1: baseline to day-8 increase (p<0.001) of 2,360 ± 2,160 cells /mcL (n=26)]. A similar pattern of somewhat smaller ALC decreases from baseline to days 3 and 4 was seen in subsequent courses; the decreases in courses 5 and 6 were not significant.

sIL2R levels: As noted previously,[35] there was a significant increase in sIL2R levels at all courses from baseline to days 4 and 8 (p< 0.0001 for courses 1-3; p<0.01 for courses 4-6). sIL2R values in courses 2, 3, 5, and 6 were higher than on corresponding days in course-1. Within the 36 patients evaluable for response, 31 reported an sIL2R level on day-4 of course-1: the 5 responders had a mean sIL2R of 17,006 ± 6,277 pg/ml vs 11,104 ± 4,372 pg/ml for the 26 not responding (p=0.015). In a comparison of sIL2R levels for the patients with a DLT versus those without a DLT, there was no association.

Anti-hu14.18-IL2 antibody response: Of 36 evaluable patients, 13 patients developed an anti-idiotype antibody against hu14.18-IL2 based on the bridging assay and 16 developed an anti-idiotypic antibody based on the “binding inhibition” assay.[39-40]

However, there was no apparent effect of this anti-idiotypic antibody response on the *in vivo* level of hu14.18-IL2. Specifically, there was no significant association of the level of anti-idiotypic antibody developed after course-1 (or after course-2) with any detectible decrease in peak hu14.18-IL2 level seen on day-1 of course-2 versus the level seen on day-1 of course-1. This is in contrast to the drop in hu14.18-IL2 levels from course-1 day-1 to course-2 day-1 for those patients with a strong anti-id response in our past phase I trials (where most patients received lower doses).[40] Furthermore there was no association of anti-idiotypic antibody response (by either of these assays) with anti-tumor effect for the 5 CRs.

All of the correlative analyses described above comparing the 5 subjects in CR to the others were repeated, comparing the 7 “improved” patients (i.e., the 5 patients with CRs plus the 2 subjects in stratum-2 that scored as SD, but showed clinical improvement in BM and or MIBG, described above) vs. the other patients. For this comparison, no statistically significant associations were found between hu14.18-IL2 levels, sIL2R levels, or anti-idiotypic antibody response with anti-tumor activity. Furthermore, no significant associations were found between response and factors at diagnosis (age, stage, MYCN, ploidy or histological grade) (Table S-1).

DISCUSSION

This study demonstrates antitumor activity of hu14.18-IL2 in relapsed/refractory neuroblastoma patients with stratum-2 disease. Five (of 23 evaluable) stratum-2 patients had a durable CR to therapy and two additional patients showed evidence of

improvement. Although this study did not collect data specifically quantifying disease burden at enrollment, there is the suggestion from their clinical descriptions that the five responders began treatment with relatively small, but clearly evaluable tumor burdens: limited MIBG-avid lesions (rather than diffuse skeletal MIBG-avidity), and partial contamination of marrow with tumor cells (rather than marrow replacement). Even so, all responders had a poor clinical prognosis after being refractory to or relapsing following frontline therapy. In contrast, none of the 15 patients entered into stratum-1 showed evidence of anti-tumor activity. This trial was not designed or powered to test for a difference in the response rate between stratum-1 and 2; however, 5 CRs out of 23 evaluable in stratum-2 compared to 0 out of 13 in stratum-1 has a p-value of 0.089. If one includes in this analysis the 2 additional stratum-2 patients with SD but descriptive improvement (patients 3 and 21 in Table S-1), the difference is significant between the strata (p=0.029). These results are consistent with preclinical data showing the efficacy of hu14.18-IL2 is best seen when used in the MRD setting.[14]

The clinical toxicities seen in this study were consistent with those previously reported for hu14.18-IL2[34-35] and for anti-GD2 mAb + IL2.[19-21,25] Most toxicities resolved within days; only three patients had their therapy discontinued due to toxicity.

Evidence for immune activation was seen as changes in sIL2R levels and lymphocytosis. Neither of these correlated with antitumor response or with toxicity. Although there was a significant increase in sIL2R levels in the 5 responders compared to the others, this correlation was not seen when the 2 “improved” patients were included in the analysis. Anti-idiotypic antibody was detected in 13 and 16 of 36 patients

using 2 different assays. This anti-idiotypic antibody did not correlate with anti-tumor activity, in contrast to clinical response correlations with human anti-mouse antibody (HAMA) detection reported in other studies.[44-45] This may be due in part to low statistical power in this study. Furthermore, the anti-hu14.18-IL2 responses we detected did not appear to have functional significance in that they were not associated with a subsequent decrease in hu14.18-IL2 levels. This suggests that the anti-idiotypic antibodies detected were not sufficiently strong to impact the function of the circulating hu14.18-IL2.

