Version 6, 1/21/2010

A Pilot Trial of Hu14.18-IL2 (EMD 273063) in Subjects with Completely Resectable Recurrent Stage III or Stage IV Melanoma

UWCCC protocol # CO05601, NCI #8701
ClinicalTrials.gov identifier: NCT00590824

Study Chairman: Mark R. Albertini, M.D.[1]

Laboratory Co-Chair: Jacquelyn A. Hank, Ph.D.[1]

Statistician: KyungMann Kim, Ph.D.[1]

Protocol Collaborators: Heidi Schalch, P.A.-C.[1, 2]
Erik Ranheim, M.D.[1]
David Mahvi, M.D.[1]
Jacek Gan, Ph.D.[1]
Ryan Cassaday, M.D.[1]
Jordan Kostlevy[1,2]
Ralph Reisfeld, Ph.D.[3]
Sharon Weber, M.D.[1]
Tracy Weigel, M.D.[1]
Greg Hartig, M.D.[1]
Jennifer Haldeman[1,2]
Heather Neuman, M.D. [1]

Protocol Consultants:

Jean Henslee-Downey, M.D..[4]

Principal Investigator: Paul Sondel, M.D., Ph.D.[1]

[1]University of Wisconsin Paul P. Carbone Comprehensive Cancer Center; Madison, WI
[2]UW Study Coordinator
[3]The Scripps Research Foundation; La Jolla, CA
[4]EMD- Serono, Inc. Rockland ,,

Mark R. Albertini, M.D.
K6/530 CSC
UWCCC
600 Highland Ave.
Madison, WI 53792
Phone: 608.263.0117
Fax: 608.265.8133
Paging: 608.262.0143
mralbert@wisc.edu

Jacquelyn A. Hank, PhD
4157 WIMR
UWCCC
1111 Highland Ave.
Madison, WI 53705
Phone: 608.263.7262
Fax: 608.263.4226
Paging: 608.262.0143
hank@humonc.wisc.edu

Paul M. Sondel, M.D., Ph.D.
4159 WIMR
UWCCC
1111 Highland Ave.,
Madison, WI 53705
Phone: 608.263.9069
Fax: 608.263.4226
Paging: 608.262.0143
pmsondel@wisc.edu

ABBREVIATIONS USED IN THE TEXT

ACTH	**adrenocorticotropin hormone**
ADCC	**antibody-dependent cellular cytotoxicity**
alb	**albumin**
alk phos	**alkaline phosphatase**
ALT (SGPT)	**alanine aminotransferase (serum glutamate pyruvate transaminase)**
AST (SGOT)	**aspartate aminotransferase (serum glutamic oxaloacetic transaminase)**
bid	**twice daily**
BUN	**blood urea nitrogen**
CBC	**complete blood count**
CDC	**complement-dependent cytotoxicity**
Ca	**calcium**
CCG	**Children's Cancer Group**
CDR	**complimentarity-determining region**
ch	**chimeric**
CR	**complete response**
CRP	**C-reactive protein**
CT	**computerized tomography**
CTCAE	**Common Terminology Criteria for Adverse Events**
CXR	**chest X-ray**
DSMC	**Data Safety Monitoring Committee**
DSMP	**Data Safety Monitoring Plan**
diff	**white blood cell differential**
DLT	**dose limiting toxicity**
ECG	**electrocardiogram**
ELISA	**enzyme-linked immunosorbent assay**
Glu	**glucose**
HAMA	**human anti-mouse antibody**
HBs Ag	**hepatitis B surface antigen**
Hgb	**hemoglobin**
HIV	**human immunodeficiency virus**
IC	**immunocytokine**
IgG	**immunoglobulin G**
IgM	**immunoglobulin M**
IL2	**interleukin-2**
IND	**investigational new drug**
INR	**international normalization ratio**
IV	**intravenous**
K	**potassium**
LDH	**lactate dehydrogenase**
LFT	**liver function tests**
mAb	**monoclonal antibody**
Mg	**magnesium**
MRI	**magnetic resonance imaging**
MTD	**maximum tolerated dose**

Na	**sodium**
NCI	**National Cancer Institute**
NK	**natural killer cells**
OS	**on study**
P	**pulse**
PBL	**peripheral blood lymphocyte**
PO	**oral**
PA	**posterior-anterior**
PHI	**personal health information**
PK	**Pharmacokinetic**
plts	**platelets**
PR	**partial response**
PRN	**as needed**
PTT	**partial thromboplastin time**
qd	**once daily**
RR	**respiration rate**
SIADH	**syndrome of inappropriate anti-diuretic hormone**
T	**temperature**
T. bili	**total bilirubin**
tid	**three times daily**
TCR	**T cell receptor**
TSH	**Thyroid-stimulating hormone**
ULN	**upper limit of normal**
WBC	**white blood cell count**
UWCCC	**University of Wisconsin Paul P. Carbone Comprehensive Cancer Center**

TABLE OF CONTENTS

OVERVIEW

Hu14.18-IL2, an immunocytokine (IC) referred to as EMD 273063 by EMD Serono, Inc., Rockland, MA, formerly by EMD Pharmaceuticals, Inc., Durham,NC, consists of the humanized 14.18 (hu14.18) monoclonal antibody (mAb) genetically linked to 2 molecules of human recombinant interleukin-2 (IL2). Hu14.18-IL2 recognizes the disialoganglioside GD2, found on melanoma cells. IL2 has been tested extensively in melanoma clinical trials and is approved as an immune activator with documented antitumor effects in patients with melanoma. The chimeric (ch) form of the hu14.18 antibody (ch14.18) has been tested as an investigational therapeutic agent in Phase I clinical trials, alone and combined with other treatments including IL2, with antitumor effects demonstrated in these studies. Preclinical studies performed *in vitro* with human cells, *in vivo* in SCID mice bearing human tumor xenografts, and *in vivo* in immunocompetent mice bearing syngeneic tumor cells transfected to express the GD2 molecule, suggest that hu14.18-IL2 may have a greater antitumor effect than either IL2, hu14.18, or a combined regimen that includes both molecules. Results in tumor bearing mice treated with this molecule and other similarly configured molecules document that the most striking antitumor effects are obtained when the tumor burden is quite low. Initial trial results of hu14.18-IL2 in subjects with $GD2^+$ tumors documented that hu14.18-IL2 is clinically safe and well-tolerated at doses that induce immunologic activation. However, despite this immunologic activation, no clear evidence of measurable antitumor effect was seen in our recent Phase I trial of hu14.18-IL2 for adults with melanoma (i.e., no subjects showed any complete or partial antitumor responses). Of 5 subjects treated in the Phase I trial with no evidence of disease at the time of treatment, 2 of these 5 subjects (treated at 0.8 and 6.0 mg/m^2/day) continue with no evidence of disease (69-102 months). The other 3 recurrenced 2, 6 and 92 months after starting hu14.18-IL2 treatment. A separate Phase II study, using this same regimen, at a dose of 6 mg/m^2/d, was recently completed. Fourteen patients with measurable disease were entered. Treatment was well-tolerated, with no protocol-defined DLTs. Of the fourteen subjects enrolled and treated in the Phase II trial, there was one confirmed partial response by RECIST criteria. Based on these initial preclinical and clinical data, this pilot protocol has been designed to evaluate the effect of 3 courses of hu14.18-IL2 treatment on the melanoma, the immune system, and the time to tumor progression for a small cohort of subjects with recurrent Stage III or Stage IV melanoma and "completely" resectable disease.

Cilengitide is an antivascular therapeutic that has been shown in animal models to have antiangiogenic effects, and is currently in clinical testing. Of great interest is the striking synergy that has been shown when cilengitide was added to treatment with ch14.18-IL2 in tumor bearing mice. Antitumor effects were noted against primary tumors, as well as against experimental and spontaneous metastases.

Initially, this protocol was designed to evaluate the effect of hu14.18-IL2 and of cilengitide, alone and in combination, for patients with Stage III or Stage IV melanoma thought to have completely resectable disease. The protocol was approved and opened in the spring of 2008, with subjects randomized to one of 4 arms. Subjects were further divided into those individuals needing extensive surgical versus non-extentive surgical procedures to achieve disease resection. Four subjects were enrolled and treated prior to the current (Version 5) protocol amendment.

UPDATE FOR PROTOCOL VERSION 5 (1/6/09)

Based on toxicities seen in one patient who received both cilengitide alone (cycle 1) and hu14.18-IL2 in combination with cilengitide (cycle 2), the decision was made to amend this protocol and to proceed using only hu14.18-IL2. The rationale for this decision, as well as the description of this serious adverse event, is described below in Section 5.4.5. All sections of the protocol that are not pertinant to subjects being enrolled following this amendment have been removed. The 4 subjects enrolled in this clinical study prior to this protocol amendment, three of whom received the combination therapy, were

monitored and managed as outlined in version 4 of the protocol. The statistical considerations section of the protocol, section 9, has been amended so that it pertains to the analysis plan following this amendment (2 treatment arms rather than 4 treatment arms) and provides the complete analysis plan for the revised protocol using hu14.18-IL2 alone,without administration of cilengitide.

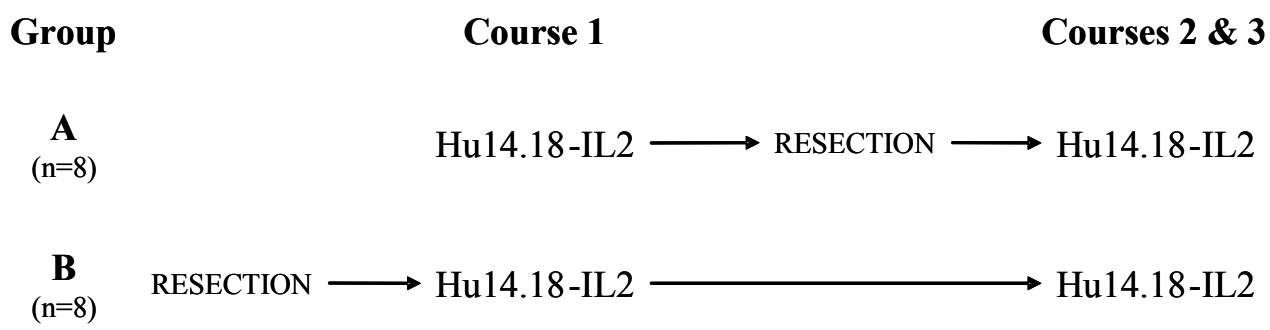
SCHEMA

Treatment groups:
At the time of study entry, subjects will be randomized to Group A or B**.** Subjects in both groups will receive 3 cycles of immunocytokine (IC) therapy. Immunocytokine therapy will be given on days 1, 2, and 3 of each 28-day treatment course. Subjects randomized to Group A will undergo surgical resection of all sites of known disease following the first course of treatment. Subjects randomized to Group B will undergo surgical resection of all sites of known disease prior to the first course of treatment. This treatment schema allows for evaluation of the biological effects on the tumor of one cycle of Hu14.18-IL2 therapy.

SURGICAL RESECTION FOR GROUP A
(Course 1 of protocol therapy prior to surgery):
Subjects randomized to **Group A** will have complete surgical resection of all detectable melanoma following Course 1 of protocol therapy. Surgical resection will be scheduled for 7-14 days following the completion of the first course of treatment (i.e., days 10-17) in order to insure reversal of toxicities prior to the surgery. Once the subject has recovered from the surgery (2-4 weeks, depending upon the nature of the surgery), the subject will begin Course 2 of treatment. Initiation of course 2 will not be sooner than 4 weeks (29 days) after day 1 of the first course of study therapy. A total of 3 courses of protocol therapy are planned.

SURGICAL RESECTION FOR GROUP B
(Surgery prior to Course 1 of therapy):
Subjects randomized to **Group B** will have complete surgical resection of all detectable melanoma upon study entry, prior to Course 1 of protocol therapy. Once the subject has recovered from the surgery (2-4 weeks, depending upon the nature of surgery), the subject will begin Course 1 of hu14.18-IL2 treatment. A total of 3 courses of protocol therapy are planned.

A summary of the treatment groups and how they differ is shown:



Study agent administration:

TREATMENT COURSE[1]										
WEEK	**1**							**2**	**3**	**4**
DAY	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8-14**	**15-21**	**22-28**
Hu14.18-IL2[2]	**X**	**X**	**X**							

[1] All subjects will be scheduled to receive a total of 3 courses of treatment. Treatment will be stopped if it can not be tolerated or if there is recurrent disease.

[2] Hu14.18-IL2 will be administered as a 4-hour IV infusion on days 1, 2, and 3 in the first week of each treatment cycle. Each treatment cycle is 4 weeks (+/- 7 days). Subjects in this study will be administered 6 mg/m^2/d of hu14.18-IL2, a dose corresponding to one dose below the maximal tolerated dose (MTD) as determined in our completed Phase I study.

1.0 BACKGROUND

1.1 Development of the hu14.18-IL2 immunocytokine

Murine models suggest two distinct mechanisms by which IL2 treatment can mediate antitumor effects (1). IL2 treatment augments antigen-specific T cell recognition and destruction of neoplastic tissue. IL2 also activates a separate population of cells referred to as natural killer (NK) cells. These NK cells, which lack mature T cell markers and antigen-specific receptors, destroy tumor targets *in vitro* and *in vivo* (2, 3). Treatment of cancer subjects with IL2 has been shown to increase the number and *in vitro* antitumor activity of these NK cells. Unfortunately, these cells are also responsible in part for the significant dose-dependent toxicity of IL2 (4, 5). A more selective induction of tumor-specific T cells, rather than activated NK cells, may provide better antitumor specificity. *In vitro* data suggests that some subjects with melanoma (and certain other cancers) have antigen specific T cells with antitumor activity that may be enhanced by treatment with IL2 (6). However, tumor-specific T cell reactivity has still been difficult to document for most human tumors, and certain data suggest that systemic *in vivo* IL2 treatment may actually cause a decrease in specific function of endogenous T cells (7).

One approach to improve the efficacy of IL2-based treatment is to focus the cytolytic activity of activated NK cells to mediate increased tumor specific destruction (8, 9). This can be demonstrated *in vitro* with the addition of mAb to facilitate antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC is primarily mediated by Fc receptor-bearing NK cells. In recent years, clinical trials have demonstrated that some mAbs effectively localize to tumors *in vivo* (10, 11). However, most mAbs induce relatively little tumor lysis and their clinical antitumor activity has been limited. Activating effector cells *in vivo* with IL2 may augment mAb mediated ADCC of tumor cells. *In vitro* studies have documented that ADCC is increased when effector cells have first been activated with IL2. Mice receiving IL2 plus tumor specific mAb show improved antitumor effects over animals treated with either agent alone (12-14). Only intact mAb, not Fab fragments, were able to induce ADCC *in vitro* or provide the antitumor effects *in vivo* (13). These results suggest that direct effector cell-tumor cell contact is involved in the antitumor mechanism and that it may be possible, with *in vivo* activation of effector cells by IL2, to improve clinical antitumor effects of mAb treatment.

This approach has been attempted in clinical trials of anti-ganglioside mAb combined with IL2 (15). GD2 is a disialoganglioside expressed on tumors of neuroectodermal origin including melanoma, neuroblastoma and certain sarcomas. Expression on normal tissues is limited to the cerebellum, peripheral nerves, and very few other tissues. The relatively tumor selective expression of GD2 makes it a suitable target for mAb treatment. *In vitro,* anti-GD2 antibodies can mediate substantial ADCC and complement-dependent cytotoxicity (CDC) against $GD2^+$ tumor target cells (9).

The original IgG3 murine anti-GD2 mAb 14.18 was developed by Dr. Ralph Reisfeld (16). An IgG2a class switch variant of 14.18, called 14.G2a, was prepared in an attempt to mediate enhanced ADCC. This antibody has been tested as a single agent and in combination with IL2 in clinical trials. When antitumor activity was noted, a human/murine chimeric mAb ch14.18 was then constructed using the murine variable genes of 14.18 and the human constant IgG1 and κ genes, which are known to be effective at CDC and ADCC (17). In creating this chimeric mAb, an attempt was made to improve the clinical utility of the antibody by reducing its immunogenicity and prolonging its half-life. The ch14.18 antibody was tested in Phase I clinical trials alone and in combination with IL2 in the treatment of subjects with melanoma.

In an effort to improve the immunologically-mediated antitumor activity, an immunocytokine (IC) composed of ch14.18 antibody and the cytokine IL2 was developed by Dr. Stephen D. Gillies *et al*. The chl4.18-IL2 IC expression vector was constructed by fusing a synthetic human IL2 gene sequence to the carboxyl end of the human Ig gene (18). A synthetic DNA linker, extending from the end of the antibody coding sequence to the IL2 sequence, was used to join the amino terminal codon of mature IL2 to the end of the CH_3 exon. The fused gene was inserted into the vector pdHL2. The plasmid was expressed in Sp2/0-Ag14 cells. For preclinical experiments, the IC was purified using protein A-sepharose as an affinity absorbent. This ch14.18-IL2 IC has undergone extensive preclinical testing, in collaboration with Dr. Ralph Reisfeld and colleagues, and has shown significant tumor reactivity and clinical potential (19-21).

The chl4.18-IL2 IC was designed to improve lysis of tumor cells by effector cells in cancer subjects. Despite the previous observation of ADCC with anti-GD2 mAbs used in combination with soluble IL2, this mechanism is potentially limited by the number and type of available effector cells. Achieving ADCC depends upon the presence of effector cells with Fc receptors and upon the number of Fc receptors on the effector cells (22). When NK cells with Fc receptors are activated and expanded with IL2 *in vivo,* they mediate dramatically augmented ADCC (9). However, many of the activated NK cells circulating in cancer subjects following *in vivo* treatment with IL2 do not have Fc receptors, in contrast to resting NK cells (23). These Fc receptor negative, activated NK cells are more cytolytic in direct lytic assays not dependent on mAb and Fc receptors. Thus, it would be advantageous to enable these activated NK cells to lyse tumor using a recognition mechanism that does not depend upon Fc receptors.

The *in vivo* activated NK cells have augmented expression of the IL2R-β (24) and demonstrate a dramatic *in vitro* response to IL2 (25). Furthermore, IL2R-bearing T cells that may not be able to specifically recognize these tumors (with their TCRs) should still be responsive to IL2. Thus, it would be advantageous to activate these IL2 receptors with a molecule that will bridge the NK cells and T cells to tumor and activate lytic interactions. This is the proposed function of the ch14.18-IL2 IC. This molecule utilizes the 14.18 anti-GD2 antibody-mediated recognition component to bind to tumor cells, the Fc component to bind to cells expressing Fc receptors, and the IL2 component to activate cells expressing IL2 receptors on lytic cells. These interactions should result in effector cell binding to tumor followed by activation of the lytic mechanisms. The laboratories of Drs. Gillies and Reisfeld, as well as our laboratory, have shown that activation of effector cell mediated lysis can occur for both T cells with IL2 receptors and NK cells (18, 26). The ch14.18-IL2 IC has also been shown to facilitate antitumor activity in a murine SCID human tumor xenograft model (27) and in conventional mice bearing

syngeneic tumors transfected to express GD2 (19). Initial studies of this IC, using an ELISA that detects the IgG component, showed chl4.18-IL2 has a markedly longer half-life than IL2 (28). Thus, it is likely that this ch14.18-IL2 IC prolongs the half-life of IL2 and concentrates IL2 in the local tumor environment.

Hu14.18-IL2 was developed to maintain the immunologic activity of the ch14.18-IL2 IC molecule and decrease IC-related human anti-chimeric antibody (HACA) responses and allergic reactions when given to humans. Our Phase I clinical trial of hul4.18-IL2 in adults with melanoma has shown that hu14.18-IL2 is clinically safe and well-tolerated at doses (0.8 – 7.5 mg/m^2) that induce immunologic activation.

1.2 Preclinical and clinical experience with 14.18-based antibodies with or without IL2

The following section first reviews clinical testing with the 14.18-based antibodies and then summarizes progress and preliminary data from our laboratory and the laboratories of our collaborators in the development and clinical testing of anti-GD2 mAbs together with IL2.

1.2.1 Prior clinical experience with 14G2a and ch14.18 plus IL2

Over the past decade, clinical trials have tested the antitumor activity, toxicity, and biologic activity of murine (14.G2a) and chimeric (ch14.18) anti-GD2 mAbs (16, 17). These antibodies have been tested as single agents, or in combination with cytokines. More recently, these antibodies have been used in association with supportive therapies (e.g., use of mAbs after high-dose chemotherapy and autologous bone marrow transplantation (29)).

Toxic effects of 14.G2a and ch14.18 have included allergic reactions (urticaria, angioedema) and neuropathic pain, particularly in the lower extremities, pelvis, and abdomen, during the antibody infusions and for a short time after the infusion. The mechanism for this pain is theorized to be binding of the antibodies to GD2 determinants expressed on peripheral nerve sheaths in certain anatomic locations, particularly on pain fibers. Although unwanted, these toxic effects have been manageable. A smaller number of subjects receiving relatively high doses of anti-GD2 antibodies have experienced more profound and longer lasting reversible neuropathic sequelae, including motor and sensory disturbance lasting up to several weeks. The mechanism of this neuropathic toxicity, its frequency, the relative role of chimeric versus murine antibody, and the potential involvement of host antibodies to the administered anti-GD2 antibody in this process, are all uncertain. However, continued studies of ch14.18 plus IL2 in children and adults with cancer have delineated safe doses of these agents combined, and antitumor responses have been observed. The following is a summary of published clinical data with these anti-GD2 mAbs.

1.2.2 Phase I testing of 14.G2a

In a trial conducted at the M.D. Anderson Cancer Center, adults with melanoma or neuroblastoma received the murine anti-GD2 mAB 14.G2a by 5-day continuous IV infusion (30). Dose-limiting toxicity (DLT) of fever, intractable diarrhea, and nausea/vomiting were seen at 40 mg/m²/d x 5 days of 14.G2a. The MTD of the antibody in this trial was 30 mg/m²/d x 5 days. At the 40 mg/m²/d dose level, two subjects developed slowly reversible hyponatremia and postural hypotension. In both of these cases, evaluation of the hyponatremia could not distinguish between salt-losing nephropathy and syndrome of inappropriate antidiuretic hormone (SIADH). The hypotension was attributed to autonomic dysfunction in both cases; other objective neurologic abnormalities were not described. In this trial, one partial response was seen in a subject with neuroblastoma, treated below the MTD.

In a separate trial conducted at the University of Alabama, adults with melanoma or neuroblastoma received the murine anti-GD2 mAB 14G2a by a 1-hour intravenous infusion on days 1, 3, 5, and 8 (31). Subjects tolerated total doses of 10 mg of 14G2a without significant side effects. However,

toxicity was more severe among 10 subjects treated with total doses of 60 mg or 120 mg. All 10 of these subjects experienced abdominal/pelvic pain requiring IV morphine during the time of 14.G2a infusion. Five of these 10 subjects had delayed neurophathic pain in the extremities. One subject developed confusion, and 2 developed hyponatremia consistent with SIADH (one complicated by seizures during hyponatremia). Hyponatremia in these subjects fully resolved within one week of cessation of 14.G2a. Three of the 10 subjects receiving 60-120 mg of 14.G2a developed sensorimotor neuropathy. Nerve conduction studies in these subjects were consistent with demyelination. Sural nerve biopsy was performed on 1 subject and demonstrated demyelination. Neuropathy persisted for several weeks after antibody administration, but fully restored in 2 subjects, with persistent dysesthesia remaining in the third subject. The onset of the neuropathy was typically within 48-72 hours after the day 8 antibody dose.

Laboratory monitoring performed with this trial demonstrated 14.G2a is highly immunogenic. Subjects developed high human anti-mouse antibody (HAMA) titers at treatment day 10, associated with disappearance of 14.G2a from the serum. A trend toward higher HAMA titers in the subjects with greater toxicity was observed. It is uncertain why the M. D. Anderson subjects tolerated higher doses of 14.G2a than did the Alabama subjects. One hypothesis is the day 8 dose of 14.G2a in the Alabama subjects allowed greater binding of 14.G2a to nerve tissue concurrent with the onset of the HAMA response, leading to greater neurotoxicity at lower doses.

Overall for this trial, one mixed and one partial response (at 60 and 120 mg total antibody dose, respectively) was seen in melanoma subjects. As some of the toxicity noted in these two trials potentially related to allergic or HAMA reactivity, subsequent testing of the chl4.18 chimeric antibody was initiated.

1.2.3 Phase I testing of ch14.18

In a Phase I trial conducted at the University of Alabama, adults with metastatic melanoma were treated with chl4.18. The first 13 subjects received a single treatment with chl4.18, administered as a single dose of 5-45 mg (IV over 4 hours), or as 2 daily doses of 50 mg each (100 mg total). Fifty mg was chosen as the maximum daily dose because abdominal/pelvic pain during the antibody infusions precluded administration of higher doses. This pain was severe in 3 of 4 subjects receiving 100 mg of antibody, but was also observed in subjects receiving 15 or 45 mg of antibody. No other neurologic side effects occurred, and no other severe toxicity was noted. Eight of 13 subjects developed antibodies to ch14.18, but the observed titers were only about one-tenth as great as those detected in the trials of murine 14.G2a. Anti-idiotype and anti-isotype antibodies were detected. Although no antitumor responses occurred, antibody was detected on tumor cells by fluorescence-activated cell sorter analysis in some subjects treated with ch14.18. Results from the 13 subjects treated on this study have been reported (32).