The results of this study support further development of hu14.18-IL2 in recurrent or refractory neuroblastoma patients with disease evaluable only by ^{123}I-MIBG scintigraphy and/or BM histology. A successor study is being planned to confirm efficacy in stratum-2 patients and quantify the disease burden in patients before and after treatment to better define which patients are most likely to respond to hu14.18-IL2 (see development plans in supplemental material).

Finally, given the efficacy recently demonstrated for the regimen of ch14.18 mAb + IL2 + GM-CSF for children with high risk neuroblastoma that have responded (CR, VGPR or PR) to their initial induction and consolidation treatment,[27] and the superiority of ch14.18-IL2 over ch14.18 + IL2 as separate molecules in preclinical studies,[28-31,46], we hypothesize that hu14.18-IL2 may be more effective than ch14.18 + IL2 in this same clinical setting. Thus the COG is planning to randomly compare a regimen of hu14.18-IL2 + GM-CSF + isotretinoin versus the now “standard” regimen of ch14.18 +

GM-CSF + IL2 + isotretinoin in its next Phase III study for newly diagnosed high-risk neuroblastoma patients that have responded to their frontline therapy.

REFERENCES:

1. Maris JM, Hogarty MD, Bagatell R, et al: Neuroblastoma. Lancet 369:2106-20, 2007

2. Matthay KK, Villablanca JG, Seeger RC, et al: Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. N Engl J Med 341:1165-73, 1999

3. Matthay KK, Reynolds CP, Seeger RC, et al: Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: a children's oncology group study. J Clin Oncol 27:1007-13, 2009

4. Mujoo K, Spiro RC, Reisfeld RA: Characterization of a unique glycoprotein antigen expressed on the surface of human neuroblastoma cells. J Biol Chem 261:10299-305, 1986

5. Cheung NK, Saarinen UM, Neely JE, et al: Monoclonal antibodies to a glycolipid antigen on human neuroblastoma cells. Cancer Res 45:2642-9, 1985

6. Chang HR, Cordon-Cardo C, Houghton AN, et al: Expression of disialogangliosides GD2 and GD3 on human soft tissue sarcomas. Cancer 70:633-8, 1992

7. Yu AL, Uttenreuther-Fischer MM, Huang CS, et al: Phase I trial of a human-mouse chimeric anti-disialoganglioside monoclonal antibody ch14.18 in patients with refractory neuroblastoma and osteosarcoma. Journal Clinical Oncology 16:2169-80, 1998

8. Cheung NK, Lazarus H, Miraldi FD, et al: Ganglioside GD2 specific monoclonal antibody 3F8: a phase I study in patients with neuroblastoma and malignant melanoma [see comments]. Journal of Clinical Oncology 5:1430-40, 1987

9. Murray JL, Cunningham JE, Brewer H, et al: Phase I trial of murine monoclonal antibody 14G2a administered by prolonged intravenous infusion in patients with neuroectodermal tumors. Journal of Clinical Oncology 12:184-93, 1994

10. Saleh MN, Khazaeli MB, Wheeler RH, et al: Phase I trial of the chimeric anti-GD2 monoclonal antibody ch14.18 in patients with malignant melanoma. Hum Antibodies Hybridomas 3:19-24, 1992

11. Handgretinger R, Baader P, Dopfer R, et al: A phase I study of neuroblastoma with the anti-ganglioside GD2 antibody 14.G2a. Cancer Immunology & Immunotherapy 35:199-204, 1992

12. Lode HN, Xiang R, Duncan SR, et al: Tumor-targeted IL-2 amplifies T cell-mediated immune response induced by gene therapy with single-chain IL-12. Proceedings of the National Academy of Sciences, U.S.A. 96:8591-6, 1999

13. Lode HN, Xiang R, Varki NM, et al: Targeted interleukin-2 therapy for spontaneous neuroblastoma metastases to bone marrow. Journal of the National Cancer Institute 89:1586-94, 1997

14. Neal ZC, Yang JC, Rakhmilevich AL, et al: Enhanced activity of hu14.18-IL2 immunocytokine against murine NXS2 neuroblastoma when combined with interleukin 2 therapy. Clin Cancer Res 10:4839-47, 2004

15. Imai M, Landen C, Ohta R, et al: Complement-mediated mechanisms in anti-GD2 monoclonal antibody therapy of murine metastatic cancer. Cancer Res 65:10562-8, 2005

16. Munn DH, Cheung NK: Interleukin-2 enhancement of monoclonal antibody-mediated cellular cytotoxicity against human melanoma. Cancer Res 47:6600-5, 1987

17. Hank JA, Robinson RR, Surfus J, et al: Augmentation of antibody dependent cell mediated cytotoxicity following in vivo therapy with recombinant interleukin 2. Cancer Res 50:5234-9, 1990

18. Barker E, Reisfeld RA: A mechanism for neutrophil-mediated lysis of human neuroblastoma cells. Cancer Res 53:362-7, 1993

19. Frost JD, Hank JA, Reaman GH, et al: A phase I/IB trial of murine monoclonal anti-GD2 antibody 14.G2a plus interleukin-2 in children with refractory neuroblastoma: a report of the Children's Cancer Group. Cancer 80:317-33, 1997

20. Albertini MR, Hank JA, Schiller JH, et al: Phase IB trial of chimeric antidisialoganglioside antibody plus interleukin 2 for melanoma patients. Clin Cancer Res 3:1277-88, 1997

21. Hank JA, Surfus J, Gan J, et al: Treatment of neuroblastoma patients with antiganglioside GD2 antibody plus interleukin-2 induces antibody-dependent cellular cytotoxicity against neuroblastoma detected in vitro. Journal of Immunotherapy 15:29-37, 1994

22. Kushner BH, Kramer K, Cheung NK: Phase II tiral of the anti-GD2 monoclonal antibody 3F8 and granulocyte-macrophage colony-stimulating factor for neuroblastoma. J Clin Oncol 19:4189-4194, 2001

23. Cheung NK, Sowers R, Vickers AJ, et al: FCGR2A polymorphism is correlated with clinical outcome after immunotherapy of neuroblastoma with anti-GD2 antibody and granulocyte macrophage colony-stimulating factor. J Clin Oncol 24:2885-90, 2006

24. Ozkaynak MF, Sondel PM, Krailo MD, et al: Phase I study of chimeric human/murine anti-ganglioside G(D2) monoclonal antibody (ch14.18) with granulocyte-macrophage colony-stimulating factor in children with neuroblastoma immediately after

hematopoietic stem-cell transplantation: a Children's Cancer Group Study. Journal of Clinical Oncology 18:4077-85, 2000

25. Gilman AL, Ozkaynak MF, Matthay KK, et al: Phase I study of ch14.18 with granulocyte-macrophage colony-stimulating factor and interleukin-2 in children with neuroblastoma after autologous bone marrow transplantation or stem-cell rescue: a report from the Children's Oncology Group. J Clin Oncol 27:85-91, 2009

26. Yu AL, Uttenreuther MM, Kamps A, et al: Combined use of human-mouse chimeric anti-GD2 (ch14.18) and GM-CSF in the treatment of refractory neuroblastoma. Antibody Immunoconjugates and Radiopharmaceuticals 8:12, 1995

27. Yu AL, Gilman AL, Ozkaynak MF, et al: Chimeric Anti-GD2 Antibody with GM-CSF, IL2 and 13-Cis Retinoic Acid for High-Risk Neuroblastoma: A Children's Oncology Group (COG) Phase 3 Study New England J. Med., *in publication*

28. Gillies SD, Reilly EB, Lo KM, et al: Antibody-targeted interleukin 2 stimulates T-cell killing of autologous tumor cells. Proc Natl Acad Sci U S A 89:1428-32, 1992

29. Buhtoiarov IN, Neal ZC, Jan J, et al: Differential internalization of hu14.18-IL2 by human NK cells and human tumor cells; influence on conjugate formation, cytotoxicity and infiltration into tumors., submitted 2009

30. Hank JA, Surfus JE, Gan J, et al: Activation of human effector cells by a tumor reactive recombinant anti-ganglioside GD2 interleukin-2 fusion protein (ch14.18-IL2). Clinical Cancer Research 2:1951-9, 1996

31. Voss SD, Robb RJ, Weil-Hillman G, et al: Increased expression of the interleukin 2 (IL-2) receptor beta chain (p70) on CD56+ natural killer cells after in vivo IL-2 therapy: p70 expression does not alone predict the level of intermediate affinity IL-2 binding. J Exp Med 172:1101-14, 1990