In a separate Phase I trial conducted in Tubingen, Germany, 9 subjects (ages 2-10) with neuroblastoma received up to 50 mg/m^2 of chl4.18 daily for 5 days. All subjects had been heavily pretreated with chemotherapy, including prior high-dose treatment and in some, autologous bone marrow transplantation. Six of the 9 subjects experienced partial tumor responses. Optic nerve atrophy was observed in 2 subjects, manifested by decreased visual acuity, and pupillary atony in 1 subject. In both cases, prior radiotherapy had been administered and was implicated as contributing to the observed toxicities. These visual adverse events gradually resolved in the six months following treatment with ch14.18. Subjects experienced pain during antibody infusion, but no other serious toxicity occurred (33). More recently, this regimen has been modified to 5 days of ch14.18 treatment at a dose of 20 mg/m^2/d, given every 6 weeks. Thirty-five courses have been given with no neurotoxicity and few side effects; all pain was transient and well controlled with morphine. Antitumor responses have been observed (personal communication, R. Handgretinger).

1.2.4 Phase I testing of IL2 plus 14.G2a or ch14.18

While the 14.G2a and chl4.18 mAbs mediate ADCC, they do this more effectively with activated effector cells. PBL from subjects treated with IL2 *in vivo* can mediate enhanced ADCC activity (7). This laboratory finding suggests anti-GD2 antibodies should be tested in subjects receiving *in vivo* IL2 treatment.

We initiated this approach through the Children's Cancer Group (CCG) in a trial treating 39 children with neuroblastoma using murine 14.G2a plus IL2 (protocol CCG-0901) (34). Subjects received 3 million units/m^2/d IL2 (Hoffman La Roche) by continuous infusion for 4 days/week for 3 weeks (i.e., beginning on days 1, 8, and 15, and ending on days 5, 12, and 19, respectively). Previous studies documented the activation of NK cells and ADCC effectors after this first week of IL2 treatment (9, 25). Thus, 14.G2a was administered over a 2-hour infusion on days 9-13, at doses of 2, 10, 15, or 20 mg/m^2/d to successive cohorts of subjects when NK activity was known to be enhanced. Because of pain (during infusion) and urticaria/angioedema observed at the 20 mg/m^2 dose level, 15 mg/m^2 was considered to be the MTD. No subject had prolonged neurotoxicity. One subject with a large retroperitoneal mass had a partial response (70% shrinkage of a large abdominal mass over three months). When the residual tumor was resected from this subject, it was found to contain a small central core of viable $GD2^+$ tumor surrounded by fibrotic scar tissue. The subject subsequently underwent high-dose chemotherapy and successful autologous bone marrow transplantation and remained disease-free after 3-plus years. Three other subjects had transient (<3 months duration) reduction in tumor as quantitated in the bone marrow.

Sera obtained from subjects treated on this study contained sufficient antibody to mediate ADCC against $GD2^+$ targets *in vitro* using subject's own circulating PBL as effector cells (35). HAMA was observed in a minority of subjects, and HAMA titers in this study were relatively low, compared with those observed in adults on other trials of 14.G2a.

A separate pilot study conducted at the University of Wisconsin treated four adults with melanoma with a similar regimen to that used in the CCG Neuroblastoma Trial. Subjects received $1.5x10^6$ U/m^2/d IL2 (Hoffman La Roche) by continuous infusion for 4 days/week, for 3 weeks. 14.G2a was given daily x 5 during the second week, at a dose of 2 or 5 mg/m^2/d. Subjects received premedication with antihistamines and morphine, and had no significant side effects attributable to 14.G2a. IL2-related side effects, commensurate with those predicted at the IL2 dose and schedule used, were observed. No antitumor responses occurred. All 4 subjects developed HAMA, as well as measurable anti-idiotype antibodies (36, 37).

In a subsequent trial, conducted at the University of Wisconsin, (N=24) subjects with melanoma received 1.5 x 10^6 U/m^2 IL2 (Hoffman La Roche) by continuous infusion for 4 days/week, for 3 weeks, as in the trial of IL2 plus 14.G2a. In this study, chl4.18 was administered daily x 5 days during the second week (days 9-13), at doses of 2, 5, 7.5, or 10 mg/m^2/d. At 10 mg/m^2/d of the antibody, DLTs included a severe allergic reaction in one subject, and weakness, pericardial effusion, and decreased performance status in another. Consequently, 7.5 mg/m^2/d ch14.18 was determined to be the MTD. A total of 15 subjects were treated at the MTD, with some variation in the sequencing of the IL2 and chl4.18 infusions. Most subjects treated at the MTD had abdominal, chest, and/or extremity pain requiring IV morphine for adequate control of their pain. One subject treated at the MTD experienced peripheral neuropathy characterized by temperature misperceptions. This was evaluated with nerve conduction velocity studies, which indicated the presence of sensory axonal/demyelination and polyneuropathy. This peripheral neuropathy clinically improved following protocol treatment, but repeat nerve conduction testing was not performed as the subject expired soon after treatment due to continued disease progression. There was no additional severe

or prolonged neuropathy in other subjects, as was observed in the trials of 14.G2a. One subject treated at the MTD had shrinkage of hepatic metastases in the face of recurrence of subcutaneous lesions; one subject had a complete response of all disease (a 1.6-cm axillary node); and one subject had a partial response in a pelvic mass. A total of 24 subjects were treated on the chl4.18 and IL2 protocol, and treatment was generally well-tolerated (37). Analyses of these subjects showed virtually no HAMA response; the HACA response observed appeared to be attenuated by IL2 pretreatment (33). More recently, a separate trial at the UWCCC added 5 days of murine R24 (anti-GD3) mAb to this same IL2 and chl4.18 regimen. Twenty subjects have completed treatment and none had clinical evidence of peripheral neuropathy.

1.3 The humanized hu14.18-IL2 immunocytokine

1.3.1 Rationale for creation of hu14.18-IL2

Despite the substantial human component of the chimeric ch14.18 antibody, several adult subjects treated with this antibody have developed strong anti-idiotype antibody responses. Furthermore, in some adults, the DLT included allergic symptoms (angioedema) (32, 36). As these reactions may be related to the murine component of this chimeric antibody, the use of the humanized form of 14.18 is being pursued. Hu14.18-IL2 is expected to behave functionally like the chl4.18-IL2 molecule, but with a decreased tendency toward induction of allergic reactions and anti-idiotypic antibody formation when given to humans.

1.3.2 Construction of hu14.18-IL2

S. Gillies and colleagues utilized the same technology used to create chl4.18-IL2 (18) for the construction of hu14.18-IL2. The hu14.18 Ab portion contains only the complimentarity-determining region (CDR) regions of the murine variable chains, grafted into the intact human IgG_1 molecule, which has an IL2 molecule at each carboxy terminus of the IgG_1 heavy chains.

1.3.3 Function of hu14.18-IL2

In vitro assays that have been performed with hu14.18-IL2 include the following:

1. Binding to $GD2^+$ tumors, detected by flow cytometry.

2. Facilitation of ADCC *in vitro* using PBL from IL2-treated subjects as effector cells.

3. Induction of proliferative responses by IL2-responsive cells, when tested as soluble IC, and when tested as IC bound to $GD2^+$ tumor cells.

In all these assays, hu14.18-IL2 showed comparable function (both quantitatively and qualitatively) to chl4.18-IL2 (J.A. Hank et al., unpublished data).

1.3.4 Preclinical experience with hu14.18-IL2

1.3.4.1 Pharmacology and specificity

Preclinical studies indicate that the hu14.18-IL2 behaves pharmacologically and toxicologically like IL2, with altered kinetics and distribution. A non-GLP pharmacokinetic (PK) and toxicity study has now been completed in non-human primates (Dahl and Gillies, unpublished data). Hu14.18-IL2 is cleared from the plasma of non-human primates with biphasic kinetics, with initial distribution followed by an elimination half-life intermediate between the free cytokine and the antibody alone. Over the course of 24 hours following IV administration, the two phases combine to remove approximately 90% or more of the protein from the plasma compartment. Tissue binding studies indicate that the normal tissue distribution of hu14.18-IL2 is the same as

that of the unfused antibody, i.e. central and peripheral nerve tissue, endothelial cells, and some lymphoid cells, with a low level of additional distribution to Fc and IL2 receptors.

1.3.4.2 Adverse events

Since IL2 effects in both mice and non-human primates are qualitatively similar to humans, preclinical toxicology studies have been conducted in both mice (non-GLP) and rhesus monkeys (GLP). In general, toxicity of hu14.18-IL2 is related to IL2 activity, with an MTD in monkeys of >16 mg/m^2/d x 5 days (These details are included in the Investigator's Brochure, included with the IND submission 1-25-05). A dose of 48 mg/m^2/d induced symptoms of anaphylaxis in monkeys on the 5th day of dosing, suggesting that for shorter courses of therapy, the MTD is greater than 48 mg/m^2/d. The most notable symptom observed in the 16 mg/m^2/d group was a slight decrease in blood pressure, consistent with known IL2 activity. The most notable finding at necropsy was reddened, enlarged lymph nodes, suggestive of inflammation, and consistent with IL2 immunological activity.

1.3.4.3 Confirmatory primate testing

Additional primate studies corroborate both the PK and toxicity findings. A 15-week toxicity study investigated potential toxicity of hu14.18-IL2 after repeated IV dosing in Cynomolgus monkeys. Three animals per sex per group received an IV infusion of hu14.18-IL-2 over one hour at doses of 0, 0.2, 0.64, and 2 mg/kg body weight.

Animals are treated for 5 consecutive days (Monday through Friday) in study weeks 1, 3, 5, 7, 9, 11, 13, and 15 (every second week). After the 5th administration, all female animals of the high dose group (approximately 60 mg/M^2/d) showed weakness and slowed movement. On study day 6, 1 female monkey (animal Number 34) of the high dose group (2 mg/kg) was found dead. This death was considered to be related to treatment with hu14.18-IL2. At gross pathological examination approximately 10-ml yellowish liquid was detected in the abdominal cavity. The ascites could be ascribed to IL-2 toxicity. On study day 34 (week 5), female monkey Number 23 treated with hu14.18-IL-2 at 0.64 mg/kg body weight was found dead after it had received a total of 14 IV infusions with hu14.18-IL2 in study weeks 1, 3, and 5. On study day 33 (day prior to death), the animal was found in lateral position, was very calm and reflexes were reduced. For this reason, treatment was skipped. Results from histopathological investigations were consistent with IL-2 toxicity, e.g. inflammation and/or histiocytosis of most organs and edema. Based on these results, the death of monkey Number 23 was probably related to treatment with hu14.18-IL2. On study day 75 (week 11), male monkey Number 28 of the high-dose group (2 mg/kg/day) was found dead after it had received a total of 27 IV infusions with hu14.18-IL-2 in study weeks 1, 3, 5, 7, 9, and 11. Treatment was skipped on Thursday and Friday in study week 9 for this animal. All other doses were administered as planned. On the basis of the results from gross necropsy, the relationship between the cause of premature death of male Number 28 and treatment with hu14.18-IL2 remains unclear.

1.3.5 Human experience with hu14.18-IL2

The initial Phase I protocol for hu14.18-IL2 (designated EMD 273063, by EMD Serono, Inc., Rockland, MA, formerly by EMD Pharmaceuticals, Inc., Durham,NC, the sponsor of that trial) entitled Phase I/Ib Trial of the EMD 273063 Fusion Protein in subjects with GD2+ tumors (CO 98901), was initiated in September 1998 at the University of Wisconsin, Madison and treatment was completed in March 2002 (38). A total of 33 subjects with melanoma were enrolled to establish the MTD. Subjects were enrolled in cohorts of 3 or 6 subjects and administered hu14.18-IL2 at one of the following dose levels: 0.8, 1.6, 3.2, 4.8, 6.0 or 7.5 mg/m^2/day. Hu14.18-IL2 was administered as a 4-hour IV infusion over 3 consecutive days during the first week of each course. Subjects with tumor regression or stable disease were given a second

course of treatment with hu14.18-IL2 four weeks after Course 1. Thirty-one subjects completed one course of treatment with hu14.18-IL2, and 19 subjects had stable disease following Course 1 and proceeded to receive a second course of treatment. The dose of 7.5 mg/m^2/day was found to be the MTD, as 2 of 6 subjects showed reversible dose-limiting toxicity at this dose level (Table 1).

Table 1.
Dose-Limiting Toxicities Observed During Course 1 in Subjects Receiving HU14.18-IL2

Dose (mg/m^2)	Total Subjects	Dose-Limiting Toxicity (number of subjects)
0.8	**3**	**None**
1.6	**6**	**AST (1)[1]**
3.2	**6**	**Hyperglycemia (2)[2]**
4.8	**6**	**Hypophosphatemia (4)[3], Thrombocytopenia (1)**
6.0	**6**	**Hypoxia (1), Hypophosphatemia (3)[3]**
7.5[5]	**6**	**Hypoxia (1), AST/ALT (1)[4], Hypotension (1), Hyperglycemia (1), Hypophosphatemia (3)[3]**

[1] Grade 3 elevation of aspartate aminotransferase (AST).
[2] Subjects with known diabetes mellitus. The protocol was amended to exclude subjects with a known history of diabetes mellitus as this therapy may alter blood glucose levels.
[3]Protocol amended to exclude grade 3 hypophosphatemia as a dose-limiting toxicity.
[4] Grade 3 elevation of AST and alanine aminotransferase (ALT).
[5] The dose of 7.5 mg/m^2/day was found to be the MTD, as 2 of 6 subjects showed reversible dose-limiting toxicity at this dose level.

Overall, hu14.18-IL2 was well tolerated, with most subjects experiencing mild to moderate adverse events (AEs), mainly known IL-2 side effects. Ten of 33 subjects (course 1) and 6 of 19 subjects (course 2) experienced pain related to the hu14.18-IL2 infusion that required opioids for pain management. Treatment related Grade 3 toxicities included hypotension (2 subjects; one subject did not require pressors), hypophosphatemia (11 subjects), hyperglycemia (3 subjects, 2 with known diabetes), transient hypoxia (3 subjects), aspartate aminotransferase elevation (2 subjects), hyper-bilirubinemia (1 subject) and transient fever >40^o C (1 subject). No Grade 4 toxicities were observed. Adverse events are summarized in table 2 and 3 on page 16 and 17. Reversible Grade 3 hypotension was only seen in the 7.5 mg/m^2 dose level and was the toxicity that primarily defined the MTD.

Laboratory analyses showed lymphocytosis, expansion of CD16+ and CD56+ NK cells, marked peripheral lymphocyte activation (evidenced by a dose-dependent elevation of circulating sIL2R alpha chain), and enhanced ability of peripheral blood mononuclear cells (PBMC) to mediate GD2+ tumor cell killing through ADCC. Thus hu14.18-IL2 has demonstrated biological activities. PK results indicate the serum $t_{1/2}$ of hu14.18-IL2 to be 3.7 hours.

Table 2. Adverse Events Observed with Hu14.18-IL2

Adverse Event[1]	Dose (mg/m^2) (course 1/course2)					
	0.8	1.6	3.2	4.8	6.0	7.5
Total Number of Subjects	3/2	6/6	6/1	6/5	6/3	6/2
Fever[2]						
39.1- 40 ºC	1/0	2/1	0/0	1/3	5/0	4/1
> 40°C	0/0	0/0	0/0	0/0	0/0	0/0
Rigors or Chills (requiring opioids)	2/1	3/2	3/0	2/2	4/1	2/0
Hypotension						
Grade 2	0/0	0/0	1/0	3/3	2/1	1/0
Grade 3	0/0	0/0	0/0	0/0	0/0	1/1[3]
Transient hypoxia (grade 3)	0/0	0/0	0/0	0/0	1/0	0/1
Pruritis (grade 2)	0/1	1/4	2/0	3/3	3/2	2/1
Severe allergic reaction	0/0	0/0	0/0	0/0	0/0	0/0
Pain[2]						
Requiring opioids	0/0	1/3	0/0	3/1	2/1	4/1
Uncontrolled with opioids	0/0	0/0	0/0	0/0	0/0	0/0
Objective peripheral neuropathy	0/0	0/0	0/0	0/0	0/0	0/0
Decline in ECOG[4] performance status >2	0/0	1/0	1/0	3/1	2/1	0/0

[1] Adverse events (all grade 3 and clinically significant grade 2 events) for all subjects for all courses were graded as defined by the NCI Common Toxicity Criteria unless otherwise indicated.
[2] Fever and pain were graded as defined by the University of Wisconsin Comprehensive Cancer Center Biologic Toxicity Criteria.
[3] Subject did not require vasopressors.
[4] Eastern Cooperative Oncology Group performance status scale.

Table 3.
Laboratory Changes Observed With Hu14.18-IL2

Laboratory Value[1]	Dose Level (mg/m^2) (course 1/course 2)					
	0.8	1.6	3.2	4.8	6.0	7.5
Total Number of Subjects	3/2	6/6	6/1	6/5	6/3	6/2
Hemoglobin 8.0 - <10g/dL (grade 2)	0/0	0/0	0/0	0/0	1/2	1/0
Absolute Neutrophil count						
1000 - 1499/μl (grade 2)	0/1	0/0	0/0	2/0	2/1	2/1
<1000/μl (grade 3)	0/0	0/0	0/0	0/0	0/0	0/0
Platelet count ≥10 - <50,000/mm^3 (grade 3)	0/0	0/0	0/0	1/0	0/0	0/0
Aspartate aminotransferase (AST)						
>2.5 - 5 times normal (grade 2)	1/0	1/2	1/0	2/1	2/0	0/0
>5 times normal (grade 3)	0/0	1/0	0/0	0/0	0/0	1/0
Total bilirubin						
>1.5 - 3 times normal (grade 2)	0/0	0/0	0/0	2/0	0/0	1/0
>3 times normal (grade 3)	0/0	0/0	0/0	1/0	0/0	0/0
Hyperglycemia						
>250 - 500 mg/dl (grade 3)	0/0	0/0	2[2]/0	0/0	0/0	1/0
Hypophosphatemia						
≥1.0 - <2.0 mg/dl (grade 3)	0/0	0/1	0/0	4/0	3/0	3/0

[1] Laboratory values (all grade 3 and clinically significant grade 2 values) for all subjects for all courses were graded as defined by version 2.0 of the NCI Common Toxicity Criteria.
[2] Subjects with known diabetes mellitus.

Although this phase I study was not designed to determine overall tumor response rate to treatment, all subjects were followed for antitumor activity. No subject showed improvement of measurable disease to qualify as a complete or partial response (CR or PR). Eight of the 33 subjects maintained stable disease (SD) after 2 courses of therapy, and 4 of these have since progressed. One subject had an objective decrease in a lung nodule following two courses of therapy, but the overall disease response was scored as disease progression due to growth in a distant node. The node was resected following hu14.18-IL2 therapy, and the subject remained free from disease progression for 3 years, after which disease progression was noted and alternate therapy begun.

Five of the 33 subjects entered the study with no measurable disease following surgical resection of recurrences or metastases. Two of these 5 subjects (treated at 0.8 and 6.0 mg/m^2/day) continue with no evidence of disease (69-102 months). The other 3 showed recurrence at 2, 6 and 92 months after starting hu14.18-IL2 treatment. One additional subject entered the study following resection of proven pulmonary and nodal metastases, but with residual small abnormalities in the lungs that were likely post-operative changes, but potentially residual melanoma. This subject had showed no evidence of progressive disease for 59 months after treatment (4.8 mg/m^2/day), when recurrent disease was noted. The findings in these 2 subjects are consistent with the hypothesis that clinical benefit from an immunotherapeutic intervention is more likely in subjects with a low tumor burden, as has been shown for tumor bearing mice treated with this

agent (39). This experience provides further clinical rationale for this Pilot Protocol for subjects with completely resectable recurrent stage III and/or stage IV melanoma.

Four other clinical trials of hu14.18-IL2 have now been completed. An EMD sponsored multi-institution phase II pilot study in adults with melanoma treated 9 adults with melanoma at 4 mg/M^2/d as a 4 hour infusion on days 1-3 of each course and for 1-4 treatment courses. Preliminary analyses indicate similar clinical tolerance and toxicities as seen in the UWCCC phase I trial (D. Wayne and S. Gillies, unpublished). A separate phase I study, using the same treatment schedule as the UWCCC melanoma study (4 hour infusions for 3 days each month, for 1-6 months) has recently been completed by the Children's Oncology Group (Protocol Chair - P. Sondel). Twenty eight children (27 with neuroblastoma and 1 with melanoma) received doses from 2.0 – 14.4 mg/M^2/d, and demonstrated a similar toxicity pattern to that seen in the UWCCC melanoma Phase I study. The MTD was determined to be 12.0 mg/M^2/d, with toxicity of hypotension and hypoxia found to be dose limiting (40). It is noteworthy that, while no definitive conclusions about dose related hu14.18-IL2 toxicities in children with melanoma can be made from the data from this pediatric study, the toxicities experienced by the one child with melanoma were similar to that seen in the 27 with neuroblastoma. This patient was treated at the initial dose level of 2.0 mg/m^2/d.

In addition, COG has recently completed a Phase II study of hu14.18-IL2 in children with neuroblastoma (Protocol ANBL0322; Protocol Chair- P. Sondel). It was being administered at 12.0 mg/m^2/d (the MTD from the preceding Phase I study in neuroblastoma). A total of 39 children were enrolled. One of these subjects experienced a serious adverse event during the second cycle of treatment as a consequence of acute vascular leak syndrome. While vascular leak syndrome is a known side-effect of hu14.18-IL2, this was unusual as it was Grade 4 and required ventilatory support. It is speculated that this subject's prior requirement for ventilatory support (following an autologous bone marrow transplant) may have predisposed her to this more serious complication. Of 24 patients enrolled with disease evaluable only by bone marrow histology or radiolabelled MIBG scanning, 5 had CRs. Finally, we have recently completed a Phase II trial of this agent, by this same schedule in adults with refractory or recurrent melanoma. Of 14 patients receiving 6.0 mg/m^2/d, one patient had a transient PR, lasting 2 months.

1.5 Study rationale

1.5.1 Rationale for schedule of administration

Administration of hu14.18-IL2

Daily administration of hu14.18-IL2 is indicated based on preclinical and clinical findings demonstrating a substantial clearance of the IC protein from the circulation within a day (approximately 10% or less remaining after 24 hours) (J. Hank, unpublished). In this study, a 3-day treatment cycle has been chosen to provide repeat, prolonged tumor exposure to hu14.18-IL2. The dose and schedule for this study (6.0 mg/m^2 given daily for 3 days every 4 weeks) was determined by our recently completed Phase I study of hu14.18-IL2 (see section 1.5.2.1).

A consecutive 3-day administration schedule was originally chosen for our Phase I study because of concern that a greater than 3 consecutive day administration would result in higher levels of neutralizing antibodies. This was based on preclinical testing with cross-species administration, where daily infusion of the IC protein was found to result in the production of neutralizing antibodies beginning on Day 4. Preliminary data from our laboratory, however, indicates that neutralizing antibodies appear at approximately Day 8 of our Phase I study,

therefore a 3-day administration schedule would be expected to provide a prolonged tumor exposure to hu14.18-IL2 without interference of substantial levels of neutralizing antibodies.

The 3-day administration schedule will be repeated on a 4-week interval, allowing for stable and responding subjects to continue receiving potentially clinically beneficial treatment. In addition, this dosing schedule will allow, in those subjects that have generated any antibody to hu14.18-IL2, the determination of neutralizing capabilities of these antibodies upon re-exposure to hu14.18-IL2. While the preclinical data reflect a cross-species immunogenic effect, a potential anti-idiotypic response is still possible, albeit at a much reduced level, and has in fact been observed in some patients in the Phase I trials (Hank et al., in preparation).

1.5.2 Rationale for dosing

Dosing of hu14.18-IL2

The hu14.18-IL2 dose for this pilot study is 6 mg/m^2 and is one dose below the MTD of 7.5 mg/m^2 established in our recently completed Phase I trial (38). This dose allows for maximal and repeated tumor exposure to hu14.18-IL2 over a 3-day period within the boundaries of the observed side-effect profile determined to be tolerable in our Phase I study. The dose of 6.0 rather than the 7.5 mg/m^2/d was chosen, as the MTD determination in the phase I study was based on DLT events in the first course of treatment. However, most subjects in this study are expected to receive 3 courses of treatment. As some subjects in the adult and pediatric Phase I studies did have episodes of DLT in subsequent courses of treatment, not necessarily seen in the first course, it was determined that starting at the 6.0 mg/m^2/d dose would be more cautious, and more likely to enable subjects to continue on treatment without requiring dose reductions. This dose also corresponds to the dose used in a Phase II study recently completed at the UWCCC for subjects with advanced melanoma with evaluable disease (58).

However, in contrast to the recent Phase II study in advanced melanoma, where subjects received 4 courses of hu14.18-IL2, we propose in this pilot study to administer 3 courses of IC therapy. This decision is based on several factors. First, subjects in this study have a lower tumor burden. Since the subjects in this study will be in a state of minimal residual disease following their resections, we feel it is appropriate to treat them with one less course of therapy than that used for subjects with bulky disease. Secondly, since a proportion of patients can achieve prolonged disease-free survival following surgery of the type proposed here (see Section 1.5.4), we feel that accrual will be challenging if potential subjects are faced with the possibility of four inpatient stays to treat a tumor that may be affected significantly by surgery alone. Lastly, given the finite amount of clinical material at our disposal and the obligation to the ongoing Phase II study in neuroblastoma using this agent (currently open and accruing patients), reducing the number of courses to 3 will increase the amount of hu14.18-IL2 that is available for future additional clinical studies.