32. Neal ZC, Imboden M, Rakhmilevich AL, et al: NXS2 murine neuroblastomas express increased levels of MHC class I antigens upon recurrence following NK-dependent immunotherapy. Cancer Immunol Immunother 53:41-52, 2004

33. Lode HN, Xiang R, Dreier T, et al: Natural killer cell-mediated eradication of neuroblastoma metastases to bone marrow by targeted interleukin-2 therapy. Blood 91:1706-15, 1998

34. King DM, Albertini MR, Schalch H, et al: Phase I clinical trial of the immunocytokine EMD 273063 in melanoma patients. J Clin Oncol 22:4463-73, 2004

35. Osenga KL, Hank JA, Albertini MR, et al: 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. Clin Cancer Res 12:1750-9, 2006

36. Seeger RC, Reynolds CP, Gallego R, et al: Quantitative tumor cell content of bone marrow and blood as a predictor of outcome in stage IV neuroblastoma: a Children's Cancer Group Study. J Clin Oncol 18:4067-76, 2000

37. Brodeur GM, Pritchard J, Berthold F, et al: Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. Journal of Clinical Oncology 11:1466-1477, 1993

38. Matthay KK, Edeline V, Lumbroso J, et al: Correlation of early metastatic response by 123I-metaiodobenzylguanidine scintigraphy with overall response and event-free survival in stage IV neuroblastoma. J Clin Oncol 21:2486-91, 2003

39. Gan J, Kendra K, Ricci M, et al: Specific enzyme-linked immunosorbent assays for quantitation of antibody-cytokine fusion proteins. Clin Diagn Lab Immunol 6:236-42, 1999

40. Hank JA, Gan J, Ryu H, et al: Immunogenicity of the Hu14.18-IL2 Immunocytokine Molecule in Adults with Melanoma and Children with Neuroblastoma. Clin Cancer Res, 2009

41. Kaplan EL, Meier P: Nonparametric-estimation from incomplete observations. Journal of the American Statistical Association 53:457-481, 1958

42. Peto R, Pike MC, Armitage P, et al: Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. Br J Cancer 35:1-39, 1977

43. Voss SD, Hank JA, Nobis CA, et al: Serum levels of the low-affinity interleukin-2 receptor molecule (TAC) during IL-2 therapy reflect systemic lymphoid mass activation. Cancer Immunol Immunother 29:261-9, 1989

44. Cheung NK, Cheung IY, Canete A, et al: Antibody response to murine anti-GD2 monoclonal antibodies: correlation with patient survival. Cancer Research 54:2228-33, 1994

45. Cheung NK, Guo HF, Heller G, et al: Induction of Ab3 and Ab3' antibody was associated with long-term survival after anti-G(D2) antibody therapy of stage 4 neuroblastoma. Clin Cancer Res 6:2653-60, 2000

46. Gubbels JA, Gadbaw B, Buhtoiarov IN, et al: AB-IL2 fusion proteins mediate NK cell immune synapse formation by polarizing CD25 to the target cell - effector cell interface. submitted 2009

Figure 1 Legend : a) EFS and OS for all patients; b) EFS for stratum-1 and stratum-2; c) OS for stratum-1 and stratum-2.

Table 1. Patient characteristics by stratum

	Stratum-1 (n=15)[1] n (%)	Stratum-2 (n=24) [2] n (%)	Total (n=39) n (%)
No. of eligible patients	15 (100)	24 (100)	39 (100)
No. of patients evaluable for toxicity	14 (93)	24 (100)	38 (97)
No. of patients evaluable for response	13 (87)	23 (96)	36 (92)
Age at diagnosis			
< 18 months	0 (0)	0 (0)	0 (0)
≥ 18 months	15 (100)	24 (100)	39 (100)
INSS stage			
1, 2, 3, 4s	0 (0)	2 (8)	2 (5)
4	11 (73)	15 (63)	26 (67)
Unknown	4 (27)	7 (29)	11 (28)
MYCN status			
Not amplified	7 (47)	11 (46)	18 (46)
Amplified	4 (27)	2 (8)	6 (15)
Unknown	4 (27)	11 (46)	15 (38)
Ploidy			
Hyperdiploid	6 (40)	10 (42)	16 (41)
Diploid	4 (27)	3 (12)	7 (18)
Unknown	5 (33)	11 (46)	16 (41)
Histology			
Favorable	0 (0)	0 (0)	0 (0)
Unfavorable	9 (60)	11 (46)	20 (51)
Unknown	6 (40)	13 (54)	19 (49)
No. of courses administered			
Total	35	76	110
Median	2	2.5	2
Range	1-6	1-6	1-6