1.5.3 Rationale for study population

Study population

Multiple approaches to treating tumor-bearing mice with hu14.18-IL2, and other similarly configured molecules, document that the most striking anti-tumor effects obtained with immunocytokines in tumor bearing mice are obtained when the tumor burden is quite low (39). Initial trial results of hul4.18-IL2 in subjects with GD2$^+$ tumors have documented that hu14.18-IL2 is clinically safe and well tolerated at doses that induce immunologic activation. However, despite this, no clear evidence of measurable anti-tumor effect was seen in the phase I trial (i.e.,

no subjects showed any complete or partial anti-tumor responses). Of 5 subjects treated in the phase I trial with no evidence of disease at the time of treatment, 2 of these 5 subjects (treated at 0.8 and 6.0 mg/m^2/day) continue with no evidence of disease (69-102 months). The other 3 showed recurrence at 2, 6 and 92 months after starting hu14.18-IL2 treatment. Therefore, this protocol is designed to obtain additional clinical experience in treating high risk melanoma subjects that have undergone complete resection of all metastatic disease.

Surgery for recurrent stage III or stage IV melanoma can provide effective palliation for patients with isolated metastases as well as for patients with a limited number of metastases (76-78). In addition, some of these patients achieve long term benefit following surgery. Median survival for patients with melanoma metastatic to a distant site is less than 12 months (76). Prognostic features are identified in the current staging system, with 1-year survival rates ranging from 59 % for patients with metastases to skin, subcutaneous tissue, or distant lymph nodes (M1a disease), to 57 % for patients with metastases to the lung (M1b disease), to 41 % for patients with metastases to other visceral sites or with an elevated LDH (M1c disease) (79, 80). Beneficial results following complete surgical resection of distant metastases have been reported from several different institutions and demonstrate median survivals that depend on the site of the resected metastases (76). The median survival reported from several institutions following resection of skin or subcutaneous disease (17 months to 31 months) is better than the median survival following resection of metastases to the lung (9 months to 19 months) or following resection of nonhepatic gastrointestinal metastases (8 months to 18 months) (76, 81-93). There is a wide range in median disease-free survival following resection of metastatic lesions, with one series reporting a median disease-free interval of 8 months (range 0.6-91.8 months) following complete metastasectomy for an initial recurrence of stage IV melanoma (77). In addition, prolonged disease-free and long-term overall survival has been reported for patients who have a second lymph node dissection after isolated nodal recurrence and recurrent stage III disease (81). However, most of these metastatic melanoma patients still die from melanoma (76). An intergroup trial (E4697; SWOG E4697; CALGB 500101) with a planned sample size of 540 eligible subjects was designed to evaluate adjuvant therapy with GM-CSF or peptide vaccination in this subject population following surgical resection of metastatic disease and is currently in progress ("A Randomized, Placebo-Controlled Phase III Trial of Yeast Derived GM-CSF Versus Peptide Vaccination Versus GM-CSF Plus Peptide Vaccination Versus Placebo in Patients with 'No Evidence of Disease' after Complete Resection of 'Locally Advanced' and/or Stage IV Melanoma"). The subject eligibility for this large intergroup study includes subjects with completely resected disease with one of the following: a) any locoregional recurrence after prior adjuvant interferon or failure on S0008; b) any local recurrence of disease after adequate surgical excision of the original primary; c) mucosal melanoma; and d) stage IV melanoma (cutaneous, ocular, mucosal, or unknown primary). The initial estimate for this subject population was for a median overall survival of 15 months (personal communication; Dr. David Lawson, study chair for E4697). The current pilot study will involve 16 subjects with completely resectable recurrent stage III or stage IV metastatic melanoma who have not yet received surgical resection of this disease.

This pilot protocol is designed to evaluate the effect of 3 courses of treatment with hu14.18-IL2 on the melanoma, the immune system and the overall survival as well as time to tumor progression for a small cohort of subjects with stage IV melanoma that have completely resectable disease. Enrolled subjects will be randomized into one of two treatment groups which differ in the timing of resection as it relates to treatment with the experimental agent (see Schema above). The resected tumors from all subjects will be evaluated histologically; immune assessments will be preformed on peripheral blood cells and serum; and subjects will be

evaluated regularly to determine the time to tumor recurrence and survival following treatment on this study.

Depending upon the results obtained in this study, this same regimen (or a modification derived from it) could be tested subsequently in a larger randomized multi-institutional Phase III trial.

1.5.4 Rationale for experimental design (see Schema for study design)

1.5.4.1 Evaluations of resected tumor

One other parameter for evaluating anti-tumor effect involves direct microscopic evaluation of the resected tumor. When this has been done, following the first course of study therapy (Group A), it will be possible to look at the tumor for evidence of tumor necrosis and apoptosis, as well as for evidence of immune activation (inflammatory infiltrate, cytokine expression). While some of these assays can be done on paraffin fixed tissues, others require analyses of fresh or freshly cryopreserved tumor (for details, see Section 7.4 [Surgical Pathology Guidelines]). For this study at the UWCCC, each tumor nodule from each patient that is obtained at the time of surgical resection will be divided into two components: (1) a component for paraffin fixation (and standard histology), and (2) a component that will be further divided for fresh tissue handling/culturing and a component cryopreserved for frozen section and molecular analyses.

1.5.4.2 Control biopsies obtained before going onto study

As an eligibility criterion, each subject with advanced disease will be required to have a tissue biopsy documenting their recurrent stage III or stage IV melanoma. Subjects must not have any systemic therapy following this biopsy prior to enrolling in this study. These biopsies will be fixed tissue, often from outside hospitals, and will not be analyzable for many of the parameters that will be evaluated on the tissue evaluated at the time of tumor resection here at the UWCCC. Nevertheless, they will provide a control (pretreatment evaluation) for all subjects from a small biopsy of tissue, for at least tumor confirmation, and for many samples allow a crude assessment of inflammatory infiltrate into the specimen. These will be compared to the detailed analyses for tumors resected following Course 1 of treatment (Group A).

1.5.4.3 Surgical resection prior to starting study treatment

While histological assessment of tumors resected after the first course of protocol treatment (Section 1.5.4.1, for patients in Group A) provides microscopic and molecular evaluations for the influence of the first course of therapy, this requires comparison for each patient to their pretreatment biopsy (Section 1.5.4.2). However, the pretreatment biopsy is not being handled in the same way [small core (or at times, fine needle) biopsy fixed in paraffin, often from outside hospitals, versus complete surgical resection of all evident disease, with fixed, frozen and fresh tissue for detailed analyses done entirely at the UWCCC]. Thus, we also wish to compare completely resected melanomas, handled at UW in an identical manner as those from patients in Group A after their first course of study treatment (Section 1.5.4.1), with tumors from subjects who have not received any such treatment at the time of their resection (Group B). Furthermore, as we will be comparing the post-treatment resected tumors from Group A (Section 1.5.4.1) to their prestudy biopsies (Section 1.5.4.2), it would be important to have pretreatment biopsies as controls for those patients having complete resections prior to receiving any study treatment (Group B). Thus, all patients in both study groups will require prestudy biopsies (can be obtained before study entry) to meet eligibility criteria, and to enable comparisons with their detailed analyses of the resected tumors evaluated here at the UWCCC. In this way, the histopathology for the resected tumors from patients in Group B (surgery before study therapy) can serve as controls for the histopathology of the resected tumors from patients in Group A.

1.5.4.4 Surgical resection randomization –Before versus After Course 1 therapy

All subjects will be followed for time to recurrence and overall survival measured from the completion of their second course of treatment. Subjects in all study groups will have spent equivalent time since study entry in getting to this point: all will have had their surgical resection and their first 2 courses of study treatment. The differences will be in the timing of their resection (pretreatment in Group B and after Course 1 in Group A). While Group A subjects also will be providing informative data regarding the influence of one course of hu14.18-IL2 on the histopathology of the resected tumor, Group B will be providing essential histopathology control data. Furthermore, any future use of this approach for patients that can achieve MRD surgically (i.e., a Phase III trial that might be based on data from this study), would be anticipated to utilize the treatment schedule for Group B, as it would allow administration of the optimal therapy (whichever this pilot trial suggests that to be) to patients that can achieve a CR prior to receiving all courses of therapy. Thus obtaining clinical and lab data from all study groups is important. Even though the numbers are small, and there will be much variability in assays and subject characteristics, it makes sense for these treatment groups to be treated at the same time (rather than sequentially), and with an effort made to keep both groups comparable in characteristics and assessments. Thus, subjects entered into this study, following confirmation of meeting all eligibility criteria and obtaining signed informed consent, will be randomized to either of the treatment groups. The randomization will stratify subjects for the single variable of "extent of surgery required". Namely, subjects needing a thoracotomy, a laparotomy, or deep soft tissue excisions in 3 anatomic locations would be considered to need "extensive resection", while those subjects requiring only superficial cutaneous excisions or deep soft tissue excision in only 1 or 2 locations would be considered to need "non-extensive resection". Randomization will be performed for all subjects entered into the study, in order to keep all treatment groups comparable to each other with respect to the number of subjects requiring extensive versus non-extensive resection.

1.5.4.5 Time to recurrence and overall survival

This study is designed to test the hu14.18-IL2 IC for clinical anti-tumor activity in the setting of minimal residual disease (MRD). Preclinical studies with this and other immunotherapies and our somewhat anecdotal experience with subjects without evidence of disease in our Phase I study of hu14.18-IL2 suggest the MRD setting has the best opportunity for inducing clinically meaningful anti-tumor effects. Thus, we will treat patients with recurrent stage III or stage IV melanoma that achieve CR by complete resection of all metastases. The absence of measurable disease for these subjects prevents direct measurement of tumor shrinkage as an indicator of "tumor response". Thus, clinical anti-tumor effects will be evaluated by determining time to relapse and overall survival. These parameters are expected to have a broad range, and data collected will be descriptive in comparing to historical controls.

1.5.4.6 Number of subjects to be entered

The number of subjects to be entered into this study is based on our desire to obtain pilot clinical experience with these regimens in melanoma subjects with MRD and our desire to have adequate tumor tissue from both groups to be able to make laboratory comparisons between them. This has lead us to study groups with 8 subjects each, for a total of 16 subjects. The statistical basis for these conclusions can be found in Section 9.0.

1.5.5 Rationale for analyses of recurrent specimens

Murine models have documented multiple molecular modifications that have allowed tumors to "escape" from immunotherapy. As most clinical trials of immunotherapy have not yet been analyzed in the MRD setting, most human subjects have not demonstrated measurable anti-tumor effect. As such, their progressive tumor had not necessarily escaped from effective immunotherapy; rather, it may not have been influenced by the therapy. We have evaluated tumor escape in mice being treated with hu14.18-IL2. In animals treated with small but established tumors, we evaluated animals that showed a CR or PR in response to the hu14.18-IL2 treatment. Some of these animals showed tumor recurrence following a clear response of their NXS2 tumor to the hu14.18-IL2 treatment. These recurrent tumors showed a slight decrease in their expression of GD2, which is the target antigen for the hu14.18-IL2. In addition, these recurrent tumors showed a > 5 fold increase in the intensity of expression of their MHC class I molecules. These latter molecules are known to activate killer inhibitory receptors on NK cells, making them less able to mediate NK killing or ADCC (39).

In this study, each subject should be receiving hu14.18-IL2 at a time when they have no measurable tumor. Our data in murine models (and similar data in several other murine models) would suggest that these subjects in MRD are likely in the best situation for having an anti-tumor effect (at the cellular level) from this therapy. Any subject who has a recurrence should thus have recurrent disease that somehow escaped from whatever *in vivo* mechanisms of anti-tumor effect might have been mediated by the hu14.18-IL2 treatment. Thus, we wish to evaluate tumors obtained from all entered subjects at the time of their surgical resection. In addition, for those subjects who have a recurrence of their tumor, we will make every effort to obtain a biopsy of the recurrent tumor to evaluate how it might have escaped from this hu14.18-IL2 treatment. This will be done by comparing the recurrent tumor to the tumor obtained at the time of initial tumor resection in this protocol for expression of GD2, HLA class I and II, and other markers relevant to immune mediated recognition and destruction.

2.0 OBJECTIVES

2.1 Primary objectives

2.1.1 Evaluate the histological evidence of anti-tumor activity (apoptosis/necrosis) of hu14.18-IL2 in subjects with advanced melanoma who have achieved a CR through surgical resection of all known metastatic disease approximately 1 week following the first course of treatment with hu14.18-IL2.

2.1.2 Evaluate the time to recurrence (duration of remission) and overall survival following treatment with hu14.18-IL2 in subjects with biopsy-proven recurrent stage III or stage IV melanoma who have achieved a CR through surgical resection of all known metastatic disease.

2.2 Secondary objectives

2.2.1 Evaluate adverse events associated with this experimental agent (3 courses of hu14.18-IL2 treatment for 3 consecutive days administered on an every 4-week basis), as well as the potential late adverse effects of this treatment as detected months or years following treatment for subjects remaining in remission following treatment.

2.2.2 Evaluate the immunologic activation induced *in vivo* by hu14.18-IL2, by evaluating standard clinical labs, and research lab evaluations of serum and PBMCs obtained by blood samplings.

2.2.3 Determine the induction of anti-idiotypic antibodies (anti-hu14.18) and antibody against the part of the compound where IL2 is linked to the Fc end of the IgG (anti Fc-IL2) from blood samples, Determine the expression of the GD2 target antigens, obtained from all subjects in this study.

2.2.4 Evaluate the density and phenotype of the cellular infiltrate, IC binding, chemokine expression, and cytokine expression in tumor specimens from all subjects receiving hu14.18-IL2 before their surgical resection (i.e., Group A), and comparing these results to these same values obtained for tumor samples obtained prior to administration of any protocol treatment (i.e., Group B).

2.2.5 For subjects in Group A with tumor that is readily accessible for biopsy, to obtain a cutaneous/subcutaneous biopsy of a single readily biopsiable lesion before starting protocol treatment in course 1, to allow histology from it to provide a "pretreatment control" for the histological analyses of the resected tumor that will be removed surgically from that same subject following course 1 of study therapy.

2.2.6 For subjects who have autologous tumor cells for functional assays, autologous tumor cell lines that are able to grow *in vitro*, or who are HLA-A2+, evaluate cryopreserved PBMC obtained before and after protocol treatment to evaluate for augmentation of T cell reactivity to autologous tumor or to known melanoma antigens (gp100).

3.0 SELECTION OF SUBJECTS

The subject population will consist of individuals with advanced melanoma. Eligible subjects will meet the inclusion criteria but not the exclusion criteria outlined in this section.

3.1 Inclusion criteria

3.1.1 Subjects must have recurrent stage III (i.e., recurrent regional metastasis), or stage IV (i.e., any distant metastasis) melanoma for which surgical resection would be clinically recommended, with biopsy proven (current or previous) Stage III or Stage IV disease. Any biopsies obtained to demonstrate recurrent regional metastasis or distant metastasis must be considered clinically appropriate for clinical management and must not be performed solely for meeting eligibility criteria. In addition, subjects must have disease that has not yet been completely excised and meets the following criteria:

3.1.1.1 Involves 3 or fewer sites. A nodal basin recurrence will be scored as one site, even if multiple nodes are positive. "Clustered" subcutaneous and/or cutaneous lesions that can be removed in a single surgical excision will be scored as one site, even if multiple subcutaneous and/or cutaneous lesions are present.

3.1.1.2 Subjects disease is determined to be completely resectable with uninvolved margins using standard surgical guidelines based on physical exam and radiographic imaging (MRI or CT of the head, and CT or MRI of the chest, abdomen and pelvis).

3.1.2 Subjects must have one of the following:

3.1.2.1 Stage III melanoma with recurrence after prior surgery, with or without subsequent adjuvant systemic (standard or experimental) and/or radiotherapy management.

3.1.2.2 Stage IV melanoma (cutaneous, ocular, mucosal, or unknown primary)

3.1.3 Subjects must be 18 years old or older **OR** if they are 15 years old or greater, considered to be mature minors, able to give adult informed consent (with parental co-signature), meet all other eligibility criteria, and also weigh at least 45 kg.. Subjects must weigh at least 45 kg in order to safely provide sufficient blood for monitoring studies (see section 7.7 for details).

3.1.4 Subjects must have an ECOG performance status of 0 or 1.

3.1.5 Subjects must have adequate bone marrow, liver, and renal function as defined by:

3.1.5.1 Total WBC $\geq$ 3500/mm^3 (or total granulocytes $\geq$ 2000/mm^3), platelets $\geq$ 100,000/mm^3, and hemoglobin $\geq$ 10.0 gm/dl.

3.1.5.2 AST/ALT < 2-times normal and a total bilirubin < 2.0 mg/dL

3.1.5.3 Serum creatinine < 2.0 mg/dl or a creatinine clearance of $\geq$ 60 ml/minute

3.1.6 Subjects with one or more of the following cardiac risk factors must complete a stress radionuclide scan with no evidence of myocardial ischemia or heart failure: (a) a history of cardiac disease, (b) age greater than 65 years old, (c) any clinically significant abnormality found on ECG (required at baseline), or (d) significant risk factors for coronary artery disease (history of significant dyslipidemia; any treatment for dyslipidemia; or two first degree relatives with a documented myocardial infarction prior to age 55).

3.1.7 Subjects with significant history of pulmonary disease, shortness of breath at rest, or known COPD must have pulmonary function tests within 35% of normal age-predicted values.

3.1.8 Subjects must be willing and able to provide informed written consent prior to any study-related procedures.

3.1.9 Subjects must have no immediate requirements for palliative chemotherapy, palliative radiotherapy, or palliative hormonal therapy.

3.1.10 Subjects must be willing and able to discontinue antihypertensive medications if advised to do so for days of hu14.18-IL2 infusion.

3.1.11 Subjects must have slides available from stage III or stage IV melanoma. Paraffin blocks are preferable, but at a minimum, slides documenting melanoma by biopsy (including fine needle cytology) must be available for pathology review, and potential restaining/staining (see Section 7.4, Surgical Pathology Guidelines). Prior histologic demonstration of metastatic melanoma (either stage III or Stage IV) may be utilized if a repeat biopsy is not clinically needed to to establish eligibility.

3.2 Exclusion criteria

3.2.1 Subjects are ineligible if they have received mAbs during biologic therapy, tumor imaging, purging of autologous marrow/stem cells for re-infusion or for any other reason unless serological testing is performed. If the absence of detectable antibody (over background) to hu14.18 is documented, the subject is eligible for the study.

3.2.2 Subjects treated with IL2 in the past that developed intolerable (Grade 4) IL2-related side effects.

3.2.3 Subjects who have received any (standard or experimental) systemic therapy for stage IV disease.

3.2.4 Women of childbearing potential will be excluded if they are pregnant, nursing, or not using effective contraception during the treatment period.

3.2.5 Subjects with symptoms of ischemic cardiac disease, congestive heart failure, myocardial infarct within the immediate preceding 6 months and/or uncontrolled cardiac rhythm disturbance.

3.2.6 Subjects with significant psychiatric disabilities or seizure disorders.

3.2.7 Subjects who have had major surgery within the past 3 weeks.

3.2.8 Subjects with clinically detectable pleural effusions or ascites.

3.2.9 Subjects with organ allografts.

3.2.10 Subjects who require or are likely to require corticosteriod or other immunosuppressive drugs or have used them within 2 weeks of registration.

3.2.11 Subjects with significant intercurrent illnesses.

3.2.12 Subjects with active infections or active peptic ulcer unless these conditions are corrected or controlled.

3.2.14 Brain metastases, whether active or inactive. A head MRI or head CT scan will be required at baseline to rule out silent metastases.

3.2.15 Active second malignancy other than non-melanoma skin cancer. Patients will be considered eligible if they have been continuously disease free for $\geq$ 5 years prior to the time of enrollment.

3.2.16 Infection with human immunodeficiency virus (HIV), hepatitis B surface antigen (HBs Ag) carrier state or with clinical evidence of hepatitis. Treatment may be initiated before laboratory confirmation of HIV and HBs Ag negativity, but will be stopped if results are positive.

3.2.17 Subjects with a clinically significant neurologic deficit or objective peripheral neuropathy (Grade $\geq$ 2) are ineligible.

3.2.18 Known hypersensitivity to the study drug, Tween-80® or to human immunoglobulin.

3.2.19 Patients with a known history of diabetes mellitus that has required systemic therapy within the past 3 months (either oral hypoglycemic agents or insulin) will be excluded, as treatment with hu14.18-IL2 may alter blood glucose levels.

3.2.20 Legal incapacity or limited legal capacity.

3.2.21 Bone metastases

4.0 REGISTRATION PROCEDURE

4.1 Subjects must not start protocol treatment prior to registration.

4.2 Subjects will be registered directly into Oncore by the Melanoma Research Staff

4.3 Subjects must meet all of the eligibility requirements listed in Section 3.0.

4.4 The following information will be requested during registration:

4.4.1 Protocol number

4.4.2 Investigator's name

4.4.3 Subject's name and medical record number

4.4.4 Subject demographics

4.4.5 Gender

4.4.6 Birthday (MM/DD/YYYY)

4.4.7 Race (according to the NCI classification)

4.4.8 Will complete surgical resection be “extensive” (i.e., require thoracotomy, laparotomy or excision of deep soft tissue metastases in 3 anatomic locations) or “non-extensive” (i.e., not require thoracotomy, laparotomy or excision of deep soft tissue metastases in 3 anatomic locations, but require only cutaneous/subcutaneous excisions and/or excision of deep soft tissue disease in only 1 or 2 locations)?

4.5 Subjects then will be randomized to one of the two study groups. (See Section 9.3.1 for more details on randomization process.)

5.0 TREATMENT PLAN

5.1 Accrual scheme

As outlined in Section 9.0 (Statistical Considerations), a total of 16 subjects will be treated with complete surgical resection and a minimum of 2 courses of protocol treatment. This may mean that additional subjects will need to be entered to account for subjects enrolled into the study and unable to

receive the complete resection and/or entered into the study and unable to receive the first two courses of protocol treatment. Accrual will be stratified to ensure that 8 evaluable subjects are entered into each arm and have received both surgery and 2 courses of study therapy. Stratification will also attempt to enroll similar numbers of subjects requiring extensive surgery and non-extensive surgery (see Section 4.4.8) into each of the treatment groups . The detailed clinical and laboratory monitoring is outlined in protocol Section 7.0 (Study Parameters), Tables 5 and 7.

5.2 Surgical resection of all detectable melanoma

5.2.1 Treatment Group A

Subjects in Group A will have complete surgical resection of all detectable melanoma following Course 1 of hu14.18-IL2. Surgical resection will be scheduled for 7-14 days following the completion of the first 3-day course of therapy (i.e., days 10-17) in order to insure reversal of hu14.18-IL2 toxicities prior to the surgery. These ranges will allow enough flexibility so that the appropriate surgical subspecialist will be available to perform the resection.

Once the subject has recovered from the surgery (2-4 weeks, depending upon the nature of surgery (Section 4.4.8), the subject will begin Course 2 of treatment. Decisions regarding whether a subject has recovered sufficiently from surgery to begin Course 2 of treatment will be left to the discretion of the treating physician(s). To aid in this decision, laboratory tests may be ordered as warranted clinically, but no specific tests are required for this assessment. Subjects undergoing "extensive surgery" (see Section 4.4.8) will not begin Course 2 of study treatment until at least 21 days after surgery. Subjects undergoing "non-extensive surgery" will not begin Course 2 of study treatment until at least 14 days after surgery (see Section 4.4.8). No course of hu14.18-IL2 therapy will be administered sooner than 4 weeks after day 1 of the prior course of hu14.18-IL2 therapy. Subjects will receive a total of 3 courses of treatment as described in Section 5.3.

5.2.2 Treatment Group B

Subjects in Group B will have complete surgical resection of all detectable melanoma upon study entry, prior to Course 1 of hu14.18-IL2. Once the subject has recovered from the surgery (2-4 weeks, depending upon the nature of surgery (Section 4.4.8), the subject will begin Course 1 of treatment. Decisions regarding whether a subject has recovered sufficiently from surgery to begin Course 1 of treatment will be left to the discretion of the treating physician(s). To aid in this decision, laboratory tests may be ordered as warranted clinically, but no specific tests are required for this assessment. Subjects undergoing "extensive surgery" (see Section 4.4.8) will not begin Course 1 of study treatment until at least 21 days after surgery. Subjects undergoing "non-extensive surgery" will not begin Course 1 of study treatment until at least 14 days after surgery(see Section 4.4.8). No course of hu14.18-IL2 therapy will be administered sooner than 4 weeks after day 1 of the prior course of hu14.18-IL2 therapy. Subjects will receive a total of 3 courses of treatment as described in Section 5.3.

5.2.3 Surgical guidelines

The surgical management of subjects in this study will vary depending on the site(s) of tumor. Two broad categories of subjects are eligible: 1) subjects with loco-regional recurrence of resected stage III disease; and 2) subjects with resectable stage IV disease. All subjects will be assessed by a surgical oncologist prior to consideration for this study. Three clinical criteria must be met: the subject must be thought to have completely resectable disease; the disease must be in 3 or fewer sites; and the subject must be able to tolerate a resective surgical procedure. Subjects with loco-regional recurrence typically have either subcutaneous in transit disease, or a nodal recurrence in the

draining nodal basin. A nodal basin recurrence will be scored as one site, even if multiple nodes are positive. "Clustered" subcutaneous and/or cutaneous lesions that can be removed in a single surgical excision will be scored as one site, even if multiple subcutaneous and/or cutaneous lesions are present. If at the time of the preoperative assessment, the oncologic surgeon believes the lesion(s) can be resected completely with negative surgical margins, the subjects will undergo surgical resection. In the event that the pathologic margins demonstrate microscopic residual disease, patients will remain on protocol provided that their evaluation following course two shows no radiographically or clinically evident disease.

Subjects with stage IV disease may have visceral disease, cutaneous disease, and/or subcutaneous disease. Subjects with subcutaneous disease will undergo resection if the clinical criteria outlined above are met. Subjects with visceral disease have significantly higher operative morbidity. The most common sites of resectable visceral disease are the small bowel and lung. Most subjects with isolated visceral disease are offered operative intervention as a standard of care. If 3 or fewer sites of disease are found, they will undergo resection. If more than 3 sites are found, based on the above guidelines, patients will undergo intervention based on their symptoms and the best clinical judgment of the treating oncologist and surgeon, but will be ineligible for this study. Eligibility decisions, based on clinical resectability, will be made by the surgical oncologist.

5.3 Treatment schedule and dose

The treatment regimens for each study group are summarized below (see also Schema above):

- **Group A**: one course of IC, followed by surgical resection, followed by two courses of IC
- **Group B**: surgical resection, followed by three courses of IC

Guidelines for initiation of therapy, following the surgical resection (Course 1 for subjects randomized to Group B and Course 2 for subjects randomized Group A) are provided in Section 5.2. Treatment Courses 1-3 (for Group B) and Courses 2-3 (for Group A) will be repeated every 28 days (+/- 7 days to allow for scheduling constraints) at the same dose, provided that there is no dose limiting toxicity (see Section 5.4) or non-DLT toxicity the treating physician feels warrents dose reduction. Subjects can receive a maximum of 3 courses of therapy. Hu14.18-IL2 will be given on days 1, 2, and 3 of each course of therapy as a 4-hour continuous IV infusion at a daily dose of 6 mg/m^2/d. (Note: given the relatively short supply of hu14.18-IL2 available, before each subject begins study treatment, we will confirm with the hospital pharmacy that there is sufficient material available in our pharmacy for that subject to receive all planned doses.) The infusions will be stopped only under the following circumstances: if dose-limiting toxicity is noted (see Section 5.4.4), if the treating physician deems it in the subject's best interest to discontinue the infusion for a significant, but non-DLT toxicity, when blood draws are needed, when other intravenous medications must be administered, if adequate intravenous access is lost (and a new IV must be placed), and/or if a subject decides to withdraw from the study.

5.3.1 Subsequent Courses:

5.3.1.1 Subjects may continue treatment if all toxicities have recovered to $\leq$ Grade 1 or the subject meets initial eligibility criteria, and there is no symptomatic disease progression (Section 6.0)

5.3.1.2 Course 3 will be administered only to subjects with a documented complete clinical response (i.e., no radiological or clinical evidence of disease) following Course 2 of treatment (see details regarding statistical evaluation in Section 9.5) provided that all toxicity has resolved to $\leq$ Grade 1 or meets initial eligibility criteria.

5.3.1.3 Subjects not showing CR following surgical resection and 2 courses of study therapy (i.e., at the evaluation following Course 2) will be considered to have progressive disease and will not be eligible for subsequent treatment. In addition, their position in the study group will be filled with a new, randomly assigned subject (see Section 9.3.2 for details). There is always the possibility that clinical or radiographic criteria indicating potential residual melanoma at this time could actually reflect post-operative changes in x-rays or scans or clinical exams, and may not necessarily reflect true progressive/residual disease. Thus, any subject that does not appear to be in CR at this time should have any site of potential recurrent, residual or progressive disease biopsied if that site is considered safely amenable to biopsy (see Section 6.0). In the interest of safety, the biopsy will be performed at least 7 days following the completion of hu14.18-IL2 and as soon after the suspect radiographic data were obtained and/or physical findings were identified (i.e., preferably within 14 days, with efforts not to exceed 21 days). If this biopsy is not consistent with melanoma, the subject may continue with Course 3 of study treatment. Treatment may be delayed to allow for recovery from the biopsy procedure (i.e., preferably no more than a 1-week delay but absolutely not longer than 3 weeks). Decisions related to the duration of time needed to recover from the biopsy, prior to resuming treatment, will be left to the discretion of the treating physician(s). To aid in this decision, laboratory tests may be ordered as warranted clinically, but no specific tests or evaluations are required for this assessment.

In the event that the area of questionable residual disease is not considered safely amenable to biopsy (for example, a small pulmonary nodule that may reflect old infection, atelectasis, or post operative scarring), and is considered potentially to reflect non-malignant post-operative change, the subject may continue with protocol treatment, and will be considered an evaluable subject for this study.

5.4 Dose limiting toxicity and subsequent courses at reduced dose

5.4.1 Definition of DLT

The definition of DLT in this study differs from the standard definition. Dose-limiting toxicity (DLT) will be defined as Grade 3 or 4 toxicity, using the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 3.0, EXCEPT as detailed below in Section 5.4.3. These toxicities will be evaluated for all subjects beginning on Course 1 day 1 during all courses of this study except for the time from the initiation of the surgical resection until recovery from surgery and initiation of Course 1 (for Group B) or Course 2 (for Group A) of protocol treatment.

5.4.2 Clarification of need for exceptions for DLT determination

Since a secondary objective of this study is to evaluate adverse events associated with this experimental agent, careful documentation of toxicity (according to the NCI CTCAE version 3.0) is essential. Though the hu14.18-IL2 fusion protein is a relatively new molecule with modest clinical testing, it is derived from the human IL2 molecule and the humanized form of the ch14.18 chimeric antibody, both of which have had extensive clinical testing. Over the past 15 years of IL2 testing and 9 years of ch14.18 testing, certain side effects have been noted to occur frequently. These toxicities are generally transient, can be well controlled clinically and/or resolve spontaneously, and have no sequelae. Therefore, though some of these frequently seen side effects do score as Grade 3 or 4 toxicities, using the NCI CTCAE 3.0 scale, these toxicities have been excluded for determination of "dose-limiting toxicity" for both IL2 or ch14.18. The list of frequently seen toxicities below will therefore not be considered as dose limiting for the purposes of drug discontinuation, dose modification, or DLT determination for this study.

In order to evaluate the clinical anti-tumor activity and duration of response of hu14.18-IL2 in subjects with advanced melanoma, subjects enrolled in this clinical study will be treated at

6mg/M^2/d, a dose below the MTD, as determined in our adult Phase I clinical trial and at a dose well tolerated (without DLT) in our recently completed Phase II trial.

5.4.3 Detailed listing of DLT exceptions

The following toxicities will be graded and recorded, but will not be used as criteria for determining DLT:

a) Grade 3 pain, requiring intravenous narcotics, provided that the narcotics are controlling the pain, and that IV narcotics for pain are not required > 48 hours after completion of hu14.18-IL2 on Day 3 of any treatment course;
b) Grade 3 nausea and vomiting that resolves within 48 hours after completion of hu14.18-IL2 on Day 3 of any treatment course;
c) Grade 3 fever (i.e., T > 40° C) lasting < 6 hours and controllable with antipyretics;
d) Grade 3 skin toxicity that improves with treatment (e.g., IV Benadryl) within 24 hrs;
e) Grade 3 metabolic/laboratory toxicity of hyponatremia, hyperglycemia, or hypophosphatemia in the absence of CNS symptoms and/or sequelae, that improves with or without treatment within 48 hrs;
f) Grade 3 sensory or motor peripheral (non-cranial) neuropathy (i.e., interference with function plus objective weakness) if transient and reversing within 3 days of completion of hu14.18-IL2 of any treatment course. Subjective findings (e.g., tingling, hot/cold hands) are expected and will not be DLT;
g) Grade 3 hematologic toxicity (or grade 4 lymphopenia – a known transient marker of immune activation by IL2) which improves to at least Grade 2 or pre-therapy baseline values without requiring transfusion within one week of completion of hu14.18-IL2 of any treatment course (see Section 10.2.1 for management of hematologic toxicity and transfusion guidelines);
h) Grade 3 infusional reactions lasting less than 24 hours, readily controlled with supportive (non-steroidal) treatments (i.e., Benadryl) and not requiring epinephrine;
i) Grade 3 fatigue or ECOG performance status that resolves in $\leq$ 1 week;
j) Grade 3 infection that resolves in $\leq$ 1 week.

The above dose limiting toxicity exceptions are based on the known, published, transient, reversible toxicities of IL2 and ch14.18. These toxicities may be expected, based on observations of hu14.18-IL2 given to 33 + 14 (phase I and II respectively) adults with melanoma, administered by the same schedule at similar doses to that being tested in this trial, and will not require interruption, modification, or discontinuation of treatment. In our experience with hu14.18-IL2 in adults with melanoma, asymptomatic hypophosphatemia is a relatively frequent occurrence. Therefore, all subjects scheduled to receive this agent will be given oral phosphate supplementation prophylactically starting on day 1 and ending on day 8 of each course (see Section 10.3.4.3 for details). **All grade 4 toxicities (except for transient lymphopenia) are considered as DLT and require stopping treatment with hu14.18-IL2. Following resolution of a DLT, and if subsequent hu14.18-IL2 treatment is given, it must be given at a reduced dose.**

5.4.4 Treatment cessation/dose modification for DLT or significant non-DLT toxicity

Any subject demonstrating DLT or significant non-DLT toxicity (see Section 10.1.2) will require that treatment with the study agents be stopped. Depending upon the nature of the toxicity and its resolution, subjects will be considered for continued treatment at a reduced dose as described below (see also Figure 1). Any planned drug administration that is withheld due to DLT will not be given on a delayed schedule to "make up" for missed drug. All dose modifications are permanent.

5.4.4.1 Dose-reduction plan for hu14.18-IL2

For toxicity associated with hu14.18-IL2, there must also be consideration of the schedule of administration. If this toxicity occurs on day 1 or 2 of any course, treatment can be resumed during that same course (i.e., on day 2 or 3) at 50% of the starting dose only if the toxicity has improved to meet eligibility criteria in time to give the day 2 or day 3 dose on schedule. If eligibility criteria are not met in time to receive additional therapy during the course in which the toxicity occurred, but are met in time to start the next course (i.e., day 1 of the next course +/- 7 days), treatment will resume at 50% of the dose that caused the DLT. If toxicity again requires treatment cessation, treatment can be restarted at 25% of initial treatment dose. If treatment at this reduced dose results in recurrence of DLT or significant non-DLT toxicity, treatment will be permanently discontinued.

Repeat lab testing will be done weekly until eligibility criteria are met, and treatment will begin at 50% of the prior dose. If after 4 additional weeks of testing, eligibility criteria still are not met, the subject will not be eligible for any additional treatment with the study agent responsible.

Subjects with Grade 4 toxicity related to protocol treatment (other than transient lymphopenia from hu14.18-IL2, see Section 5.3.3.g) will not receive additional protocol treatment during that treatment course. Patients experiencing Grade 4 neutropenia or leukopenia that lasts $\leq$ 4 days; Grade 4 thrombocytopenia that can be corrected with platelet transfusion (see Section 10.2.1); or Grade 4 nausea, vomiting, anorexia or fatigue lasting less than 2 days, and resolves to meet eligibility criteria, may be eligible to receive continued study therapy (provided that all other criteria for continued treatment are met) at 50% of the dose received prior to the onset of this toxicity. Subjects with any other Grade 4 toxicity related to the protocol treatment will not receive additional protocol treatment during that course and will be withdrawn from the study. Subjects experiencing Grade 4 events that are clearly unrelated to the study treatment, last $\leq$ 3 days, and improve to meet eligibility criteria, may be eligible to receive continued study therapy (provided that all other criteria for continued treatment are met) at 50% of the daily dose received prior to the onset of this toxicity.

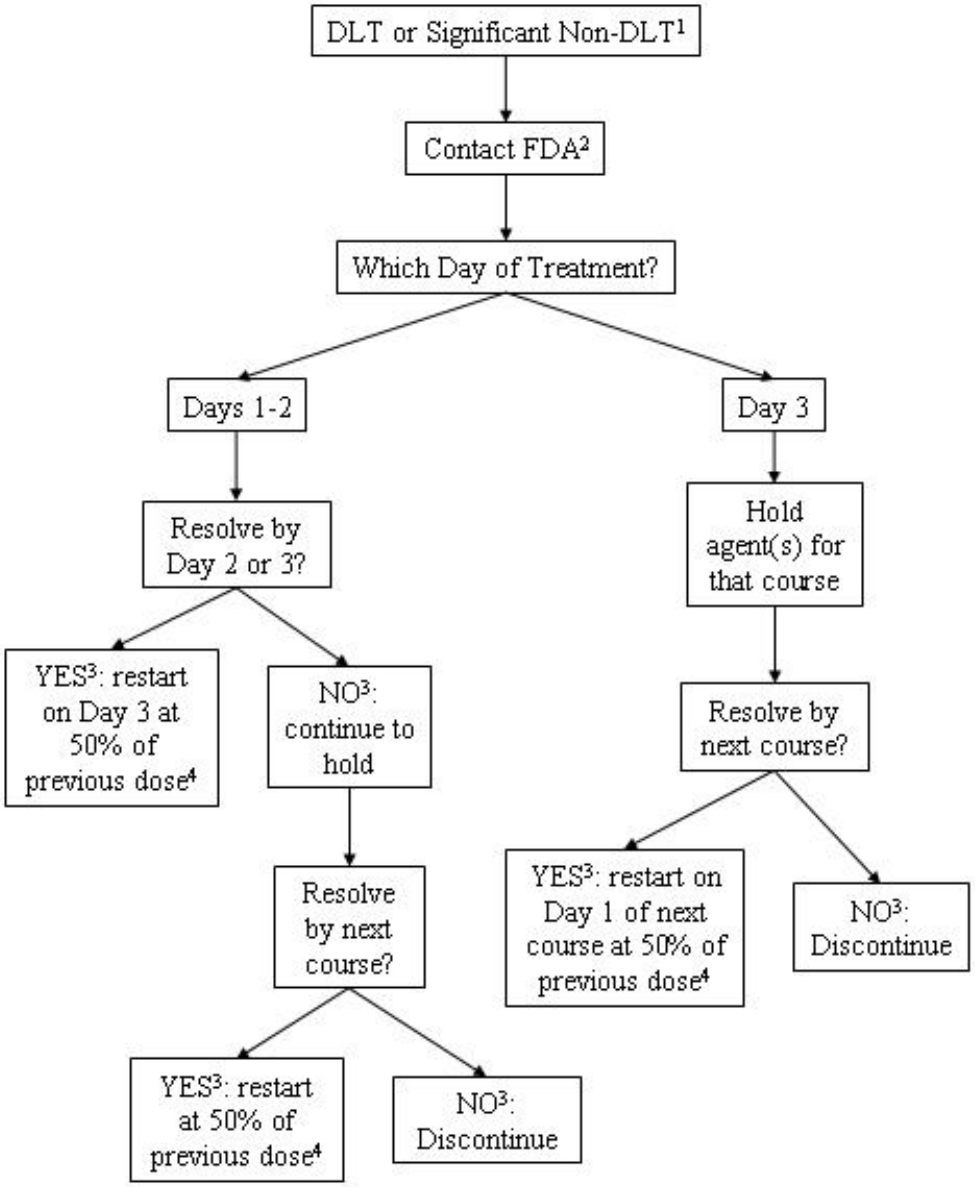
5.4.5 Description of serious adverse event seen in Version 4

Because of a serious adverse event seen with the combination of hu14.18-IL2 and cilengitide, this protocol (Version 5) was amended to exclude further enrollment and treatment with cilengitide. The event occurred in a subject who was enrolled in Group D. What follows is a summary of this subject's clinical course (updated as of 9/19/08): The morning after discharge, following administration of Cycle 2 of therapy, the subject fell and showed neurologic changes. A CT scan showed that he has developed a subdural hematoma which required a craniotomy for evacuation of blood. The neurosurgeon felt that there was evidence of new bleeding, as well as some chronic bleeding. The head CT scan revealed both acute and chronic bleeding that was new since the beginning of protocol therapy. The subdural hematoma was thus possibly associated with either Cilengitide or the combination of hu14.18-IL2 and Cilengitide. After a 10-day hospitalization, the subject was medically stable and was transferred to an assisted living facility (rehabilitation program). While at the assisted living facility, the subject later underwent inferior venacavogram and inferior vena cava filter placement due to the development of a deep venous thrombosis with pulmonary embolus.

Because of this experience, we held accrual to this study, notified the FDA and our IRB as well as other patients on this study. A more detailed summary of this patient's history and clinical course were provided to the FDA and Merck Serono as well as our IRB. While this event can not be completely attributed to the experimental agents, the timing of the event suggst that it is possibly associated with the treatment, and in particular, possibly associated with the cilengitide. Thus we have amended this protocol to cease accrual into Groups C and D. As a consequence, no more

subjects will receive the combination of hu14.18-IL2 and cilengitide on this protocol. We wish to continue our investigation of the activity of hu14.18-IL2 in adults with melanoma that is considered completely resectable, so we will continue to accrue subjects into Groups A and B. We plan to develop an independent Phase I protocol specifically to study the toxicity of hu14.18-IL2 in combination with cilengitide.

FIGURE 1. ALGORITHM FOR MANAGEMENT OF DOSE-LIMITING TOXICITIES



[1] See Section 5.4.3 for a list of DLT exceptions.
[2] See Section 14.2 for instructions for contacting the FDA.
[3] The "yes" or "no" will be based on the FDA's decision after consulting with them on a case-by-case basis, both at the time of onset and time of resolution of the toxicity.
[4] If this is the first time the DLT was noted, reduce dose to 50% of starting dose. If this is the second time the DLT was noted, reduce to 25% of starting dose. If this is the third time, treatment must stop—no further dose reductions are allowed.

5.5 Removal from protocol therapy and off study criteria

Microscopic residual disease at the pathologic margins of the resected tissue is not grounds for exclusion of subject from protocol therapy. At the time of disease evaluation (following course two) if subject remains without radiographical or clinically evidence of disease, investigational therapy will continue.

5.5.1 Criteria for removal from protocol therapy

a) Progressive disease. Subjects with symptomatic disease recurrence confirmed clinically or radiologically at any time will be removed from protocol therapy. Subjects showing asymptomatic disease recurrence at any of the scheduled clinical monitoring times will be removed from protocol therapy. See Section 6.0 for criteria for recurrent disease.
b) Drug intolerance (i.e., subjects who experience DLT) at 25% of the starting dose.
c) Treatment-related toxicities that have not recovered to ≤ Grade 2 in 2 weeks following day 3 of any treatment course of hu14.18-IL2.
d) Refusal of further protocol therapy by subject.
e) Completion of the 3 allowable courses of therapy.
f) Physician determines that removal from protocol therapy is in subject's best interest.

Subjects who are off protocol therapy are to be followed until they meet the criteria for Off Study (see Section 5.5.2). Follow-up data will be required unless consent was withdrawn.

5.5.2 Off study criteria

a) Death.
b) Lost to follow-up.
c) Withdrawal of consent for any further data submission.

5.6 Late toxicity

Subjects who have completed protocol therapy, without evidence of recurrence, will be evaluated regularly for status of remission (see Section 6.0), and for any evidence of late toxicity. Subjects with a global deterioration of health status should be checked carefully for any evidence of disease recurrence, as well as for all other clinical processes that may account for the symptoms. These subjects will continue to be followed according to protocol, and the responsible physician will need to assess whether the non-recurrent disease process is the result of, possibly related to, or unrelated to the prior study treatment.

6.0 DETECTION AND CONFIRMATION OF RECURRENT DISEASE

6.1 Biopsy confirmation of recurrence

Whenever recurrent disease is identified, especially if disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology. If easily accessed by biopsy, this should be done. If not accessible by biopsy, fine needle aspiration should be strongly considered. If the treating physician feels it is not in the subject's best interests to confirm the neoplastic nature by cytology/histology, the physician can let the subject know that he/she is recommending not proceeding with the biopsy. If a biopsy of potentially recurrent disease is obtained, the pathologist will be asked to save some of the biopsy tissue (if available) for research analysis (see pathology evaluation, Section 7.4). Should this recurrence be noted while the subject is still receiving study therapy, any biopsy will be scheduled with the same timing as that for the surgical resection with respect to administration of study therapy (see Sections 5.2 and 5.3.1.3).

6.2. Symptomatic recurrence

If, at any time, the subject is having symptoms that may be clinically attributable to recurrent disease, appropriate clinical and radiological evaluation should be performed to identify and localize potentially recurrent disease. If recurrent disease is identified clinically or radiologically, efforts should be made to confirm its neoplastic nature (see Section 6.1)

6. 3 Asymptomatic recurrence

Clinical and radiological evaluations for recurrent disease will be performed following Course 2 and 1 and 3 months after Course 3. They then will be performed every 3 months from month 6 through month 24, after which they will be performed every 6 months. If, at any of these evaluations, a potential for recurrent disease is identified, appropriate clinical studies (additional X-rays, scans, and/or lab tests, as clinically indicated) will be performed to identify and localize potentially recurrent disease. If recurrent disease is identified clinically or radiologically, efforts should be made to confirm its neoplastic nature by biopsy (see Section 6.1)

6.4 Time to recurrence following "study evaluability"

As all subjects on this study have disease deemed to be completely resectable, all subjects are expected to be going into complete remission following surgical resection. Uniformity of follow up time is best provided by clarifying the time after "study evaluability" that recurrence is first identified (and confirmed whenever possible). As such, the time point of "study evaluability" will be designated the day of evaluation following Course 2 of study treatment, when CR is first demonstrated clinically and radiologically. Subjects in all treatment groups will each have spent equivalent time since study entry in getting to this point: each will have had their surgical resection and their first 2 courses of protocol treatment. The only difference is that surgery is before Course 1 for Group B and after Course 1 for Group A.

6.5 Overall survival

This will measure the interval from the date of study evaluability (see Section 6.4) to the time of death, regardless of intervening therapy that may be given for the treatment of recurrent disease.

6.6 Management of recurrence

Once recurrent disease is identified (and preferably confirmed histologically or cytologically), the responsible physician should initiate whatever treatment is indicated clinically. Subject survival data will still be collected, as will clinical evaluations for potential long-term toxicity.

7.0 STUDY PARAMETERS

7.1 Guidelines

7.1.1 All CT and MRI scans should be done within 3 weeks prior to registration. (**NOTE:** No study specific procedures should be done prior to subject's inclusion in study and obtaining Informed Consent.) All areas identified as malignant disease, or suspicious for malignant disease, must be resectable. The surgical resection must remove these sites, as confirmed by the surgeon.

7.1.2 CBC with differential, platelets (plts), AST, ALT, alkaline phosphatase, bilirubin, albumin, LDH, Na, K, glu, phos, Mg, Ca, Cr, BUN, HgbA1C, C-reactive protein (CRP), TSH, INR, PTT, urinalysis, HIV, and HBsAg should be done $\leq$ 2 weeks before randomization and meet eligibility criteria.

7.2 Clinical Assessment

The detailed schedules for treatment administration and clinical monitoring are included below in Tables 5, 6A, and 6B.

TABLE 5. CLINICAL ASSESSMENT FOR EACH TREATMENT COURSE

		Week 1							Weeks 2-4	Week 5[1]
DAY	**Base Line[2]**	**1**	**2**	**3**	**4**				**8**	**29/1[1]**
Hu14.18-IL2		**X**	**X**	**X**						**X**
Complete history/PE	**X**									
Physical Exam		**X**	**X**	**X**						**X[3]**
Adverse event assessment		**X**	**X**	**X**	**X**				**X**	**X[3]**
Weight	**X**	**X**	**X**	**X**	**X**				**X**	**X[3]**
Performance Status	**X**	**X**	**X**	**X**	**X**				**X**	**X[3]**
Pregnancy Status	**X**									
ECG	**X**									
Tumor Assessment	**X[6]**									**X[4]**
Vital signs (T, BP, P, RR)	**X**	**X[5,6]**	**X[5,6]**	**X[5,6]**	**X**				**X**	**X[3]**
CBC, diff, plts	**X**			**X**					**X**	**X[3]**
AST, ALT, T bili, alk phos, alb, LDH, Na, K, Ca, Mg, Cr, BUN, CRP[7]	**X**			**X**					**X**	**X[3]**
Serum phosphate[8] and glucose[9]	**X**			**X**					**X**	**X[3]**
INR/PTT	**X**			**X**						**X[3]**
Urinalysis	**X**									**X[3]**
Hgb A1C, TSH	**X**									
HIV/HBsAg	**X**									
Head MRI or Head CT	**X**									
Biopsy	**X[10]**									

[1] Day 29 of each course (+/- 7 days leeway to facilitate clinical scheduling) = day 1 of subsequent course.

[2] Baseline tests/exams, with the exception of laboratory assessments, may be performed on or before day 1 but must be performed ≤ 3 weeks prior to randomization. Laboratory assessments must be done ≤ 2 weeks prior to randomization.

[3] These tests may be performed up to 1 week prior to initiating the next course of therapy, or day 22-29 of the present course if no more study therapy is to be administered.

[4] Tumor assessments will take place at baseline and after Course 2. (Long-term follow-up will include further tumor measurements; see Table 8.) The assessment after Course 2 will be performed no earlier than day 8, but can be performed out to day 29 (+/- 7 days leeway to facilitate clinical scheduling), thus allowing adequate time to obtain biopsies of any questionably recurrent disease to determine if the subject is eligible to continue on study (see Section 5.3.1.3 for details regarding these biopsies). Baseline radiological evaluation will include CT scans of the chest, abdomen, and pelvis, and head MRI. Assessments after Course 2 and during long-term follow-up will include CT scans of areas of known prior disease. Other examinations can be performed as warranted clinically. Consistency will be maintained in the imaging modality used to measure tumor while patient is on study.