[1]Stratum-1: Disease measurable by standard radiographic criteria
[2]Stratum-2: Disease evaluable only by ^{123}I-MIBG and/or bone marrow histology

Table 2. Response Summary

Stratum	Number of evaluable patients	Number of Responders	Level of Response				
			CR	VGPR	PR	SD	PD
1 (n=15)	13	0	0	0	0	3	10
2 (n=24)	23	5	5	0	0	4	14

Table 3. Response Details

Patient	Disease at Study Entry	Courses	Dose Reduction Required	Response	Time to Event[1]
2	Bone marrow	6	No	CR	13 months[2]
10	Bone marrow	4	Yes	CR	9 months
22	MIBG (1 site)	6	Yes	CR	20 months[2]
27	Bone marrow	6	No	CR	30 months
29	Bone marrow, MIBG (multiple sites)	6	No	CR	No event[3]

[1]Time to progression from start of therapy
[2]Patient received cis-retinoic acid following the completion of hu14.18-IL2
[3]Patient in remission for 35 months at last follow-up

Table 4. Grade 3 and 4 toxicities for all courses of therapy

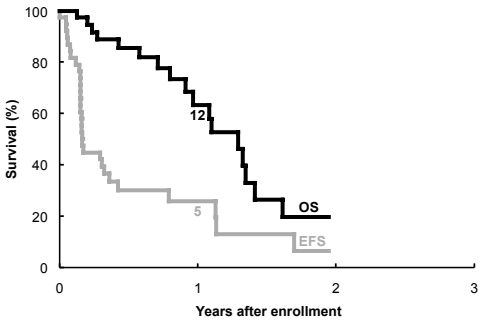
Toxicity	Count[1, 2]	Incidence of toxicity (%)
Acute vascular leak syndrome	12	31.6
Allergic reaction/hypersensitivity	4	10.5
ALT (SGPT) elevation	8	21.1
AST (SGOT) elevation	9	23.7
Bilirubin	8	21.1
Fever (without neutropenia)	15	39.5
Hemoglobin	9	23.7
Hypokalemia	4	10.5
Hyponatremia	2	5.3
Hypotension	6	15.8
Infection (catheter related) with ANC > 1000/mm^3	5	13.2
Leukocytes	9	23.7
Lymphocytes	15	39.5
Neutrophils	13	34.2
Pain (head/headache)	4	10.5
Pain (other)	12	31.6
Platelets	16	42.1
Pleural effusion (non-malignant)	2	5.3
Pneumonitis/pulmonary infiltrates	2	5.3
Rash	2	5.3
Urticaria	2	5.3

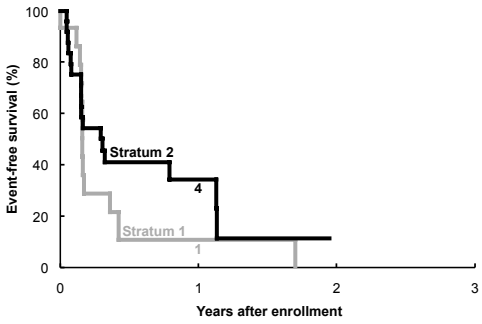
[1]Number of patients reporting at least one grade 3 or 4 toxicity over all courses
[2]38 patients were evaluable for toxicity (treatment not initiated in 1 patient)

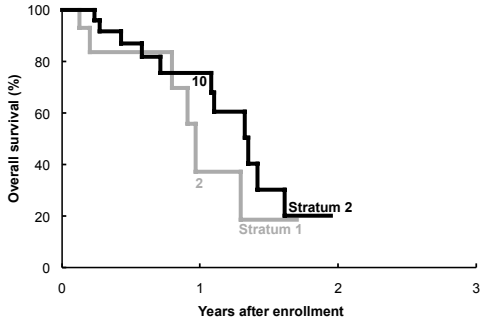
Table 5. Dose-Limiting Toxicity

Patient	Course	Toxicity	Result
3	2	Grade 3 hypoxia, pneumonitis/pulmonary infiltrates	Tolerated courses 3-6 at 50% dosing
4	2	Grade 4 acute vascular leak	Therapy discontinued due to toxicity
10	3	Grade 3 acute vascular leak	Course 4 at 50% dosing, discontinued day 2
13	1	Grade 3 acute vascular leak and hypotension	Therapy discontinued due to toxicity
14	2	Grade 3 hyperbilirubinemia	Course 3 50% dosing, course 4 25% dosing
18	1	Grade 3 transaminitis	Tolerated course 2 at full dose[1]
19	3	Grade 3 transaminitis	Tolerated course 4 at 50% dosing
21	4	Grade 3 transaminitis	Repeat toxicity course 5 at 50% dosing
22	4	Grade 3 hyperbilirubinemia	Tolerated course 5 at 50% dosing and course 6 at 75% dosing
24	1	Grade 3 transaminitis	Tolerated course 2-4 at 50% dosing
26	1	Grade 3 hyperbilirubinemia	Tolerated course 2 at 50% dosing
31	2	Grade 3 hypotension	Off study end of course due to PD
32	1	Grade 4 allergic reaction	Therapy discontinued due to toxicity
34	2	Grade 3 hypotension	Tolerated course 3 at 50% dosing and course 4 at 75% dosing
37	1	Grade 3 transaminitis	Tolerated course 2 at 50% dosing
38	2	Grade 3 acute vascular leak	Tolerated courses 3-6 at 50% dosing

[1]Dosing in violation of the protocol


Survival (%)
100
80
60
40
20
0
0
1
2
3
Years after enrollment
12
OS
5
EFS


Event-free survival (%)
100
80
60
40
20
0
0
1
2
3
Years after enrollment
Stratum 2
4
Stratum 1
1


Overall survival (%)
100
80
60
40
20
0
0
1
2
3
Years after enrollment
10
2
Stratum 2
Stratum 1

SUPPLEMENTAL MATERIAL FOR ON-LINE PUBLICATION:

Anti-tumor activity of hu14.18-IL2 in relapsed/refractory neuroblastoma patients: a Children's Oncology Group (COG) phase II study. Shusterman S, London WB, Gillies SD, et al. Hank JA, Voss S, Seeger RC, Reynolds CP, Kimball J, Albertini MA, Wagner B, Gan J, Eickhoff J, DeSantes KD, Cohn SL, Hecht T, Gadbaw B, Reisfeld RA, Maris JM, Sondel PM.

Supplemental Table S-1: Additional clinical details for 5 patients that experienced CR and 2 patients that scored as SD, but had descriptive evidence of anti-tumor activity

Pt. #	Response	Tumor characteristics	Description
2	CR	MYCN:amplified Ploidy:hyperdiploid Histology:unfavorable	Prior History: Relapse noted following ASCT. Study Entry: BM disease only detected at study entry. Study Response: BM clear and ICC negative following course 2. Completed 6 courses of treatment at full dose with no evidence of disease. CRA given post treatment. Recurred with BM and abdominal disease after 10 mo of CR.
10	CR	MYCN: not amplified Ploidy:diploid Histology: unfavorable	Prior History: Relapse in BM noted following ASCT. Study Entry: BM disease only at study entry. Study Response: BM clear following course 2. Completed 4 courses with no evidence of disease. No further treatment given due to hypotension at 50% dose. Recurred in BM and by bone scan after 8 mo of CR.
22	CR	MYCN: not amplified Ploidy: hyperdiploid Histology:unknown	Prior History: Persistent MIBG detected disease in tibia after ASCT with new faint MIBG lesion seen in liver (possible progressive disease). Study Entry: Disease detected only by MIBG (tibia and possibly liver). Study Response: MIBG clear after course 2. Competed 6 courses of treatment with no evidence of disease. Recurred at tibial site after 18 mo of CR.
27	CR	MYCN: not amplified Ploidy: hyperdiploid Histology: unfavorable	Prior History: Relapse in BM after ASCT. Study entry: BM disease only detected at study entry. Study Response: BM clear following course 2. Completed 6 courses of treatment with no evidence of disease. Recurred in scalp after 28 mo of CR.