[5] Vital signs will be obtained q 4 hours while in hospital and prior to initiation of each hu14.18-IL2 infusion. After initiation of the hu14.18-IL2 infusion, continue q 15 min x 2hr, then q 30 min x 2 hr, then q hr x 2 hr (or more frequently if medically indicated).

[6] Pulse oximetry will be checked on all patients prior to initiation of each hu14.18-IL2 infusion on days 1-3 of each course.

[7] C-Reactive Protein (CRP) will not be obtained on day 8.

[8] If Grade 3 hypophosphatemia is observed, additional inpatient monitoring of subjects will include daily serum phosphate, electrolytes, and creatinine from the time the hypophosphatemia is observed until it resolves to < Grade 3. All subjects scheduled to receive hu14.18-IL2 will be given oral phosphate supplementation prophylactically starting on day 1 and ending on day 8 of each course of hu14.18-IL2 (see Section 10.3.4.3 for details).

[9] If Grade 3 hyperglycemia is observed, additional monitoring of subjects will include cutaneous skin puncture for glucometer monitoring of blood glucose levels hourly during hu14.18-IL2 infusion, 4 hours following hu14.18-IL2 infusion, and then q 6 hours. If Grade 3 hyperglycemia is noted in the outpatient setting, it will be rechecked within 24 hours, and appropriate intervention initiated.

[10] Biopsy confirmation must be considered clinically appropriate for clinical management (e.g., confirmation of diagnosis) and must not be performed solely for meeting eligibility criteria. For disease recurrence at a site of known prior biopsy-proven disease, another biopsy need not be performed. For recurrence at a new site or for a *de novo* diagnosis, a biopsy must be performed prior to enrollment onto this study.

TABLE 6A.

CLINICAL ASSESSMENT GUIDELINES FOR THE SURGICAL RESECTION AND POST-OP PERIOD: GROUP A (SURGERY AFTER COURSE 1 OF STUDY THERAPY)

NOTE: Please look at requirements pre and post surgery (ie PE <1 week prior….)

	≤ 1 week before surgery[1]	**Pre-Operative[2]**	**Immediate Post-operative[3]**	**≤ 1 week before starting study therapy[4]**
Physical Exam	**X**		**X**	**X**
Weight	**X**	**X**	**X**	**X**
Performance Status	**X**	**X**	**X**	**X**
Tumor Assessment	**X[5]**			**X[6]**
Vital signs (T, BP, P, RR)	**X**	**X**	**X**	**X**
CBC, diff, plts	**X**			**X**
AST, ALT, T bili, alk phos, alb, LDH, Na, K, Cl, HCO_3, Cr, BUN, Glucose, Ca, Mg, Phosphate	**X**			**X**
INR/PTT	**X**			**X**
Urinalysis	**X**			**X**

[1] The exact tests to be ordered at this time will be left to the discretion of the treating physician(s) as they are warranted clinically to assess if the patient is fit for surgery. With the exception of INR/PTT, monitoring labs are routinely obtained on cycle 1 day 8. It is anticipated that the surgery will be performed 7-14 days after the completion of the 3-day course of treatment for Group A. If any of these lab tests do not meet the initial eligibility criteria by the day range 22-29 of Course 1, these tests should be repeated weekly until they meet eligibility criteria, at which point the patient may undergo surgical resection. If more than 8 weeks pass following the treatment of Course 1 and eligibility criteria still are not met, the subject will not receive further study therapy.

[2] The pre-operative studies indicated here can be done the day of or the day before surgery

[3] Post-operative management and tests required should be those felt to be needed for subject care by the surgical team.

[4] The exact tests to be ordered will be left to the discretion of the treating physician(s) as they are warranted clinically to assess recovery from surgery. It is anticipated that these tests will be obtained 1-3 weeks post-operatively, depending on the nature and extent of the surgery, and the post-operative recovery. If the results of these tests suggest the subject has not fully recovered from surgery, additional clinical evaluation may be required to identify the cause of the abnormality and to provide treatment if indicated. Tests may be repeated as needed until adequate resolution so the patient may begin Course 2 of protocol treatment, preferably within 4 weeks of surgery. If 8 weeks pass post-operatively, and the treating physician(s) still feel(s) that the subject is not fit to continue, the subject will not receive further study therapy.

[5] Tumor assessment will only be done if toxicity from the study treatment is so prolonged that it delays surgery by 4 or more weeks from the anticipated range (see Section 5.2.1). If a delay of 4 or more weeks occurs, then radiographic assessment of tumor status is required pre-operatively (within 2 weeks of surgery). The presence of new, resectable disease at this time

will not be considered as disease progression, and the subject can continue on study, provided the identifiable melanoma is completely resectable, and the subject has not had irreversible DLT from the protocol therapy that delays the surgery to 8 weeks after administration of cycle 1 protocol therapy.

[6] Tumor assessment will only be done if toxicity from the surgery is so prolonged that it delays starting the second course of study therapy by 4 or more weeks from the anticipated time (post-op day 14 for non-extensive surgery or post-op day 21 for extensive surgery). If initiation of course 2 therapy is delayed by $\geq$4 weeks, radiographic assessment of tumor status is required within 2 weeks of starting Course 2 of study therapy.

TABLE 6B.

CLINICAL ASSESSMENT GUIDELINES FOR THE SURGICAL RESECTION AND POST-OP PERIOD: GROUP B (SURGERY BEFORE COURSE 1 OF STUDY THERAPY)

	Pre-Operative[1]	**Immediate Post-operative[2]**	**$\leq$ 1 week before starting hu14.18-IL2[3]**
Physical Exam		**X**	**X**
Weight	**X**	**X**	**X**
Performance Status	**X**		**X**
Tumor Assessment			**X[4]**
Vital signs (T, BP, P, RR)	**X**	**X**	**X**
CBC, diff, plts			**X**
AST, ALT, T bili, alk phos, alb, LDH, Na, K, Cl, HCO_3, Cr, BUN, Glucose, Ca, Mg, Phosphate			**X**
INR/PTT			**X**
Urinalysis			**X**

[1] The pre-operative studies indicated here can be done the day of or the day before surgery

[2] Post-operative management and tests required should be those felt to be needed for patient care by the surgical team.

[3] The exact tests to be ordered will be left to the discretion of the treating physician(s) as they are warranted clinically to assess recovery from surgery. It is anticipated that these tests will be obtained 1-3 weeks post-operatively, depending on the nature and extent of the surgery, and the post-operative recovery. If the results of these tests suggest the subject has not fully recovered from surgery, additional clinical evaluation may be required to identify the cause of the abnormality and to provide treatment if indicated. Tests may be repeated as needed until adequate resolution so the patient may begin Course 1 of hu14.18-IL2, preferably within 4 weeks of surgery. If 8 weeks pass post-operatively, and the treating physician(s) still feel(s) that the subject is not fit to continue, the subject will not receive hu14.18-IL2.

[4] Tumor assessment will only be done if toxicity from the surgery is so prolonged that it delays starting the hu14.18-IL2 by 4 or more weeks from the anticipated time (post-op day 14 for non-extensive surgery or post-op day 21 for extensive surgery). If a delay of 4 or more weeks from the time scheduled to start hu14.18-IL2 occurs, radiographic assessment of tumor status is required within 2 weeks of starting hu14.18-IL2 for Course 1.

7.3 Immunological monitoring (Table 7A)

These *in vitro* studies will be done to monitor various aspects of the immune response to hu14.18-IL2. The time points mentioned in this section assume that treatment with hu14.18-IL2 will begin on day 1 and end on day 3.

7.3.1 Detection of anti-hu14.18-IL2 antibodies (anti-idiotypic and anti-FcIL2) will be performed on subjects' serum obtained at baseline and approximately 10 minutes prior to initiation of treatment, and serum samples on Days 3, 4, 8, and 29/1. Blood will be drawn in a red top tube, and kept refrigerated until serum is separated and stored frozen at -20º C.

7.3.2 As part of a laboratory substudy, additional biopsies will be requested from consenting subjects assigned to Group A (surgical resection after Course 1 of protocol therapy) who have readily accessible cutaneous or subcutaneous lesions. A "pre-treatment" biopsy will be a control tissue sample. In addition, we will ask for a "post-treatment" biopsy after receiving therapy for Course 1 but before surgical resection; according to the Schema above, this would be at approximately days 3-5. This tissue sample would depict any early inflammatory changes to the tumor that may not be present several days later at the time of surgery.

Readily accessible biopsies (cutaneous or subcutaneous lesion) will be obtained using 1% lidocaine for local anesthesia. The pretreatment biopsy of a cutaneous lesion will be compared both to the post-treatment biopsy and to tumor that is resected following treatment Course 1. The biopsies will consist of approximately 0.3 cm skin or subcutaneous samples (using a 3 or 6 mm punch). In the event the tumor nodules are larger than accessible using a 6 mm punch biopsy, a surgical consult will be obtained for excision of the nodule(s) using local anesthesia. These tumor biopsies, as well as all resected melanoma, will be evaluated as described in the Pathology Guidelines in Section 7.4.

7.3.3 Other *in vitro* immunological monitoring, including soluble IL2 receptor α levels, will be performed on subjects as per the assessment times in Table 7. Again, blood will be drawn using a red top tube, **and kept** refrigerated until the serum is separated, then stored frozen at -20º C.

7.3.4 NK and ADCC testing will be done on selected patients with freshly collected PBMC at baseline (as a control), on day 8 of Courses 2 and 3, and on day 29/1 of Courses 2 and 3. The time point at day 8 was shown to be the peak for lymphocytosis and NK/ADCC function in our completed Phase I studies.

7.3.5 Lymphocyte cryopreservation for genetic testing will be done with lymphocytes obtained on day 8 of Course 2 and Course 3 in 7.3.4. As these time points are associated with lymphocytosis, the 10 cc of heparinized blood collected for NK/ADCC testing on those days will provide adequate PBMC numbers for the NK/ADCC assays and for cryopreservation of extra cells. These cryopreserved cells will be used for pilot analyses of DNA markers that may be relevant to anti-tumor effects or anti-id responses [e.g., analyses of immunoglobulin allotype alleles (93) of Fc receptor phenotype (94) or MHC-KIR gene mismatching].

7.3.6 Pilot studies of *in vitro* proliferative and cytotoxic responses of T-cells will be performed on selected subjects with 50 ml samples of heparinized blood obtained at baseline prior to Course 1, once within one week of the end of Course 2 (i.e., one week prior to starting Course 3), and once in the range of days 22-29 of Course 3. The lymphocytes will be obtained using 50 ml of heparinized blood left at **room temperature** until lymphocytes are separated, cryopreserved and stored in liquid nitrogen or in a –140º C ultracold freezer. When possible (based on availability of autologous tumor lysate for functional studies, growth of autologous melanoma cells, and/or HLA-A2 phenotype), subjects' PBMC will be evaluated for cytotoxicity against their own autologous melanoma cells,

tested for reactivity to the melanoma associated gp100 antigen by intracytoplasmic induction of IFNγ, and tested for reactivity to allogeneic melanoma lines. These studies will determine whether the 2 or 3 courses of hu14.18-IL2 treatment have induced detectable increases in T cell reactivity to autologous melanoma or to melanoma-associated antigens.

7.4 Determination of Peak Serum hu14.18-IL2 concentration. (Tables 7A and 7B)

7.4.1 Hu14.18-IL2 levels

Analysis of hu14.18-IL2 serum levels will be performed on samples from all groups for all treatment courses. On days 1, and 3, blood will be obtained just prior to starting the hu14.18-IL2 ("pre"), and within 10 minutes of stopping the 4-hour infusion ("post" or peak). This is shown in Table 7A below.

Blood to test hu14.18-IL2 levels will be drawn in a 10 ml red top tube at the times states above relative to the hu14.18-IL2 infusion, and kept refrigerated until serum is separated and stored frozen at -20º C. Measurement of hu14.18-IL2 will occur in the Immunomonitoring Laboratory of the UW Paul P. Carbone Comprehensive Cancer Center.

7.4.2 Anti-hu14.18-IL2 antibody levels

Serum specimens will be obtained at the indicated times shown in Table 7A and cryopreserved. These will then be tested in ELISA assays currently in use (36) and now being developed to detect and quantitate antibody against the hu14.18-IL2 IC molecule.

TABLE 7A.
IMMUNOLOGICAL AND PHARMACOKINETIC ASSESSMENT

	Base Line[1]	Week 1				Week 2	Week 5
DAY		**1[2]**	**2**	**3**	**4**	**8**	**29(1)[2,3]**
Hu14.18-IL2		**X**	**X**	**X**			
Serum hu14.18-IL2 levels[4]		**X/X**	**X/X**	**X/X**			
Soluble IL2 receptor	**X**	**X/C1**		**X/ C1**	**X**	**X**	**X**
Anti- hu14.18-IL2 Ab	**X**	**X/ C1**		**X/ C1**	**X**	**X**	**X**
Red top tubes (mL)[4]	**2x10**	**2x10/1x10; 3x10**	**1x10/1x10**	**2x10/1x10**	**1x10**	**1x10**[11]	**1x10**[10]
In vitro NK/ADCC studies[5]	**X**					**X C2+3**	**X C2+3**
Green top tubes (mL) [5]	**1x10**					**1x10 C2+3**	**1x10 C2+3**
In vitro T cell studies[6,7]	**X [6,7]**						**X[6,7] C2+3**
Heparinized syringe (mL) [6,7]	**50**						**50 [6,7] C2+3**
In vivo binding[8]	**X**				**X**		

X/ = pre-IC 9
/X = post-IC[9]

[1] Studies scheduled for "Baseline" can be obtained at any time from 3weeks prior to randomization until just prior to (within 10 minutes) beginning study treatment.
[2] Day 29 of each course (+/- 7 days leeway to facilitate clinical scheduling) = day 1 of subsequent course.
[3] These tests may be performed up to 1 week prior to initiating the next course of therapy (day 22-29 of current course).
[4] To better understand the pharmacokinetics of hu14.18-IL2 serum will be obtained from subjects in both groups for all courses to measure hu14.18-IL2 levels at the following times: pre and post the 4 hr IC infusion and again 2, 4, and 8 hours after completion of the 4-hr IC infusion on days 1 and 3; on day 2, only the pre- and post-IC samples will be collected.

[5] *In vitro* testing of NK and ADCC function will be performed on selected patients five times with 10 mL of heparinized blood: at baseline, on day 8 of Courses 2 and 3 (C2+3) and within one week of the end of Courses 2 and 3. Additional lymphocytes obtained at the day 8 time points will be cryopreserved for subsequent DNA analyses.

[6] Lymphocytes will be cryopreserved for subsequent T cell function testing from 50 mL of heparinized blood. Plasma collected with this sample also will be cryopreserved for subsequent immunological assays. This sample will be obtained three times: once at baseline, and within one week of the end of both Courses 2 and 3 (C2+3).

[7] For subjects weighing between 45 and 50 kg, and for subjects who experience anemia (i.e., documented hemoglobin < 10.0 gm/dl), the volumes of these syringes will be reduced to 10 ml.

[8] Biopsies will be performed during Course 1 only on selected, consenting subjects in Groups A who have readily accessible cutaneous lesions. (Group B is excluded because they will have no detectable disease at this time.) In addition to pilot studies analyzing for hu14.18-IL2 *in vivo* binding, immunohistologic components, including lymphocyte infiltration, target antigen expression, and assessing for T cell responses (including $CD8^+$, $CD4^+$ and NK phenotypes), will be performed on the biopsy samples. Depending on the number of cutaneous lesions, biopsies will be done at baseline and then once again between days 4-7 (following completion of cycle 1 therapy).

[9] Just prior to (within 10 minutes) starting the infusion and within 10 minutes prior to stopping the infusion, for the 4-hour hu14.18-IL2 infusion, as indicated.

[10] Ten ml Red top tube will be obtained on days 8 and 22/29 of each cycle regardless of the protocol therapy to which subject is randomized.

Version 6, 1/21/2010

TABLE 7B.
ADMINISTRATION OF HU14.18-IL2 AND BLOOD SAMPLING FOR PK ASSAYS ORGANIZED BY AGENTTO BE ADMINISTERED

Hu14.18-IL2 Only:

Time (h)[1]	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
hu14.18-IL2[3] (4 h)	|----------	----------	----------	----------	----------|									
hu14.18-IL2 Samples[4]	X/ pre				/X post		•		•				•		
Sample # [2]	IC1.1				IC1.2										

[1] Time is listed with the reference point (i.e., time 0) as the start of treatment on a given day.
[2] Samples will be labeled with the following information: an abbreviation of the agent "IC" for hu14.18-IL2), an integer representing the day in a given treatment course (day 1 as shown in the table above), a decimal point, and a second integer representing the time point as depicted in the table.
[3] Hu14.18-IL2 is given as a 4-hour infusion. [4] Hu14.18-IL2 PK samples (10 ml red top tube, 5 ml of serum per sample) are obtained just before (pre) and just at the end of the hu14.18-IL2 infusion (post).

7.5 Surgical pathology guidelines

7.5.1 Review of outside diagnostic material

Prior to surgery at the University of Wisconsin, slides of patients' prior diagnostic material will be reviewed by a pathologist to ensure accuracy of diagnoses and presence of melanoma at the resection site, as appropriate. As many of the biopsies demonstrating recurrence will likely be small needle cores or fine needle aspirates, extensive analysis of any inflammatory infiltrate prior to enrollment is unlikely to be possible in most cases. As described above, in a subset of patients from Groups A that have accessible lesions, we will attempt to obtain two core biopsies of these sites to later be fully resected: one biopsy prior to initiation of study treatment, and the second biopsy within one week (i.e., approximately days 5-7) of initiating study treatment. These biopsies will be bisected, with half submitted for paraffin sections and half frozen in OCT embedding media for sections (to be assessed as described in Sections 7.4.2 and 7.4.3).

7.5.2 Biopsy and resection specimen handling and pathologic analysis

All biopsies and all freshly resected tumor specimens at UW will be taken immediately to the surgical pathology suite adjacent to the operating room. The specimen will be inked in order to provide subsequent pathologic evaluation of presence or absence of residual tumor at the resection margins. The specimen will then be serially sectioned with submission of the appropriate tissue for permanent, paraffin embedded sections to ensure accurate pathologic analysis of resection margins, as well as high-quality hematoxylin and eosin stained sections for morphologic analysis of the tumor and for evaluation of necrosis, apoptosis, and any inflammatory infiltrate. These sections also will be assessed by immunohistochemical analysis for the presence of CD4 and CD8 positive T cells, NK cells (CD56 and CD16), and macrophages (CD68) (the antibodies for which all function well on formalin-fixed tissues).

7.5.3 Handling of fresh tissue samples.

The remaining unfixed tissue will be divided into specimens that will be sent to our research lab for derivation of autologous cultured tumor cell lines and bulk tumor lysate (for use as pulsed antigen for *in vitro* immunologic assays), and tissue that will be frozen in OCT for frozen sections or cryopreserved for pilot molecular studies. The standard protocol for frozen section analysis will include assessment of activation markers on infiltrating T cells (CD69) and for evidence of regulatory T cells (CD25 and FoxP3) by double immunolabeling with CD3, CD4, or CD8, as appropriate. In addition, we will stain for HLA class I and II to assess changes in HLA level on tumor cells, as enhanced class I expression was noted in the mouse studies. Finally, we will assess the expression of the respective target antigens of these experimental agents (i.e., GD2) on tumor cells before and after study treatment. For all histologic analyses, it is likely that inflammatory infiltrates will be patchy, making precise quantification difficult. Nonetheless, a semiquantitative grading scheme will be developed in order to compare the composition and degree of inflammatory infiltrates between patients and, where possible, in pre- and post-treatment specimens from the same patient.

7.5.4 Quantification of the histologic analyses.

For the primary parameters to be assessed on the resected melanoma (i.e., necrosis, apoptosis, and cellular infiltrate), an objective scoring system will be established by a pathologist, grading each specimen with a score of 0, +, ++, +++. The specific criteria for each category will be determined based on the appearance of specimens from the initially enrolled patients in order to provide a spectrum from which meaningful comparisons can be derived. In addition, purely quantitative assessment of necrosis of tumor cells also will be measured and scored with a value ranging from 0% - 100% of tumor area. These and all pathology analyses will be performed by a pathologist in a non-blinded fashion, but will be repeated, using the scoring system thus established,

by a second blinded pathologist. For quantitation of expression levels of HLA class I and II and the GD2 antigen on a per cell basis, it may be useful to use flow cytometric analyses in addition to attempting to qualitatively compare "brightness" of staining between histologic frozen sections in different tumors. As such, fresh tissue will be disaggregated into a single-cell suspension, and these parameters will be evaluated by flow cytometry where possible.

7.5.5 Additional preliminary analyses.
For select samples that do show a cellular infiltrate, *in situ* hybridization will be performed on frozen sections to determine the expression of intracellular gamma interferon in T cells and NK cells infiltrating into the tumor. Depending upon what is observed, other cytokines may be evaluated selectively by *in situ* hybridization.

7.5.6 For patients with accessible recurrences following completion of protocol therapy, we will attempt to obtain core needle biopsies of the recurrent lesions and apply similar analyses to those previously described.

7.6 Late evaluation for recurrence or late toxicity

The following schedule (Table 8) describes clinical evaluations, lab testing and immunological samplings suggested for subjects following completion of all study treatment, to be evaluating for late effects of the treatment as well as time to recurrence. The exact tests to be ordered will be left to the discretion of the treating physician(s) as they feel appropriate, given each subject's clinical history and presenting symptoms and signs. Subjects who have recurrence of disease will be followed for survival only.

TABLE 8.
CLINICAL ASSESSMENT GUIDELINES FOR RECURRENCE OR LATE TOXICITY

	2 & 4 months after treatment completion of cycle 3 therapy (months 5 & 7)	Every 3 months (months 10-25)	Every 6 months (starting at month 31)	At time of recurrence[1]
Interim complete history and Physical Exam	X	X	X	X
Adverse event assessment	X	X	X	X
Weight	X	X	X	X
Performance Status	X	X	X	X
Tumor Assessment	X[2]	X[2]	X[2]	X[2,3]
Vital signs (T, BP, P, RR)	X	X	X	X
CBC, diff, plts[4]	X	X	X	X
AST, ALT, T bili, alk phos, alb, LDH, Na, K, Cl, HCO_3,Cr, BUN, Glucose, Ca, Mg, Phosphate[4]	X	X	X	X
INR/PTT[4]	X			X
Urinalysis[4]	X			X
Biopsy				X[5]

[1] These evaluations will be performed only at the time that disease recurrence is noted. Following this time, subjects will be followed only for survival.

[2] These evaluations should include CT scans of all areas of known prior metastatic disease. Other examinations can be performed as warranted clinically. If, at any of these evaluations, a potential for recurrent disease is identified, appropriate clinical studies (additional X-rays, scans, and/or lab tests, as clinically indicated) will be performed to identify and localize potential recurrent disease.

[3] If recurrent disease is identified clinically or radiologically, efforts should be made to confirm its neoplastic nature by biopsy.

[4] The exact laboratory tests to be obtained at follow-up visits shall be left to the discretion of the treating physician(s) as they are indicated clinically.

[5] Recurrence should be confirmed by biopsy whenever possible. If possible and clinically indicated, the biopsy should be a core or excisional biopsy performed at the UWCCC. In doing so, fixed and fresh tissue can be evaluated with the same techniques used to evaluate the surgically resected tissue from this same subject as part of this study.

7.7 Blood drawing totals

All subjects entered into this study will participate in all specified treatment, evaluations, specimen collection and blood testing, with their written consent. A subject may choose to withdraw consent and leave the study at any time. To minimize the amount of blood withdrawn, we will be collecting all required blood tests in pediatric tubes. For Groups A (the subjects who will undergo the greatest degree of monitoring), the blood drawing requirements (utilizing blood sparing microtubes and smallest possible volumes for all clinical tests) for the first 2 courses of treatment for all studies included in Tables 5, 6A, 6B, and 7A are as follows:

Courses 1 and 2 and surgery clinical labs ----------	80 ml	
Courses 1 and 2 hu14.18-IL2 PK and serological assays ----------	190 ml	Courses 1 and 2
NK/ADCC ----------	30 ml	
Courses 1 and 2 T cell studies ----------	100 ml	
Total blood for Courses 1 and 2 and surgery ----------	430 ml	

As the removal of 480 ml of blood (1 unit) is considered acceptable for individuals > 55 kg, this corresponds to 8.7 ml/kg over this initial 2 month period. The required volume of 430 ml needed for full participation in the first 2 courses of this study is below this limit (7.8 ml/kg). Blood drawing requirements during Course 3 are less than during Course 1 and 2 because there is no need to repeat the "baseline samples" obtained prior to Course 1. Thus, insuring safe blood volumes are obtained for Courses 1 and 2 will insure the same for Course 3. With this level of monitoring, an individual weighing as little as 50 kg could provide sufficient blood and still fall below the limit of 8.7 ml/kg. As such, for any subjects weighing between 45 and 50 kg, we will reduce the volume of heparinized blood collected at the end of Courses 2 and 3 from 50 ml to 10 ml (see Table 7A). This reduces the total blood volume for Courses 1 and 2 to 390 ml, which would allow subjects with weights as low as 45 kg to give less than the 8.7 ml/kg limit. Since the risks from obtaining adequate monitoring blood samples becomes greater in subjects with lower weights, we shall exclude any subject < 45 kg. This is unlikely to exclude any otherwise eligible subjects, as any subject healthy enough to enroll in this study very likely would weigh in excess of 45 kg.