29	CR	MYCN: not amplified Ploidy: hyperdiploid Histology: unfavorable	Prior History: Refractory disease in BM and by MIBG scan 2 months after ASCT with ^{131}I-MIBG treatment. Study Entry: BM biopsy and MIBG (4 sites) detectable disease. Study Response: After course 2, BM morphology negative and MIBG cleared, but ICC slightly positive. All clear after courses 4 and 6. Has continued in CR through last follow up (35^{+} mo CR).
3	SD	MYCN:unknown Ploidy:unknown Histology: unfavorable	Prior History: After ASCT, MIBG remains positive (never cleared), but BM did clear. Then BM showed relapse. Study Entry: BM biopsy positive with MIBG positive at multiple sites. Study Response: After course 2, BM biopsies are negative, and MIBG unchanged. Following 4 courses, MIBG read as PR locally, with marrow remaining negative. Same status after 6 courses. Scored as PR overall locally, but central radiology review considered MIBG not sufficiently improved to be PR. Patient's response thus scored as SD. Because of improved but persistent MIBG+ disease, child then received ^{131}I-MIBG treatment with improvement but not clearing.
21	SD	MYCN: not amplified Ploidy: hyperdiploid Histology: unfavorable	Prior History: Primary refractory diseaseafter chemotherapy, received ablative chemotherapy + ^{131}I-MIBG and ASCT. Three months later, not responding, and entered this study. Study Entry: MIBG positive disease with 10-15% replacement of marrow on biopsy at study entry. Study Response: After 4 courses, MIBG scan is clear and marrow biopsies are clear bilaterally, with aspirates clear save for a single clump of 6 neuroblastoma cells on one aspirate cover slip. Child received course 5 and was scored as SD as marrow did not completely clear. Child then received a separate Phase I agent and marrow then becomes clear, and child has remained in CR for 32^{+} months

Additional clarifications and tests for associations amongst responding patients and 2 patients scored as SD with descriptive evidence of improvement:

Table S-1 provides the MYCN, ploidy and Shimada histology classification for the 5 patients that showed CR, and for the 2 additional patients that were scored as SD but descriptively judged to have shown some improvement, in response to the hu14.18-IL2 treatment. In addition, the clinical sequence for each of these 7 patients is briefly summarized.

There was no statistical association of response (CR vs. <CR) with factors at the time of diagnosis (age, stage MYCN, ploidy or histological status). Similarly, when response was categorized as (CR+SD vs. <SD), there was also no significant association. However, this should not be considered proof of lack of association(s), as these tests were unplanned and underpowered.

Note that, of the 5 patients with CR, patient #29 had refractory rather than relapsed or progressive disease. Thus it remains possible that patient #29 may potentially have shown an unusual delayed response to the MIBG treatment received 2 months prior to going onto this trial. For the following reasons, such a delayed response to prior ^{131}I-MIBG treatment seems quite unlikely. Resolution of measurable disease (CT or MRI detected) may take months to confirm radiologically following effective treatment (regardless of the treatment used). This likely reflects the time required for normal tissue to remodel (especially for bony or large soft tissue lesions) following effective destruction of viable tumor cells at a site of measurable disease. In contrast, the 5 responders, and 2 "improved" patients described in this study had no disease detectible by CT or MRI when they entered this study. Their disease was evaluable at that time only by BM histology and/or by MIBG scintigraphy. The latter requires tumor specific uptake of MIBG by viable neuroblastoma cells to give a specific signal (Taggart et al, JCO, 2009). The localized scar tissue at sites of sterilized (ie: non-viable) neuroblastoma would not be expected to still concentrate MIBG in the absence of viable neuroblastoma cells. Thus the presence of MIBG detectible disease should indicate residual viable neuroblastoma at that site. Similarly, standard microscopic histology is able to distinguish morphology of viable cells from those that are necrotic. The identification of neuroblastoma cells in the marrow, by standard histology, and the identification of areas of ^{123}I-MIBG uptake at the time of study entry (2 or more months following prior treatment, as in patient #29) would not be anticipated if the prior treatment actually destroyed all viable neuroblastoma cells.

Table S-2: Duration of toxicities in Adverse Events Expedited Reports (ADEERS) attributed as definitely, probably or possibly related to hu14.18-IL2 .

Toxicity	# Reported	Grade	Median duration (range) [1]
Acute Vascular Leak[2]	11	2 (n=1)	3 (0-20)
		3 (n=9)	
		4 (n=1)	
AST	1	3	2
Dyspnea	1	3	2
Edema	1	3	2
Fever	1	3	2

Hemoglobin	1	2	2
Hypoalbuminemia	1	2	6
Hypocalcemia	1	2	6
Hypokalemia	1	3	1
Hypotension	1	2	6
Hypoxia[3]	3	2 (n=2) 3 (n=1)	6 (0-9)
Infection with normal ANC	2	3	19 (7-31)
Lymphopenia	1	4	7
Platelets[5]	1	3	11
Pleural effusion	1	3	2
Pneumonitis	1	3	2

[1] Median duration of toxicity in days following the completion of hu14.18-IL2. These data were collected from all ADEERS reports submitted for this study. Each report reflects a separate event; a given patient could have multiple reports.