In addition, there is a risk of additional blood loss during the surgical resection that is scheduled as part of this study. It is difficult to anticipate how much blood will be lost from subjects during the resection. Therefore, we will rely upon the hemoglobin concentration (obtained with a CBC) that will be checked regularly in the context of clinical monitoring (see Tables 5, 6A, and 6B) to assess for anemia due to excess blood loss. Subjects whose hemoglobin concentrations fall below the level that is set as an inclusion criterion (i.e., 10 gm/dl) will not receive the next scheduled course of protocol treatment until this parameter is again above 10 gm/dl. As described in Tables 6A and 6B, this test may be repeated as needed until the result meets this inclusion criterion. Upon re-initiation of treatment, to reduce the volume of blood drawn from these particularly susceptible subjects, we will reduce the volume of heparinized blood collected at the end of Courses 2 and 3 from 50 ml to 10 ml (see Table 7A). If recovery of anemia delays the start of the subsequent course of protocol treatment by more than 8 weeks, the subject will not be allowed to receive any further study treatment.

7.8 Analyses to be performed in laboratory correlate studies

In addition to the clinical outcome data from this study, and in addition to the laboratory studies planned for each patient based on analyses of required blood and tissue samples, we also plan to perform two elective laboratory correlate studies using blood and tissue from selected subjects. (These subjects will give informed consent to participate in these studies in addition to the main clinical study.) One of these laboratory correlate studies will enable us to obtain an elective biopsy prior to receiving treatment with the experimental agent(s) for subjects randomized to Group A that have subcutaneous lesions which are

readily accessible to punch or excisional biopsy. These biopsy specimens would come only from readily accessible lesions in a manner similar to that described previously so as not to put these subjects at additional undue risk. No subjects in Group B will be entered into this elective biopsy study since all of their clinically detectable disease will be resected prior to administration of hu14.18-IL2. From these pre-treatment research biopsy samples, we will evaluate the general histological appearance, and presence of any immune-cell infiltrate within the tumors before study treatment. More importantly, these elective pretreatment biopsies will enable us to use these for comparison purposes, when we evaluate the resected tumor obtained after the first course of treatment for subjects in Group A.

The other laboratory correlate study will obtain blood and tissue samples from subjects who have recurrence of their disease. These research samples will be subjected to the following analyses: the influence of the prior treatment on the immune system, the subjects' immune response against the agents *per se*, and histological studies similar to those described for the preceding correlate study, in order to evaluate potential changes in tumor biology that may be associated with recurrent disease.

In addition to these correlate studies, subjects also may volunteer to have residual blood and tissue samples (which were taken during the course of the main clinical study, yet not completely consumed for these studies) stored for use in future experiments further evaluating the immune response to melanoma, the activity of the experimental agents used in this study, or other related cancer research. These specimens will remain at the University of Wisconsin and will be coded. This coding will enable linkage of these samples to clinical treatment from this particular clinical study. Investigators not working on this study will not be able to recognize any patient identification with these samples. The code for these samples will remain in a secure laboratory in the research files of the Protocol Chair and Principal Investigator. They will not be sold for commercial use.

8.0 EXPERIMENTAL AGENT FORMULATION

8.1 Hu14.18-IL2 formulation

8.1.1 Treatments administered

Subjects in both study groups will receive hu14.18-IL2 at 6 mg/m^2 as a 4-hour daily infusion on 3 consecutive days each 28-day cycle; this includes 3 total courses for all subjects. Treatment may be stopped for severe or dose-limiting toxicity. Hu14.18-IL2 may be discontinued or dose-reduced following resolution of the toxicity that prompted the interruption in dosing. See Section 5.3 for the definition of DLT and the list of DLT exceptions.

8.1.2 Investigational product

This agent has been produced for the prior Phase II studies (COG-ANBL0322 and NCI-6304/CO04601) and this study by the Pharmaceutical Development Program (BDP), SAIC-Frederick for the NCI-Developmental Therapeutics Program (NCI-DTP), based on a Material Transfer Cooperative Research and Development Agreement (M-CRADA) with EMD Serono, Inc. pursuant to an intra-company assignment from EMD Pharmaceuticals Inc.(see section 8.1.3 below). All information in this section has been provided and/or reviewed by NCI-DTP staff for accuracy.

8.1.2.1 Identity of this investigational product

This investigational product is an immunocytokine (IC) referred to as "hu14.18-IL2". This agent has been shown, by release assays, to be equivalent to a prior investigational product identified as Merck KGaA product number EMD lot 99M738 hu14.18-IL2 (BB IND-9798). The product is a sterile solution for IV infusion following dilution, consisting of hu14.18-IL2, at pH 7.0, in a vehicle of mannitol, arginine, hydrochloride, citrate, and polysorbate 80 (Tween-80®).

8.1.2.2 Description of hu14.18-IL2

The final drug product, hu14.18-IL2, is a frozen, sterile, clear, colorless to slightly opalescent concentrated solution for IV infusion following dilution. It is available in single-dose glass vials stoppered with rubber septa. Each 5 ml vial contains a total of 2 mg of hu14.18-IL2 in a total volume of 2 ml (final concentration is 1 mg/ml). Hu14.18-IL2 is dissolved in a stabilizing formulation buffer of mannitol, arginine hydrochloride, citrate, and Tween 80® at pH 7. Frozen hu14.18-IL2, once thawed in the vial, requires dilution with 0.9% Sodium Chloride Injection and 0.25% Human Serum Albumin (HSA) prior to infusion. Hu14.18-IL2 sterile solution **MUST** be stored frozen (-10 to -30º C) until it is thawed for use.

8.1.2.3 Packaging and labeling of hu14.18-IL2

Hu14.18-IL2 will be provided to the trial site in open-label, supplies for admixture and administration at the site. No drug will be dispensed directly to subjects. Hu14.18-IL2 was manufactured according to current Good Manufacturing Practices (cGMP).

The information on the vial label will be in accordance with all applicable regulatory requirements. The vial label will include the drug name and concentration, volume of drug contained in the vial, lot number, and storage conditions, as well as a notice that federal law restricts the use of the product to investigational use.

The Investigator/Designee is advised to place a similar label on the infusion bag after admixture. In accordance with applicable regulatory requirements, it is recommended that the infusion bag label identify the investigational drug, lot number, the date and time of admixture, the admixture expiration date and time (i.e., maximum of 12 hours after admixture = 4 hours of infusion plus 8 hours of storage), amount of drug added, infusion rate, and storage conditions prior to administration. The infusion bag label should include the UW protocol study number (CO05601), the subject's initials, and the subject's study identification number.

8.1.2.4 Shipping, storage, issue, and return of hu14.18-IL2

Hu14.18-IL2 will be provided by the NCI, DTP, BRB. This frozen material is shipped to the commercial storage facility (arranged by the PI, and shipping performed upon approved release criteria by NCI-DTP). Frozen vials of hu14.18-IL2 are then shipped from the storage facility to the Investigator on dry ice at approximately -80º C when the UW experimental pharmacy supply is becoming low (approximately quarterly), upon request to the commercial storage facility by the UW experimental pharmacy. Upon receipt of the medication, the Investigator, or the responsible pharmacist, will inspect the medication and send back the acknowledgement of receipt form that is enclosed with the parcel, duly completed and signed, confirming that the material arrived frozen and the vials were intact. A copy of the signed acknowledgement of receipt must be kept in the study files.

All study medication will remain frozen and will be stored safely and separately from other drugs in a limited access room under the responsibility of the Investigator/Designee. Frozen hu14.18-IL2 will be stored by the Investigator/Designee in a continuously monitored non-frost-free freezer (reserved for medication storage only). Hu14.18-IL2 **MUST** remain frozen (-10 to -30º C) until it is thawed for use. The Investigator/Designee will be responsible for the storage, dispensing, inventory, and accountability of all clinical supplies, and will exercise accepted medical and pharmaceutical practices. An accurate, timely record of the disposition of all clinical supplies will be maintained. The supplies and inventory must be available for inspection by the designated representatives of the UWCCC and NCI upon request.

The Investigator/Designee must maintain records of the delivery of the study medication to the trial site, the inventory at the site, the use by each subject, and the return to NCI or destruction of the medication at the site. The drug dispensing log must be kept current and must include the following information:

- The identification of the subject to whom the drug was dispensed
- The date(s) and quantity of the drug dispensed to the subject
- The product lot number and the release date

Under no circumstances will the Investigator allow the investigational drug to be used other than as directed by this protocol. The preparation of the study drug should be documented on the Drug Preparation and Drug Dispensing Log form. The inventory of study medication must be available at the treatment center for monitoring, auditing, or inspection.

The Investigator/Designee must retain and maintain complete records of the disposition of all study medication not used during the study period, as well as used vials of study medication. After the monitor has verified drug accountability, the Investigator/Designee will initiate destruction of all used investigational materials (medication and packaging) and will initiate destruction of or return of all unused hu14.18-IL2. Instructions will be provided separately.

A copy of the inventory record and a record of clinical supplies destroyed must be maintained and be available for review upon request by the UWCCC or NCI. This form shall include information on:

- All administered units
- All unused units
- All units destroyed
- The date of destruction and location

Records shall be maintained by the Investigator/Designee of any alternate disposition of the study medication. These records must show the identification and quantity of each unit disposed of, the method of destruction (taking into account the requirements of local law), and the person who disposed of the test substance. Such records shall be submitted to the study monitor for forwarding to Sponsor.

8.1.2.5 Stability of hu14.18-IL2

From the time of manufacture, frozen hu14.18-IL2 solution is stable for at least 24 months if appropriately stored. It is continuously monitored as part of an on-going stability program. If stability testing allows, the release date of hu14.18-IL2 may be extended. NCI will provide immediate notification to the Investigator should the product not meet stability specifications and should no longer be used for the clinical study.

The greatest concern to product stability is the potential for aggregation of the protein once it is thawed. Aggregation may occur when the thawed solution in a vial is handled roughly or shaken. This is exacerbated by dilution of the stabilizing formulation buffer when the thawed hu14.18-IL2 solution is admixed into 0.9% Sodium Chloride Injection with 0.25% HSA in a 250 mL 0.9% Sodium Chloride infusion bag. For this reason, freezing is required for storage and handling of the hu14.18-IL2 solution until it is thawed for admixture. Do not use a filter in dose preparation.

8.1.2.6 Preparation of hu14.18-IL2 into the final diluted solution

Aseptic technique must be used to prepare hu14.18-IL2 infusions. Hu14.18-IL2 infusions must be prepared only on the day of administration to subjects. The vials of hu14.18-IL2 must be thawed at room temperature. Depending on the ambient temperature, thawing can take up to one hour. Thawed hu14.18-IL2 vials must be *gently* mixed by inverting the vials 10 times. **Vials must not be shaken.** The preparation must be carefully inspected. Thawed product should be a homogeneous, clear, and colorless to slightly opalescent solution free of particles.

To prepare the dosing solution, dilute 5% HSA 1:20 into a 250 mL infusion bag of 0.9% Sodium Chloride Injection for a final HSA concentration of 0.25%. To do this, first remove 12.5 mL of the 0.9% Sodium Chloride Injection solution from the 250 mL infusion bag and then add 12.5 mL of 5% HSA to the bag. Mix the solution by gently kneading the infusion bag (**do NOT shake**). Once the frozen hu14.18-IL2 solution is thawed, use a large bore needle (e.g., an 18 gauge needle) to draw and add the appropriate amount of hu14.18-IL2 from the vials to 250 mL infusion bags containing 0.25% HSA in 0.9% Sodium Chloride Injection. Glass infusion bottles must not be used. In-line filters must not be used. Hu14.18-IL2 must then be mixed gently by inverting the infusion bags 10 times. The bags should not be shaken in order to avoid frothing or excessive shearing of the protein solution. This procedure should result in a homogeneous, clear solution free of particles. If necessary, gentle mixing can be continued for another 5 inversions to produce a homogeneous solution free of particles. If this does not occur or if a precipitate is noted at any time during the preparation and storage of hu14.18-IL2 in the infusion bags prior to administration to subjects, discard the solution and prepare a fresh dose.

After the dilution, the headspace air in the infusion bag must be completely removed by an 18-gauge needle. This ensures safe transport from the pharmacy to the site for administration. Because of the possibility for aggregation of hu14.18-IL2 during transport, the pharmacy must be in close proximity to the clinic where the hu14.18-IL2 is to be administered.

The ready-to-use hu14.18-IL2 infusion must be maintained at room temperature and administered to subjects as soon as possible (within 12 hours of admixture including infusion time). Hu14.18-IL2 infusion must be prepared on the day of administration. **No other drugs** (except for HSA) **can be added to the infusion bag containing hu14.18-IL2.** In-line filters must not be used during administration.

Any stock solution remaining after the subject's dose is taken from the vial may not be used to prepare another dose or used for any other purpose.

8.1.2.7 Stability of hu14.18-IL2 as the final diluted product

Hu14.18-IL2 in the infusion bags should be maintained at room temperature after dilution. **It must be handled gently without shaking.** It must not be exposed to direct sunlight or heat. Hu14.18-IL2 solutions should not be used if the product has aggregated or precipitated.

All reconstituted hu14.18-IL2 should be used as soon as possible after mixture. Hu14.18-IL2 infusions must only be prepared on the day of infusion and should be administered to subjects within 12 hours of mixture (i.e., within 8 hours of storage plus 4 hours of infusion).

8.1.2.8 Administration of hu14.18-IL2

The subject's height and weight determination at the baseline prestudy evaluation ($\leq$ 7 days before starting the first cycle of treatment) will be used to calculate the body surface area (BSA) for treatment cycle one. Calculation of the body surface area will be repeated prior to each subsequent treatment cycle ($\leq$ 7 days before starting each subsequent treatment cycle)

The BSA determination will be multiplied by the dose (mg/m^2) to determine the subject's daily dose of hu14.18-IL2. The actual dosage of hu14.18-IL2 administered will be documented in the dispensing document in the subject file by the Investigator/Designee.

The dose of hu14.18-IL2 will be infused intravenously over 4 hours. Hu14.18-IL2 should be administered via a peripheral or central IV catheter and an 18 to 20-gauge needle. In-line filters must not be used when administering the diluted solution. Prior to and following each dose, the IV catheter should be flushed with 0.9% Sodium Chloride Injection.

Subjects will be treated in the hospital for the duration of infusions (Days 1 through 3 of each cycle) through the safety monitoring period, after which they will be released from the hospital. Subjects will be under close observation by the Investigator/physician or a designated skilled staff member during the infusion of hu14.18-IL2. Vital signs will be evaluated during infusion. (See Clinical Assessment [Table 5] for details.)

The Investigator will use clinical judgment, depending on the subject's status, in releasing the subject from close monitoring.

8.1.2.9 Activity

The activity assays have shown that 1 mg of the hu14.18-IL2 fusion protein is approximately equivalent to 1-3 x 10^6 Units of IL2 (26, 78) (J. Hank and S. Gillies, unpublished data), depending on the assay used.

8.1.3 Material Transfer Cooperative Research and Development Agreement (M-CRADA)

The agent used in this protocol, hu14.18-IL2, is supplied by NCI. The agent is provided to the NCI under a M-CRADA (hereinafter referred to as Collaborative Agreement) between EMD Pharmaceutical Company, hereinafter referred to as Collaborator, and the NCI Developmental Therapeutics Program (NCI-DTP). Therefore, the following obligations/guidelines, in addition to the provisions in the Intellectual Property Option to Collaborator contained within the terms of award, apply to the use of the Agent in this study:

1. Agent may not be used for any purpose outside the scope of this protocol, nor can Agent be transferred or licensed to any party not participating in the clinical study. Collaborator data for Agent are confidential and proprietary to Collaborator and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from http://ctep.cancer.gov.

2. Clinical Trial Data and Results and Raw Data developed under a collaborative agreement will be made available exclusively to Collaborator, the NCI, and the FDA, as appropriate. All data made available will comply with HIPAA regulations.

3. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigator (principle investigator for the study) of Collaborator's wish to contact them.

4. Any manuscripts reporting the results of this clinical trial must be provided to CTEP and to NCI-DTP for immediate delivery to Collaborator for advisory review and comment prior to submission for publication. Collaborator will have 30 days from the date of receipt for

review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator's intellectual property rights, are protected. Copies of abstracts must be provided to CTEP and to NCI-DTP for forwarding to Collaborator for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP and to NCI-DTP prior to release. Copies of any manuscript, abstract and/or press release/media presentation should be sent to:

Regulatory Affairs Branch, CTEP, DCTC, NCI
Executive Plaza North, Suite 7111
Bethesda, MD 20892
FAX: 301-402-1584
E-mail: anshers@ctep.nci.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator. No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

9.0 STATISTICAL CONSIDERATIONS

9.1 Overview

The main goal of this study is to obtain pilot data on the effect of hu14.18-IL2 in subjects with advanced melanoma that are able to achieve CR through complete surgical resection of all disease. In essence, we seek to evaluate whether treatment with hu14.18-IL2 in the minimal residual disease setting (where it is most active in preclinical murine models) has sufficient histological and clinical activity to warrant further evaluation. We are unable to directly measure anti-tumor effect in subjects receiving hu14.18-IL2 following achievement of surgically induced CR. Instead we are able to evaluate the histological evidence of anti-tumor activity indirectly by evaluating the tissue samples from surgical resection. In order to do so, we will randomize eligible subjects into two groups where subjects in group A will receive hu14.18-IL2 following their surgical resection and subjects in Group B will receive their surgical resection after the 1st course of hu14.18-IL2, and then receive subsequent courses of hu14.18-IL2 following the surgical resection. Under these conditions we will be able to evaluate the histological evidence of anti-tumor activity of hu14.18-IL2 by comparing apoptosis, necrosis and cellular infiltrates in tissues samples from group A (as control) and group B. We will also evaluate the time to recurrence (duration of remission) and the overall survival time following treatment with hu14.18-IL2 for these subjects.

9.2 Definition of Study Endpoints

9.2.1 Primary Endpoints

Pathology evaluations of necrosis, apoptosis, and cellular infiltrate:
Pathology evaluations of necrosis, apoptosis, and cellular infiltrate will be performed on a standard scale with 4 categories: "0" (none), "+" (low), "++" (medium), or "+++" (high). These evaluations will be performed independently by two pathologists in a blinded fashion. In the case of a disagreement, the tumor tissues will be evaluated by a third pathologist.

Time to recurrence (duration of remission):

For a given subject, time to recurrence will be defined as the number of days from the day of evaluation following course 2 of IC treatment to the day the subject experiences an event of recurrence or death, whichever comes first. All subjects on this study have disease deemed to be completely resectable, and all subjects are expected to be going into complete remission following surgical resection. Uniformity of follow up time is best provided by clarifying the time after "study evaluability" that recurrence is first identified (and confirmed whenever possible). As such, the time point of "study evaluability" will be designated the day of evaluation following course 2 of IC treatment, when CR is first demonstrated clinically and radiologically. Subjects in Group A and Group B will each have spent equivalent time since study entry in getting to this point: each will have had their surgical resection and their first 2 courses of IC treatment. The only difference is that surgery is before course 1 for Group A and after it for Group B.

If a subject has not experienced an event of recurrence (or death) at the time of analysis, then the subject's data will be censored at the date of the last available evaluation

Overall survival:
Overall survival will be defined as the number of days from the randomization to the date of the subject's death. If a subject is alive at the time of analysis, then the subject's data will be censored at the date of the last available evaluation

9.2.2 Secondary Endpoints

Adverse events:
Adverse events and toxicities (associated with 4 course of IC treatment for 3 consecutive days administered on an every 4-week basis) observed will be categorized according to version 3.0 of the NCI Common Toxicity Criteria.

Biological endpoints:
Biological endpoints include immunological parameters (lymphocyte counts, NK/ADCC, soluble IL2, anti-idiotypic antibodies, and anti Fc-IL2), expression of the GD2 target antigen, density and phenotype of the cellular infiltrate, vascularity, IC binding, IFN-γ expression and T cell reactivity.

9.3 Study Design

This is a two-arm randomized study. At the time of study entry, eligible subjects will be randomized to either Group A or Group B.

Group A: Subjects will have surgery prior to starting course 1 of IC treatment.
Group B: Subjects will have surgery after course 1 and before course 2 of IC treatment.

The rationale for the study design is described in detail in Section 1.3.4.

9.3.1 Randomization/Stratification

The randomization will be based on a permuted block of size 4, stratified by type of surgical resection ("extensive" vs. "non-extensive"). "Extensive" surgical resection is defined as: Resection requires thoracotomy, laparotomy or excision of deep soft tissue metastases in 3 or more anatomic locations. "Non-extensive" surgical resection is defined as: Resection requires cutaneous/subcutaneuous excision and/or excision of deep soft tissue disease in only 1 or 2 locations.

9.3.2 Sample Size/Accrual Rate

The first primary objective of this study is to evaluate the histological evidence of anti-tumor activity of hu14.18-IL2 by comparing pathological endpoints between the two groups after dichotomizing the evaluation of apoptosis, necrosis and cellular infiltrate into *positive* (i.e., the microscopic evaluation is classified as "low", "medium", or "high") or *negative* (i.e., the evaluation is classified as "none") using Fisher's exact test. With eight subjects per group, a difference of 70% in the proportions of subjects classified as *positive* for necrosis, apoptosis, or cellular infiltrate between the two study arms will be detected with at least 82% power at a two-sided significance level of 0.1. A difference of 60% will be detected with at least 64%. Smaller differences are unlikely to be detected as statistically significant. The sample size chosen is sufficient to provide accuracy in the estimated rate of subjects classified as *positive*. Specifically, with eight subjects per group, the standard error of the proportions of subjects classified as *positive* necrosis, apoptosis, or cellular infiltrate for each group will be at most 0.18.

The second primary objective of this study is to evaluate the time to recurrence (duration of remission) and overall survival relative to historical control data described in Section 1.3.3. This study is not designed to detect differences in time to recurrence or overall survival between the two study arms. Instead, time to recurrence and overall survival time of both arms combined will be compared to historical controls qualitatively. Time to recurrence and overall survival time will be summarized using point estimates of the median time to recurrence/overall survival time and associated 90% confidence intervals. Furthermore, the two-year recurrence free survival rate and the two-year survival rate will be reported along with the corresponding 90% confidence intervals. The following table gives (two-sided) Wilson's score 90% confidence intervals for the two-year survival/two-year recurrent-free survival rates, assuming a total sample size of 16 subjects.

Table 9: Ninety percent confidence bounds for two-year survival/two-year recurrent-free survival rates

Observed number of subjects alive/recurrent-free after 24 months	Proportions of subjects alive/recurrent-free after 24 months	Lower 90% confidence limit	Upper 90% confidence limit
2	0.12	0.04	0.32
4	0.25	0.12	0.45
6	0.38	0.21	0.58
8	0.50	0.31	0.69
10	0.62	0.42	0.79
12	0 .75	0.55	0.88
14	0.88	0.68	0.96

Therefore, with a sample size of 16 subjects, the 90% confidence interval for the two-year survival/two-year recurrent-free survival rate will be no wider than 38%.

Secondary endpoints of this pilot study include adverse events, immunological parameters, density & phenotype of cellular infiltrate and expression of GD2 target antigen, and IFN-γ expression. Assuming a total sample size of 16, the probability of observing at least one incidence of Grade ≥ 3 toxicity is 81%, if the true toxicity rate is 10%. Analogously, the probability of observing at least one incidence of Grade ≥ 3 toxicity is 93%, if the true toxicity rate is 15%. Immunological parameters will be measured on a continuous scale. Assuming a sample size of eight subjects per

study arm, a difference of 1.5 standard deviation units in magnitude will be detected with 80% power at a two-sided significance level of 0.05 (based on a two-sample t-test). A difference of only 1.25 standard deviation units will be detected with 63%. Smaller differences are unlikely to be detected as statistically significant.

In order to account for "unevaluable" subjects and drop-outs (e.g., patients without an evaluation following course 2 of IC treatment), we will accrue up to 20 eligible subjects (10 per arm) in total. Based on our past experience with advance melanoma subjects treated at UWCCC, we expect to accrue 20 eligible subjects within 24 months. The minimum follow-up period will be 12 months after the last subject has entered the study.

9.4 Statistical Analysis Plan

9.4.1 General

Descriptive statistics will primarily be generated to summarize the data. For continuous variables, e.g., immunological parameters, GD2 target antigen expression etc., descriptive statistics may include the number of subjects reflected in the calculation (n), mean, standard deviation, median, minimum, and maximum; frequencies and percentages may be displayed for categorical data (e.g., toxicities, phenotype of cellular infiltrate, etc.). All data analyses will be performed using SAS® (SAS Institute Inc., Cary, North Carolina) version 8.2 or greater and R version 1.91 or greater, a publicly available statistical analysis environment (http://www.r-project.org).

9.4.2 Baseline Comparability

All measurements (variables) collected at baseline will be summarized and compared between study arms. If the pre-treatment value is missing, the measurement recorded at the screening visit will be substituted. Significant unbalances found between study arms may be considered as covariates in exploratory analyses of primary endpoints (i.e., pathology evaluations of necrosis, apoptosis, and cellular infiltrate).

9.4.3 Demographics

All demographic variables (e.g., gender, age, weight, etc.) will be summarized by standard descriptive statistics, i.e., in terms of means, standard deviations, medians, and ranges for variables on a continuous scale, and in terms of frequency tables for variables on a categorical scale.

9.4.4 Primary Endpoints

Pathology evaluations of necrosis, apoptosis, and cellular infiltrate will be summarized in frequency tables (for each study arm). The proportions of subjects with tumor tissues classified as *positive* (i.e., the microscopic evaluation is classified as "low", "medium", or "high") will be reported along with the corresponding 90% confidence intervals. The comparisons of pathology evaluations between the two study arms will be performed by Fisher's exact test. For subjects in treatment group B, tumor tissues from biopsies obtained at study entry (pre-study biopsies) will be compared to surgically resected tumor tissues obtained after the first course of IC treatment. Paired comparisons of necrosis, apoptosis, and cellular infiltrate pathology evaluations will be performed using McNemar's test.

Time to recurrence and overall survival will be summarized using point estimates of the median time to recurrence/overall survival and associated 90% confidence intervals. The data will be presented graphically using Kaplan-Meier plots. The two-year recurrence free survival rate and two-year survival rate will be reported along with the corresponding 90% confidence interval. Time to recurrence and overall survival will be contrasted to historical controls (47,49-53) as well as data from comparable subject groups being observed following surgically induced CR that may be published now and when the data from the present study are being analyzed.

9.4.5 Secondary Endpoints

The number and severity of toxicity incidents will be analyze descriptively in tabular format. Immunological parameters, i.e., lymphocyte counts, NK/ADCC, soluble IL2, anti-idiotypic antibodies, and anti Fc-IL2, will be summarized by standard descriptive statistics in terms of means, standard deviations, medians and ranges for each measurement time point (see Section 7.3) and group separately. Within each group, differences between pre-IC treatment values and post-IC treatment values will be evaluated by paired t-tests and/or non-parametric Wilcoxon test.

Comparisons of immunological parameters between the two study arms will be performed by two-sample t-tests. Furthermore, longitudinal data analyses of immunological parameters will be performed to incorporate the time effect. Specifically, linear and/or nonlinear mixed effect models with subject specific random effects and generalized estimating equations (GEE) models with identity link and exchangeable (working) correlation structures will be used to account for the correlation between repeated measurements of immunological parameters. Missing data may be imputed using regression type imputation techniques. For immunological parameters not conforming to the assumptions of parametric analysis, the non-parametric Wilcoxon Rank Sum test will be used for comparing the two study arms. The association between the various immunological parameters will be examined by computing Spearman's rank correlation coefficients and linear or nonlinear regression techniques. Immunological parameters will be correlated with clinical endpoints by fitting Cox regression models (for clinical endpoints overall survival & time to recurrence) and logistic regression analysis models (for clinical endpoints toxicity and histological evidence of anti-tumor activity). The data of all immunological parameters will be presented graphically where possible (boxplots, histograms, profile plots and scatterplots).

The analysis of GD2 target antigen expression, density and phenotype of cellular infiltrate, vascularity, IC binding, and IFN-γ expression will be exploratory by necessity. Expression levels for these will be summarized in terms of means, medians, standard deviations and ranges where possible. Comparisons of expression levels between the two groups will be performed using two-sample t-tests (after appropriate transformations) and/or non-parametric Wilcoxon Rank Sum tests. Phenotypes of cellular infiltrate will be summarized in tabular format. Differences of phenotype frequencies between the two groups will be evaluated using Fisher's exact tests.

Augmentation of T cell reactivity to autologous tumor or to known melanoma antigens will be analyzed descriptively. The level of significance of each hypothesis test involving secondary endpoints will be 0.05. Due to the exploratory nature of these analyses, there will be no multiplicity adjustment.

9.4.9 Stopping rules for excessive toxicity

Many of the dose limiting toxicities (DLTs) that occur due to hu14.18-IL2 therapy are not unexpected. However, a subset of the DLTs described in Section 5.0 of the protocol are considered unacceptable. Those DLTs are:

1. Toxicity requiring the use of vasopressors, including Grade 4 vascular leak syndrome and/or grade 4 hypotension, and
2. Toxicity requiring ventilatory support, including Grade 4 respiratory toxicity.

If at any time a patient requires ventilatory support, new accrual to the study will be temporarily stopped, and the study will be reviewed for safety, dose modification and safety modification.

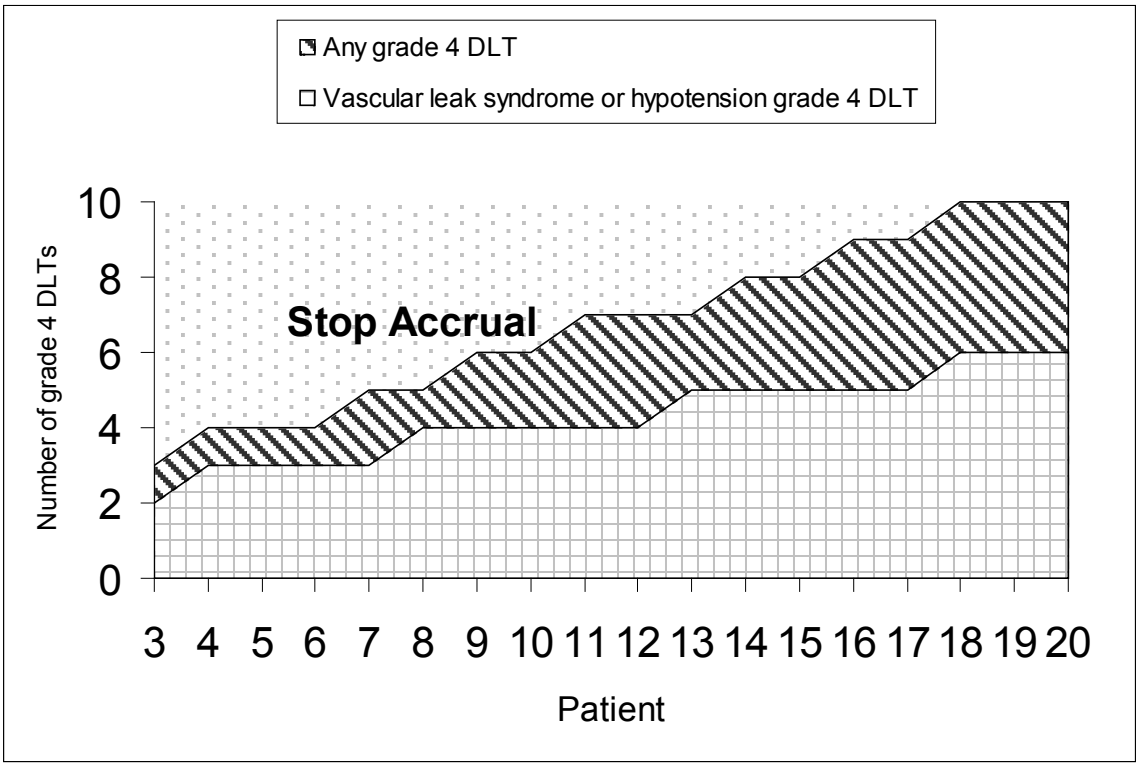
Based on this, a decision regarding reopening the study, likely with an appropriate amendment, would be made by the DSMC, working with the study committee.

Furthermore, accrual to this study will be temporarily suspended at any stage during the accrual period if an excessive toxicity rate is observed. An excessive toxicity rate is defined as follows:

1. At least 15% for Grade 4 vascular leak syndrome or grade 4 hypotension toxicities, or
2. At least 33% for any Grade 4 DLTs.

Evidence that the toxicity rate is excessive will be considered sufficient if the lower limits of the 90% one-sided confidence intervals for the estimates of the true toxicity rates exceed the toxicity limits defined above (i.e., 15% for Grade 4 vascular leak syndrome or hypotension, 33% for any grade 4 DLTs).

Operationally, this will occur if any of the following events occur: Grade 4 vascular leak syndrome or Grade 4 hypotension toxicities are present in at least 2 out of 3 (or less) patients, 3/7, 4/12, 5/17, or 6/20 of the patients accrued, or if any Grade 4 DLTs are present in at least 3 out of 3, 4/6, 5/8, 6/10, 7/13, 8/15, 9/17, or 10/20 of the patients accrued. If one of these thresholds is reached, accrual will be temporarily suspended and the study will be reviewed for safety, dose modification and safety modification. The study would only be reopened if modifications can be made, and approved by the DSMC and the UW-IRB, to assure that patient safety is reestablished. If not, the study would be closed. The stopping rule is illustrated in the following figure:



10.0 EVALUATION AND MANAGEMENT OF TOXICITY AND SUPPORTIVE CARE

10.1 Grading of toxicity and dose modification

Grading of toxicities on this study will be with NCI CTC, version 3.0. All significant adverse events will be followed until they resolve to baseline or < Grade 2, or are deemed irreversible.

10.1.1 Dose modification for DLT

Dose limiting Toxicity (DLT) criteria and exclusions are described in protocol section 5.3. For any occurrence of DLT, treatment with protocol therapy will be stopped. Treatment may not be resumed until after toxicity has resolved to meet original eligibility criteria. Resuming treatment following any DLT at a reduced dose (according to the guidelines below) requires contact (by phone or e-mail) with the FDA on a case-by-case basis. The first contact will be to the FDA Oncology Drug Products review division in the Center for Drug Evaluation and Research at 301-796-2320. An alternate contact is at the following: Melanie.pierce@fda.hhs.gov; (301) 796-2320, (301) 796-1273.

For toxicities occurring during treatment with hu14.18-IL2, if DLT occurs on day 1 or 2 of any course, treatment can only be resumed during that same course (i.e., on day 2 or 3) at 50% of the starting dose, and only if the toxicity has resolved to meet eligibility criteria in time to give the day 2 or day 3 dose on schedule. If eligibility criteria are not met in time to receive additional therapy during the course in which the toxicity occurred, but are met in time to start the next course (i.e., day 1 +/- 7 days), treatment will resume at 50% of the dose associated with the DLT. Any planned drug administration that is withheld due to DLT will not be given on a delayed schedule to "make up" for missed drug. If toxicity again requires treatment cessation, treatment can be restarted at 25% of initial treatment dose. If treatment at this reduced dose results in recurrence of DLT or significant non-DLT toxicity, treatment will be permanently discontinued. Treatment will be resumed, as described above, using only 50% of the starting dose of hu14.18-IL2, and only if the toxicity has resolved to meet eligibility criteria.

10.1.2 Dose modification for toxicity that is not graded as DLT

It is the intention of this pilot study to treat all patients, at all courses, at the starting doses of 6 $mg/m^2/d$ for hu14.18-IL2, or the maximum dose that each patient demonstrates is tolerable for them. However, if severe toxicity is observed, such that the investigator feels discontinuation of treatment at the dose level causing the toxicity is in the patient's best interest, temporary or permanent cessation of treatment at that dose level, or a 50% dose reduction for subsequent infusions (in the absence of DLT) is permissible. Justification for discontinuation of therapy or dose reduction must be clearly documented. At no time will the dose level be escalated.

10.2 Supportive care

10.2.1 Hematologic toxicity

All subjects will be transfused as needed to maintain an adequate Hgb level ($\geq$ 8.0) and platelet count ($\geq$ 40,000) during Days 1-3 of treatment each month. Hematologic DLT criteria are detailed in Section 5.3. As there may be an association of vascular leak syndrome with co-administration of monoclonal antibody-IL2 and transfusions, it is recommended to avoid transfusions during the 3 days of hu14.18-IL2 administration, and particularly during the daily 4-hour infusions. If transfusions are needed, it is recommended that that they be given at least 12 hours before starting treatment on day 1 of each course and > 12 hours after finishing the day 3 infusion. If transfusions must be given on day 3 to maintain Hgb >8.0 and plts > 40,000, these should be given 12 hours after completing the prior 4-hr infusion of hu14.18-IL2. If the subject experiences neutropenia (ANC < 1000) while receiving the fusion protein, treatment would not be interrupted since neutropenia is common and rapidly reversible upon stopping IL2 treatment. For days 8-29 of each course of therapy, transfusions should be given, if needed, to maintain a platelet count of >20,000, and a Hgb of > 7.0.

10.2.2 Hepatic Toxicity

No dose interruption or dose modifications are required for Grade ≤ 2 transient hepatic toxicity. Increases in bilirubin and transaminases are routinely seen with this IC, and are usually quickly resolved within 2-3 days of completing (or stopping) hu14.18-IL2 treatment. Grade 3 toxicity will require cessation of agent and treatment at 50% dose when toxicity has resolved and entry criteria are met.

10.2.3 Cardiac toxicity and/or dyspnea

10.2.3.1 Cardiac abnormalities

Any evidence of cardiac abnormalities will require an immediate ECG evaluation. Evidence of ischemia will require immediate discontinuation of therapy. Subjects with evidence of asymptomatic atrial irregularities (including supraventricular tachycardia) related to an elevated temperature but no evidence of ischemia or clinically significant hypotension will be monitored but continue therapy.

10.2.3.2 Blood pressure and fluid overload

10.2.3.2.1 Fluid overload

Complications of fluid overload may be seen with IC treatment. Patients with clinical problems related to fluid overload can be treated with furosemide (20 mg IV) provided they have < 40 mm Hg decrease in systolic blood pressure from day 1 of cycle, obtained prior to initiation of IC therapy, and a systolic BP > 90 mm Hg.

10.2.3.2.2 Hypotension

Mild hypotension with systolic blood pressure 10-15% below baseline is common in patents receiving IL2.

a. Asymptomatic patients with mild hypotension should be monitored closely

b. Symptomatic patients or those with systolic blood pressure < 85 mmHg or < 100 mmHg if representing a decrease in systolic blood pressure >20% from the lowest value of any documented systolic blood pressure obtained since study consent was signed should be treated for hypotension. (Do not include blood pressures obtained on days of hu14.18-IL2 treatment after hu14.18-IL2 infusion started for purposes of getting this lowest usual blood pressure).

   Patients meeting the above guidelines should be given a 500 ml IV bolus of normal saline over 15 minutes. If unresponsive:

   - Stop the hu14.18-IL2 infusion
   - Repeat the fluid bolus: 500 ml IV bolus of normal saline over 15 minutes. Fluid bolus may be repeated once or twice as needed. May be limited by symptomatic fluid overload (i.e. pulmonary edema, tense ascites).
   - If unresponsive to fluid bolus, may give Albumin (25%) 50 gm IV, then q 6-12 h PRN if serum albumin ≤ 3.0.
   - RBC Transfusion to maintain Hgb ≥ 10.0g/dL
   - If responsive, (i.e., blood pressure increases to a systolic blood pressure over 100 mm Hg or to the patient's blood pressure prior to administration of hu14.18-IL2 for that day if the baseline systolic blood pressure was less than 100 mm Hg), may reinitiate the hu14.18-IL2 treatment on the following day (if scheduled) at

50% reduced dose. If hypotension is recurrent, treatment will be stopped and patients managed as outlined above in this section. Treatment can be restarted (at their next scheduled infusion day) at 25% of the initial dose, if systolic blood pressure is responsive to the fluid management described above. If hypotension is recurrent at this 25% dose, treatment will be discontinued and the patient will be off protocol therapy.

c. Symptomatic hypotension that does not respond to these measures requires:

- IV vasopressors, (i.e., phenlyephrine, dopamine, etc.)
- If responsive (i.e., blood pressure increases to a systolic blood pressure over 100 mm Hg or to the patient's blood pressure prior to administration of hu14.18-IL2 for that day if the baseline systolic blood pressure was less than 100 mm Hg and no longer requiring vasopressor support), may reinitiate the following day (if scheduled) at 50% reduced dose. If hypotension is recurrent, treatment will be stopped and patients managed as outlined above in this section. Treatment can be restarted (at their next scheduled infusion day) at 25% of the initial dose, if systolic blood pressure is responsive to the fluid management described above. If hypotension is recurrent at this 25% dose, treatment will be discontinued and the patient will be off protocol therapy.
- For Grade 3 hypotension, see dose modification guidelines in section 10.1
- Additional boluses of 500 ml IV Normal Saline can be administered as clinically indicated

10.2.3.3 Acute vascular leak syndrome

High dose IL2 can induce a capillary leak syndrome and sepsis-like physiology (decreased systemic vascular resistance, increased cardiac output and some degree of peripheral or pulmonary interstitial edema and/or ascites). Cardiovascular toxicity may be additive when IL2 is combined with hu14.18. For Grade 3 acute vascular leak syndrome, see dose modification guidelines in Section 10.1

10.2.3.4 Dyspnea

Subjects experiencing dyspnea and whose oxygen saturation is less than 90% may receive brief oxygen supplementation. Subjects who experience this toxicity are often fluid overloaded and will need furosemide, provided that they are not hypotensive (see management of hypotension, above). Therapy will be discontinued and not restarted until the oxygen saturation is above 90% without oxygen supplementation. Once the oxygen saturation is above 90% without oxygen supplementation, treatment with hu14.18-IL2 may be reinitiated on the following day (if scheduled) at 100% dose (if recovery occurred in less than 1 hour) or at 50% reduced dose (if recovery took longer than 1 hour to resolve). If they are fluid overloaded, hypoxic and hypotensive, treatment should be stopped and vasopressor support initiated, as needed. Once toxicities resolve, subsequent treatment will be at the 50% dose level.

If dyspnea with oxygen saturation below 90% is recurrent, treatment will be stopped and patients managed as outlined above in this section. Treatment can be restarted (at their next scheduled infusion day) at 50% of the prior dose, if oxygenation is responsive to the management described above. If dyspnea with oxygen saturation below 90% is recurrent at 25% of the initial dose, treatment will be discontinued and the patient will be off protocol therapy.

If the oxygen saturation does not then improve to over 90% off of oxygen supplementation within 24 hours, treatment will be discontinued and restarted at 50% of the previous dose of

hu14.18-IL2, provided that the toxicity resolves. If they are fluid overloaded, hypoxic and hypotensive, treatment should be stopped and vasopressor support initiated, as needed. Once toxicities resolve, subsequent treatment will be at the 50% dose level.

10.2.4 Neurotoxicity

Patients experiencing Grade 3 or worse neurotoxicity except confusion (i.e., objective peripheral neuropathy or prolonged weakness) for > 3 days (see Section 5.3.3f) will have treatment held. Treatment will not be restarted until that toxicity returns to ≤ Grade 1. A dose reduction of 50% will be required for any subsequent course of treatment. In the event of Grade 3 or worse neurotoxicity, repeated neurological exams will be performed regularly, and documented, until symptoms resolve or irreversibility is documented.

10.2.5 Metabolic/laboratory

Symptomatic hyponatremia (sodium < 125 nmol/L, > 48 hours) or severe hyponatremia without symptoms (sodium < 120 nmol/L on 2 consecutive samples) will require permanent discontinuation of protocol therapy. Since asymptomatic hypophosphatemia was observed with relatively high frequency in our Phase II study of hu14.18-IL2 in adults with melanoma, all subjects due to receive this agent will be given oral phosphate supplementation prophylactically starting on day 1 and ending on day 8 of each course (see Section 10.3.4.3 for details). Intravenous phosphate supplementation will be considered in the event of symptomatic hypophosphatemia or persistent (> 24 hrs) asymptomatic hypophosphatemia (i.e., < 1.5 mg/dL) despite oral prophylaxis in order to prevent the serum phosphate from remaining < 2.0 mg/dL for > 48 hours. Glucose values greater than 250 mg/dL that are persistent for > 12 hours may be treated with parenteral insulin. If glucose values greater than 250 mg/dL are noted in the outpatient setting, the level will be rechecked within 24 hours, and appropriate management will be initiated.

10.2.6 Performance status

Treatment will be stopped for Grade 4 fatigue or an ECOG performance status of 4. If performance status improves to ≤ 2 in < 1 week, fusion protein treatment dosing in the subsequent cycle can be restarted at 50% of the dose which caused this toxicity.

10.2.7 Temperature elevations

Fusion protein treatment will be held for persistent temperature elevations (> 6 hours) of 40º C or greater not responding to symptomatic treatment with antipyretics. Provided that renal function remains stable (i.e., creatinine does not increase more than 50% over baseline), all patients will receive indomethacin 25 mg/dose every 6 hours, starting 1 hour before the first dose of hu14.18-IL2 and continuing 12 hours after the last dose on day 3 (see premedication, Section 10.3.3). As most patients experience fevers, it is recommended to begin acetaminophen (325-650 mg) as soon as any elevated temperature (> 37.5° C) develops after the first dose of hu14.18-IL2, and to continue it on a scheduled basis for the next 60-72 hours (not to exceed 4 gm per day). Rigors, which may accompany temperature changes, can be treated with meperidine IV. Persistent temperature elevations > 39° C which are not well controlled with acetaminophen and indomethacin may also be treated with a cooling blanket. No dose modifications will be made for temperature elevations (unless the temperature persists > 40º C for > 6 hours) despite treatment with the above antipyretic approaches. If fever does persist for > 6 hours despite antipyretics, then hu14.18-IL2 treatment will be stopped. Treatment can be restarted within a 3-day course of treatment once the temperature is ≤ 38º C. Treatment will then be at 50% of the dose which caused this toxicity.

Use of prophylactic antibiotics for fever:

Although the IL2 component of hu14.18-IL2 is known to, and expected to cause fever, IL2 is also known to cause some neutrophil dysfunction and predispose to bacterial infections, including central

catheter related infections. Thus, for fever > 38.5° C, it is recommended that blood cultures be drawn, and a broad-spectrum antibiotic be considered if fever persists or the patient is clinically unstable.

10.2.8 Confusion

Confusion related to temperature elevations will be managed by aggressive use of antipyretics and cooling blankets, and will not require dose reduction or drug stoppage, if the confusion normalizes with initiation of aggressive antipyretic measures.

Persistent confusion (> 12 hours) which is clearly not temperature related or related to supportive care medicines (Benadryl, morphine, etc.) will require stopping study drug. Subsequent re-initiation of treatment at one-half the prior dose will proceed if reversal of this toxicity occurs prior to the next scheduled dose.

10.2.9 Pain

Subjects experiencing pain due to hu14.18-IL2 will be treated with morphine or similar analgesics as needed and have their pain graded according to the CTCAE. It is recommended to have morphine available, but not given as a premed, on the first day of treatment. If pain is noted and morphine is needed on that first day, then including morphine as a premed for all subsequent doses may be helpful. Subjects with pain that is not controlled with IV narcotics or that requires IV narcotics > 48 hours after completion of hu14.18-IL2 on day 3 of any treatment course will not receive additional protocol treatment at that dose. Provided that the pain resolves, and eligibility requirements are met, subsequent courses would be given at ½ the prior dose.

10.2.10 Nephrotoxicity

High dose IL2 is associated with nephrotoxicity, but this has been minor in subjects receiving IL2 doses similar to those to be administered in this study. Adequate renal function is an eligibility requirement. If renal function is worsening (ie: > 2 times the upper limits of normal but not yet Grade 3), nephrotoxic drugs (including indomethacin) should be avoided.

10.2.11 Infusional reactions

Hu14.18-IL2 will be held for any infusional reactions requiring additional supportive anti-allergic (non-steroidal) treatments (i.e., Benadryl, epinephrine) and will not be restarted until that toxicity resolves. Then, hu14.18-IL2 will be restarted at 100% dose. Patients who experience Grade 3 allergic reactions that are considered true allergic reactions (i.e., IgE-mediated and usually worsen with re-exposure) will not be retreated.

10.2.12 Surgical wound care

Should any subject in any of the treatment groups develop complications related to their surgical wound(s) (e.g., dehiscence, infection, etc.), a General Surgery consult will be obtained (preferably from the surgeon who performed that subject's resection) to manage such complications appropriately.

10.3 Concomitant Therapy

10.3.1 No other anti-neoplastic agents or growth factors can be used.

Radiotherapy may be given to surgical sites with close or microscopically positive surgical margins. Radiotherapy to these sites will be planned to occur immediately upon completion of all protocol therapy.

10.3.2 Prohibited medications: therapeutic anticoagulation with Vitamin K antagonists (e.g., coumadin); therapeutic-dose anticoagulation with heparin, resulting in a prolonged PTT; or therapeutic-dose anticoagulation with low molecular weight heparin (i.e., at doses that usually decrease Factor Xa). Low (prophylactic) dose low molecular weight heparin or coumadin (i.e., 1 mg/d) may be used.

10.3.3 Premedication and ancillary medications for management of common symptoms related to hu14.18-IL2

Appropriate antibiotics, blood products, antiemetics, fluids, electrolytes and general supportive care are to be used as necessary.

10.3.3.1 Premedication prior to hu14.18-IL2 administration on day 1-3

Preclinical data suggest that concomitant use of indomethacin may enhance the antitumor effect of immunocytokine treatment in mice (S. Gillies, unpublished), and that indomethacin can ameliorate clinical toxicity of IL2, it is recommended that indomethacin (25 mg/dose every 6 hours) be started as a premed 1 hour before administration of hu14.18-IL2 on day 1, and continue for 12 hours after the last dose of hu14.18-IL2 during each treatment cycle. Indomethacin should not be given if renal function has worsened from baseline (> 50% increase over baseline serum creatinine) or if the platelet count is ≤ 50,000/µl. Other anti-inflammatory drugs, such as ibuprofen, are permitted as a substitute for indomethacin if, in the investigator's judgment, indomethacin will not be well tolerated.

Any patient showing allergic symptoms with hu14.18-IL2 should receive diphenhydramine, acetaminophen, and an H_2 blocker (e.g., ranitidine 150 mg PO) as premedication before all subsequent doses.

Any patient experiencing pain with a dose should receive premedication with morphine (or an appropriate narcotic) before all subsequent hu 14.18-IL2 doses.