[2] One patient had acute vascular leak syndrome lasting 9 days with a maximum toxicity grade 2. Another patient had grade 4 acute vascular leak syndrome lasting for 20 days and is described in the text of the manuscript in detail (patient with an unacceptable dose limiting toxicity requiring ventilator support for 14 days).

[3] One patient had transient grade 2 hypoxia (and acute vascular leak) that resolved the same day the antibody was stopped. A second patient had a grade 2 intermittent oxygen requirement that persisted for 9 days following the completion of treatment. This patient had intermittent desaturations, mainly at night, that resolved with diuresis. A third patient developed acute vascular leak syndrome and a pleural effusion 24 hours after completing hu14.18-IL2 and required oxygen for 6 days.

Supplemental Figure S-1: Absolute lymphocyte counts from days 1, 3, 4 and 8 of each treatment course

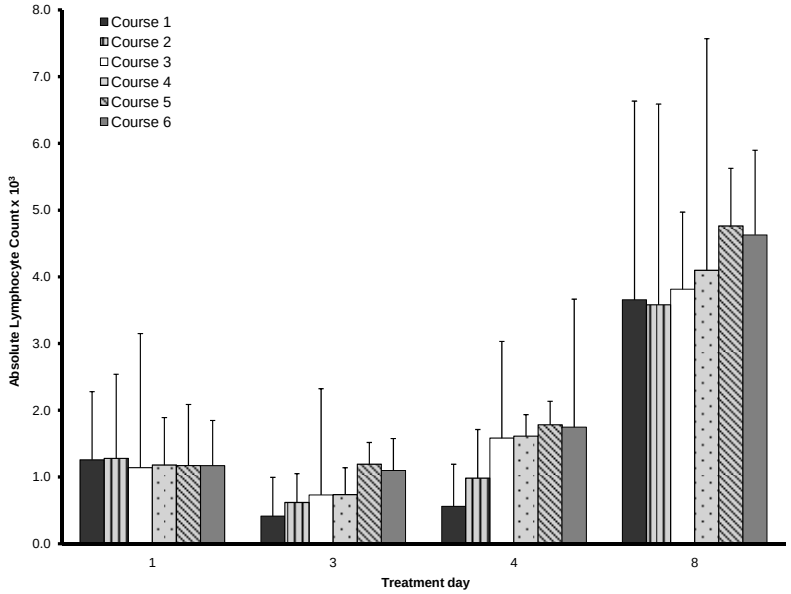


Figure S-1: Absolute lymphocyte counts obtained for each course. For each course, the absolute lymphocyte count (lymphocytes/mcL $x10^{-3}$) were measured in peripheral blood on day 1 (just prior to starting day 1 of hu14.18-IL2), on day 3 (just prior to starting the day 3 infusion of hu14.18-IL2), on day 4 (20 hours after completing the day 3 hu14.18-IL2) and on day 8 (5 days after completing the day 3 hu14.18-IL2 infusion). The transient lymphopenia seen on day 3 and 4 is more severe for courses 1 and 2. By day 8, for each course, there is a prominent lymphocytosis. Data shown are the means for all treated patients.

Plans for further testing and development:

Our goal in this current study was to design a Phase II trial that would enable detection of clinical activity for an agent that was predicted (from preclinical data) to have activity against microscopic residual disease rather than bulky disease. Given the activity seen in stratum-2 patients in this Phase II trial, we hypothesize that this agent may be most helpful in preventing relapse for patients in remission but at high risk for relapse. This is the rationale for COG's plans to perform a randomized comparison of ch14.18 + GM-CSF + IL2 +CRA vs. hu14.18-IL2 + GM-CSF + CRA as part of its next COG Phase III randomized trial of children with newly diagnosed high risk NBL following front-line chemotherapy, surgery, ASCT and radiotherapy. In addition, the data from this trial suggest that this agent warrants additional testing as potential treatment for patients that have relapsed or progressive refractory disease after completing front-line treatment, provided that their disease is not bulky (ie: meets stratum-2 criteria).

Additional reference noted above but not included in the manuscript itself

Taggart DR, Han MM, Quach A, Groshen S, Ye W, Villablanca JG, Jackson HA, Mari Aparici C, Carlson D, Maris J, Hawkins R, Matthay KK. Comparison of iodine-123 metaiodobenzylguanidine (MIBG) scan and [18F]fluorodeoxyglucose positron emission tomography to evaluate response after iodine-131 MIBG therapy for relapsed neuroblastoma. J Clin Oncol. 27:5343-9. 2009