10.3.3.2 Medications acceptable for symptom management*

- Acetaminophen 650 mg PO q4 hrs PRN, fever
- Acetaminophen 325 mg/oxycodone 5 mg (Percocet) 1-2 PO q4-6 hrs PRN, pain
- Diphenhydramine 25-50 mg PO/IV q6 hrs PRN, pruritis, urticaria
- Hydroxyzine 50-100 mg PO/IM q6 hrs PRN, pruritis, urticaria or anxiety
- Dolasetron 100 mg PO/IV qd PRN, nausea/vomiting
- Granisetron 1 mg PO/IV qd PRN, nausea/vomiting
- Loperamide 2 mg tablets, 4 mg PO initially followed by 2 mg after each unformed stool; maximum of 16 mg qd PRN
- Lorazepam 0.5-1 mg PO/IV q2-4 hrs PRN, anxiety or nausea/vomiting
- Meperidine 25 mg IV q3-4 hrs PRN for chills/rigors; patients developing chills may be treated for possible infection with broad spectrum antibiotics (Section 10.2.6)
- Morphine sulfate 1-4 mg IV q2-4 hrs PRN, pain
- Naproxen 250-500 mg PO bid PRN**
- Ondansetron 24 mg IV prior to treatment/8 mg PO tid PRN, nausea/vomiting
- Oxycodone 5-10 mg PO q2-4 hrs PRN, pain
- Prochlorperazine 10 mg PO q6 hrs PRN or 25 mg PR q12 hrs PRN
- Pseudoephedrine 60 mg PO q6 hrs PRN
- Ranitidine 150 mg PO bid PRN

***Dexamethasone and other corticosteroids MAY NOT BE GIVEN (see Section 10.3.3.4).**

**Care should be taken when using non-steroidal, anti-inflammatory drugs (NSAIDs) secondary to potential nephrotoxicity. Anti-inflammatory drugs such as naproxen are permitted.

10.3.3.3Hypophosphatemia prophylaxis
Given the relatively frequent occurrence of asymptomatic hypophosphatemia in our Phase II study of hu14.18-IL2 in adults with melanoma, all subjects due to be treated with this agent will be given oral phosphate supplementation prophylactically starting on day 1 and ending on day 8 of each course. This regimen initially will consist of KPhos Neutral 2 tabs PO BID (or a similar preparation). Dosage will then be individualized and titrated to maintain serum phosphate levels of > 2.0 mg/dL, and preferably within the normal range. This KPhos supplementation will be decreased or held in the event of any hyperkalemia or nephrotoxicity, as detected by our clinical monitoring labs (see Table 5).

10.3.3.4 Other supportive medicines
Standard preventative treatments (including prophylactic-dose anticoagulation) may be continued per the discretion of the treating physician, provided that they are not growth factors, steroids, or myelosuppressive anti-neoplastic drugs.

10.3.3.5 Severe allergic reaction (anaphylactic precautions should be taken).
Treatment can include:
Dexamethasone 10 mg IV
Diphenhydramine 50 mg IV
Epinephrine 3-5 ml IV [1:10,000]; 0.5 ml SC [1:1000]
Equipment for assisted ventilation
A free-flowing IV line must be established at all times

NOTE: Treatment with corticosteroids will result in withdrawal from **protocol therapy.**

11.0 RECORDS TO BE KEPT

11.1 Case Report Form

11.2 Study Drug Administration Form

11.3 Course Assessment Forms

11.4 Surgical Assessment Form

11.5 Off Treatment Summary

12.0 SUBJECT CONSENT AND PEER REVIEW

All NCI, federal, and institutional guidelines concerning informed consent will be followed, including Title 21 CFR 312.68 regarding access to records. Consent forms will be provided to all subjects for review prior to entry on the study. The appropriate consent form must be signed prior to enrollment in the study.

13.0 DATA AND SAFETY MONITORING PLAN

13.1 Oversight and monitoring plan

The UWCCC Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and subject safety for all UWCCC clinical studies. A summary of DSMC activities follows:

Review of all clinical trials conducted at the UWCCC for data integrity and safety
Review of all serious adverse events requiring expedited reporting as defined in the protocol
Review of reports generated by the UWCCC data quality control review process
Submit recommendations for corrective action to the Clinical Research Committee (CRC)
Notify the Study Chair of the DSMC recommendation to the CRC
The committee ensures that notification is provided to external sites participating in multiple-institutional clinical trials coordinated by the UWCCC of serious adverse events requiring expedited reporting.

13.2 Monitoring and reporting guidelines

Data related to these trials are discussed at regularly scheduled Disease Oriented Working Group meetings where the result of each subject's treatment is discussed and the discussion is documented in the minutes. The discussion will include for each treatment arm/dose level, the number of subjects, significant toxicities as described in the protocol, dose adjustments, and responses observed. Twice yearly, summaries will be submitted to the Data and Safety Monitoring Committee for review.

13.3 Review and oversight requirements

13.3.1 Adverse event – reported within 24 hours

Serious Adverse Events requiring expedited reporting within 24 hours (as described in the protocol) will also be reported to the Data and Safety Monitoring Committee (DSMC) Coordinator via saenotify@uwcarbone.wisc.edu within one working day. Confirmation that all appropriate parties were notified will be done at this time. Hardcopies or electronic versions of NCI ADEERS form (#3500) and/or any other documentation available at that time will also be reviewed by the Committee Chair who will determine if immediate action is required. Within ten working days all subsequent SAE documentation that is available will be submitted with a completed UWCCC SAE Routing Form to the designated parties on the form and Committee Chair who will determine if further action is required. All information will be tracked in the UWCCC database.

If the SAE occurs on a multiple-institutional clinical trial coordinated by the UWCCC, the Outreach Coordinator will ensure that all participating sites are notified of the event and resulting action within one working day of the determination.

13.3.2 Adverse event – reported within 10 days

Serious Adverse Events requiring expedited reports in writing within 10 working days (as described in the protocol) will be sent to the UWCCC DSMC Coordinator via datasafetymonitoring@uwcarbone.wisc.edu. Hardcopies or electronic versions of NCI ADEERS form or other required forms will be submitted along with a copy of the UWCCC SAE Routing Form. The Committee Chair will review these forms and determine if further action is required. This information will be tracked in the UWCCC database.

If the SAE occurs on a multiple-institutional clinical trial coordinated by the UWCCC, the Outreach Coordinator will ensure that all participating sites are notified of the event and resulting action within one working day of the determination.

13.3.3 Study Progress Review

Cumulative reports of serious adverse events requiring expedited reporting and any new serious adverse event requiring expedited reporting are also reviewed at the committee's bimonthly meetings.

An overall assessment of accrual, toxicities as described in the protocol, and responses will enable the committee members to assess whether significant benefits or risks are occurring that would warrant study closure. This information is provided by Disease Group meeting minutes, internal audit and/or response review reports. The committee may request external DSMB reports or further information from the Disease Groups, or Study Chair.

The Data and Safety Monitoring Committee recommendations for modifications to the trial are forwarded to the Clinical Research Committee (CRC), composed of Cancer Center senior leaders that oversee all aspects of clinical research conducted at the UWCCC and makes final decisions on all issues related to clinical trials. The Study Chair is notified of this recommendation in order that he/she may alert all investigators, at the UWCCC and at external sites involved in the trial, about the potential action. At this time the Study Chair may submit to the CRC additional information that could affect the Committee's decision. The CRC will notify the Study Chair if they concur with the Data and Safety Monitoring Committee recommendations, including suspension or closure. The Study Chair will notify all investigators involved with the study at UWCCC and external sites, the IRB, the sponsor and the funding agency and provide written documentation of these notifications to the CRC.

14.0 EXPEDITED REPORTING OF ADVERSE EVENTS

14.1 Definitions

14.1.1 Adverse events (or adverse experience):

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

An Adverse Event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a pharmaceutical product, whether or not considered related to the pharmaceutical product. Grade 3, 4 or 5 toxicities will be recorded from the time of study enrollment (randomization) until 30 days following the last administration of study drug regardless of causation. Any toxicity which persists beyond 30 days following the final administration of study agent thought by the study team to be possibly, probably, or definitely related to study participation will be followed until the time of resolution or until such time as it has been deemed to be permanent.

14.1.2 Adverse drug reaction (ADR)

In the pre-approval clinical experience with a pharmaceutical product or its new usages, particularly as the therapeutic dose(s) may not be established, all noxious and unintended responses to a pharmaceutical product related to any dose should be considered adverse drug reactions. The phrase "responses to a pharmaceutical product" means that a causal relationship between a pharmaceutical product and an Adverse Event is at least a reasonable possibility (i.e., the relationship cannot be ruled out). "Toxicity" is a common term for ADRs in oncology studies.

Investigators are asked to assess every AE as to the relationship between the investigational product and the AE.

14.1.3 Serious adverse event/reaction/experience (SAE)

A serious Adverse Event (experience) or reaction is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
- requires in-patient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room, or at home for allergic bronchospasm; blood dyscrasias; convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

SAEs are a subset of AEs, which meet the regulatory criteria for events that are considered to be serious. Hospitalization is not an SAE; the event that caused the subject to be hospitalized is the SAE.

Unexpected AE: Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure or updated clinical information included in this protocol.

Associated with the use of the drug: there is a reasonable possibility that the experience may have been caused by the drug (i.e., possibly related to investigational product).

14.2 How to report

Depending on the nature, severity, and attribution of the event, an ADR report will be phoned in, submitted in writing, or both according to the Table below. Expedited Serious Adverse Events must also be reported to the UWCCC Data Safety Monitoring Committee Chair within one business day of the event. All expedited serious adverse events must also be reported to the UW IRB (if applicable) and any sponsor/funding agency not already included in the list.

Initial reports to:

- Project manager of the FDA Oncology Drug Products review division in the Center for Drug Evaluation and Research, responsible for review of the IND. Telephone: 301-796-2320 within 24 hours.

UWCCC Data and Safety Monitoring Committee Coordinator at

saenotify@uwcarbone.wisc.edu within one working day of the event

Written reports, (FDA MedWatch form 3500A) within 15 calendar days to:

◆Project manager of the FDA Oncology Drug Products review division in the Center for Drug Evaluation and Research, responsible for review of the IND.
FAX: 301-594-0498

◆UWCC Data and Safety Monitoring Committee Coordinator **– Email datasafetymonitoring@uwcarbone.wisc.edu,** FAX 608-265-5676 or deliver to CSC K4/6 Mailcode 6160

- ♦ UW Institutional Review Board (IRB).

EMD Pharmaceuticals Medical Product Safety Desk using FAX +49(0) 6151 726 914
and email : GlobalDrugSafety@merckserono.net and regina.schick@merck.de

Expedited Reporting Table

Summary Of Reporting Requirements For Adverse Events On Trials Where The Investigator Holds IND			
EXPEDITED REPORTING FOR PHASE II AND PHASE III STUDIES			
Unexpected Event		**Expected Event**	
GRADES 2 - 3 Attribution of Possible, Probable, or Definite	**GRADES 4 and 5 Regardless of Attribution**	**GRADES 1 - 3**	**GRADES 4 and 5, Regardless of Attribution**
Expedited report within 15 working days to FDA. (Grade 1 - Adverse Event Expedited Reporting NOT required.)	Report by phone to FDA within 24 hrs. Expedited report to follow within 15 working days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributed to the agent (possible, probable, or definite) should be reported within 15 working days.	Adverse Event Expedited Reporting NOT required.	Expedited report, including grade 5 Aplasia in leukemia subjects, within 10 working days. Report by phone to FDA within 24 hrs. Expedited report to follow within 15 working days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributed to the agent (possible, probable, or definite) should be reported within 15 working days. Grade 4 Myelosuppression or other Grade 4 events that do not require expedited reporting will be specified in the protocol.

For Hospitalization Only – Any medical event equivalent to the CTC Grade 3,4,5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of requirements for phase of study, expected or unexpected and attribution.

Expedited reporting may not be appropriate for specific expected adverse events for certain later phase 2 and phase 3 protocols. In those situations the adverse events that will not have expedited reporting must be specified in the text of the approved protocol. An expected Grade 3 event that is using the generic reporting criteria, for instance. In a trial of investigational agents where grade 3 diarrhea requiring hospitalization is expected, only diarrhea requiring ICU care (Grade 4) might be designated for expedited reporting.

14. 3 Exceptions to reporting requirements

The adverse event exceptions listed in this section do NOT require reporting. The exceptions for this protocol are those common or expected toxicities listed herein and clarified in this protocol, including those not being criteria for dose limiting toxicity determinations. These exceptions are listed below.

14.3.1 Grade 3 pain, requiring intravenous narcotics, provided that the narcotics are controlling the

pain, and that IV narcotics for pain are not required > 48 hours after completion of hu14.18-IL2 on Day 3 of any treatment course.

14.3.2 Grade 3 nausea and vomiting that resolves within 48 hours after completion of hu14.18-IL2 on Day 3 of any treatment course.

14.3.3 Grade 3 fever (i.e., T > 40° C) lasting less than 6 hours and controllable with antipyretics.

14.3.4 Grade 3 skin toxicity that improves with treatment (e.g., IV Benadryl) within 24 hours.

14.3.5 Grade 3 metabolic/laboratory (including hyponatremia or hypophosphatemia), in the absence of CNS symptoms and sequelae, that improve with or without treatment within 48 hrs (see Section 10.2.5 for reporting requirements of symptomatic or severe hyponatremia).

14.3.6 Grade 3 hypotension and hypertension that resolves within 48 hours after completion of the hu14.18-IL2 on Day 3 of any treatment course.

14.3.7 Grade 3 hepatic toxicity that resolves in < 4 days following Day 3 of any treatment course.

14.3.8 Grade 3 hematologic toxicity or Grade 4 lymphopenia that improves to at least Grade 2 or pre-therapy baseline values within 1 week of completion of the hu14.18-IL2 of any treatment course.

14.3.9 Infusional reactions readily controlled with supportive anti-allergic (non-steroidal) treatments (i.e., Benadryl, epinephrine).

14.3.10 Grade 3 performance status that resolves in < 1 week.

14.3.11 Grade 3 capillary leak syndrome that resolves in < 1 week.

14.3.12 Grade 3 infection, which resolves in < 1 week.

These toxicities are exempt from reporting requirements as outlined in the above Expedited Reporting table as they represent known, published, transient, reversible, and non-dose limiting toxicities of IL2 and ch14.18. Based on preliminary observations of 33 adults with melanoma and 28 children with neuroblastoma or melanoma given similar doses of hu14.18-IL2 by the same schedule tested in this trial, these toxicities may be expected.

15.0 REFERENCES

1. Mule, J.J., J.C. Yang, R.L. Afreniere, S.Y. Shu, and S.A. Rosenberg. 1987. Identification of cellular mechanisms operational in vivo during the regression of established pulmonary metastases by the systemic administration of high-dose recombinant interleukin 2. *J Immunol* 139:285-294.
2. 1995. Biological Therapy of Cancer. V.T. Devita, S. Hellman, and S.A. Rosenberg, editors. J. B. Lippincott Co., Philadelphia, PA.
3. Mitchell, M.S. 1993. Biological approaches to cancer treatment: biomodulation. McGraw-Hill, Inc.,
4. Sondel, P.M., J.A. Hank, P.C. Kohler, B.P. Chen, D.Z. Minkoff, and J.A. Molenda. 1986. Destruction of autologous human lymphocytes by interleukin 2-activated cytotoxic cells. *J Immunol* 137:502-511.
5. Damle, N.K., L.V. Doyle, J.R. Bender, and E.C. Bradley. 1987. Interleukin 2-activated human lymphocytes exhibit enhanced adhesion to normal vascular endothelial cells and cause their lysis. *J Immunol* 138:1779-1785.
6. Kawakami, Y., S. Eliyahu, C.H. Delgado, P.F. Robbins, L. Rivoltini, S.L. Topalian, T. Miki, and S.A. Rosenberg. 1994. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proc Natl Acad Sci U S A* 91:3515-3519.
7. Hank, J.A., J.A. Sosman, P.C. Kohler, R. Bechhofer, B. Storer, and P.M. Sondel. 1990. Depressed in vitro T cell responses concomitant with augmented interleukin-2 responses by lymphocytes from cancer patients following in vivo treatment with interleukin-2. *J Biol Response Mod* 9:5-14.
8. Harel, W., H. Shau, C.G. Hadley, A.C. Morgan, Jr., R.A. Reisfeld, D.A. Cheresh, and M.S. Mitchell. 1990. Increased lysis of melanoma by in vivo-elicited human lymphokine-activated killer cells after addition of antiganglioside antibodies in vitro. *Cancer Res* 50:6311-6315.
9. Hank, J.A., R.R. Robinson, J. Surfus, B.M. Mueller, R.A. Reisfeld, N.K. Cheung, and P.M. Sondel. 1990. Augmentation of antibody dependent cell mediated cytotoxicity following in vivo therapy with recombinant interleukin 2. *Cancer Res* 50:5234-5239.
10. Yeh, S.D., S.M. Larson, L. Burch, B.H. Kushner, M. Laquaglia, R. Finn, and N.K. Cheung. 1991. Radioimmunodetection of neuroblastoma with iodine-131-3F8: correlation with biopsy, iodine-131-metaiodobenzylguanidine and standard diagnostic modalities. *J Nucl Med* 32:769-776.
11. Schlom, J. 1995. Monoclonal antibody targeted diagnosis and therapy of cancer: defined principles and progress. In Biological Therapy of Cancer. V.T. Devita, S. Hellman, and S.A. Rosenberg, editors. J.B. Lippincott, Co., Philadelphia, PA.
12. Shiloni, E., A. Eisenthal, D. Sachs, and S.A. Rosenberg. 1987. Antibody-dependent cellular cytotoxicity mediated by murine lymphocytes activated in recombinant interleukin 2. *J Immunol* 138:1992-1998.
13. Berinstein, N., C.O. Starnes, and R. Levy. 1988. Specific enhancement of the therapeutic effect of anti-idiotype antibodies on a murine B cell lymphoma by IL-2. *J Immunol* 140:2839-2845.
14. Schultz, K.R., J.P. Klarnet, D.J. Peace, M.A. Cheever, C.C. Badger, I.D. Bernstein, and P.D. Greenberg. 1990. Monoclonal antibody therapy of murine lymphoma: enhanced efficacy by concurrent administration of interleukin 2 or lymphokine-activated killer cells. *Cancer Res* 50:5421-5425.
15. Bajorin, D.F., P.B. Chapman, G. Wong, D.G. Coit, J. Kunicka, J. Dimaggio, C. Cordon-Cardo, C. Urmacher, L. Dantes, M.A. Templeton, and et al. 1990. Phase I evaluation of a combination of monoclonal antibody R24 and interleukin 2 in patients with metastatic melanoma. *Cancer Res* 50:7490-7495.
16. Mujoo, K., D.A. Cheresh, H.M. Yang, and R.A. Reisfeld. 1987. Disialoganglioside GD2 on human neuroblastoma cells: target antigen for monoclonal antibody-mediated cytolysis and suppression of tumor growth. *Cancer Res* 47:1098-1104.
17. Gillies, S.D., K.M. Lo, and J. Wesolowski. 1989. High-level expression of chimeric antibodies using adapted cDNA variable region cassettes. *J Immunol Methods* 125:191-202.

18. Gillies, S.D., E.B. Reilly, K.M. Lo, and R.A. Reisfeld. 1992. Antibody-targeted interleukin 2 stimulates T-cell killing of autologous tumor cells. *Proc Natl Acad Sci U S A* 89:1428-1432.
19. Becker, J.C., J.D. Pancook, S.D. Gillies, K. Furukawa, and R.A. Reisfeld. 1996. T cell-mediated eradication of murine metastatic melanoma induced by targeted interleukin 2 therapy. *J Exp Med* 183:2361-2366.
20. Lode, H.N., R. Xiang, N.M. Varki, C.S. Dolman, S.D. Gillies, and R.A. Reisfeld. 1997. Targeted interleukin-2 therapy for spontaneous neuroblastoma metastases to bone marrow. *J Natl Cancer Inst* 89:1586-1594.
21. Lode, H.N., R. Xiang, T. Dreier, N.M. Varki, S.D. Gillies, and R.A. Reisfeld. 1998. Natural killer cell-mediated eradication of neuroblastoma metastases to bone marrow by targeted interleukin-2 therapy. *Blood* 91:1706-1715.
22. Berke, G. 1993. The functions and mechanisms of action of cytolytic lymphocytes. In Fundamental Immunology. W.E. Paul, editor. Raven Press, New York, NY. 965-1014.
23. Weil-Hillman, G., P. Fisch, A.F. Prieve, J.A. Sosman, J.A. Hank, and P.M. Sondel. 1989. Lymphokine-activated killer activity induced by in vivo interleukin 2 therapy: predominant role for lymphocytes with increased expression of CD2 and leu19 antigens but negative expression of CD16 antigens. *Cancer Res* 49:3680-3688.
24. Voss, S.D., R.J. Robb, G. Weil-Hillman, J.A. Hank, K. Sugamura, M. Tsudo, and P.M. Sondel. 1990. 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* 172:1101-1114.
25. Sondel, P.M., P.C. Kohler, J.A. Hank, K.H. Moore, N.S. Rosenthal, J.A. Sosman, R. Bechhofer, and B. Storer. 1988. Clinical and immunological effects of recombinant interleukin 2 given by repetitive weekly cycles to patients with cancer. *Cancer Res* 48:2561-2567.
26. Hank, J.A., J.E. Surfus, J. Gan, P. Jaeger, S.D. Gillies, R.A. Reisfeld, and P.M. Sondel. 1996. Activation of human effector cells by a tumor reactive recombinant anti-ganglioside GD2 interleukin-2 fusion protein (ch14.18-IL2). *Clin Cancer Res* 2:1951-1959.
27. Sabzevari, H., S.D. Gillies, B.M. Mueller, J.D. Pancook, and R.A. Reisfeld. 1994. A recombinant antibody-interleukin 2 fusion protein suppresses growth of hepatic human neuroblastoma metastases in severe combined immunodeficiency mice. *Proc Natl Acad Sci U S A* 91:9626-9630.
28. Gillies, S.D., D. Young, K.M. Lo, and S. Roberts. 1993. Biological activity and in vivo clearance of antitumor antibody/cytokine fusion proteins. *Bioconjug Chem* 4:230-235.
29. Ozkaynak, M.F., R. Seeger, K. Bauer, P.M. Sondel, B. Blazar, and G. Reaman. Opened 1998. A Phase I study of ch14.18 with GM-CSF in children with neuroblastoma following autologous BMT. In Children's Cancer Group Study CCg-0935.
30. Murray, J.L., J.E. Cunningham, H. Brewer, K. Mujoo, A.A. Zukiwski, D.A. Podoloff, L.P. Kasi, V. Bhadkamkar, H.A. Fritsche, R.S. Benjamin, and et al. 1994. Phase I trial of murine monoclonal antibody 14G2a administered by prolonged intravenous infusion in patients with neuroectodermal tumors. *J Clin Oncol* 12:184-193.
31. Saleh, M.N., M.B. Khazaeli, R.H. Wheeler, E. Dropcho, T. Liu, M. Urist, D.M. Miller, S. Lawson, P. Dixon, C.H. Russell, and et al. 1992. Phase I trial of the murine monoclonal anti-GD2 antibody 14G2a in metastatic melanoma. *Cancer Res* 52:4342-4347.
32. Saleh, M.N., M.B. Khazaeli, R.H. Wheeler, L. Allen, A.B. Tilden, W. Grizzle, R.A. Reisfeld, A.L. Yu, S.D. Gillies, and A.F. LoBuglio. 1992. Phase I trial of the chimeric anti-GD2 monoclonal antibody ch14.18 in patients with malignant melanoma. *Hum Antibodies Hybridomas* 3:19-24.
33. Handgretinger, R., K. Anderson, P. Lang, R. Dopfer, T. Klingebiel, M. Schrappe, P. Reuland, S.D. Gillies, R.A. Reisfeld, and D. Neithammer. 1995. A phase I study of human/mouse chimeric antiganglioside GD2 antibody ch14.18 in patients with neuroblastoma. *Eur J Cancer* 31A:261-267.
34. Frost, J.D., J.A. Hank, G.H. Reaman, S. Frierdich, R.C. Seeger, J. Gan, P.M. Anderson, L.J. Ettinger, M.S. Cairo, B.R. Blazar, M.D. Krailo, K.K. Matthay, R.A. Reisfeld, and P.M. Sondel. 1997. 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* 80:317-333.
35. Hank, J.A., J. Surfus, J. Gan, T.L. Chew, R. Hong, K. Tans, R. Reisfeld, R.C. Seeger, C.P. Reynolds, M. Bauer, and et al. 1994. Treatment of neuroblastoma patients with antiganglioside GD2 antibody plus interleukin-2 induces antibody-dependent cellular cytotoxicity against neuroblastoma detected in vitro. *J Immunother* 15:29-37.
36. Albertini, M.R., J. Gan, P. Jaeger, J.A. Hank, B. Storer, K. Schell, T. Rivest, J. Surfus, R.A. Reisfeld, J.H. Schiller, and P.M. Sondel. 1996. Systemic interleukin-2 modulates the anti-idiotypic response to chimeric anti-GD2 antibody in patients with melanoma. *J Immunother Emphasis Tumor Immunol* 19:278-295.
37. Albertini, M.R., J.A. Hank, J.H. Schiller, M. Khorsand, A.A. Borchert, J. Gan, R. Bechhofer, B. Storer, R.A. Reisfeld, and P.M. Sondel. 1997. Phase IB trial of chimeric antidisialoganglioside antibody plus interleukin 2 for melanoma patients. *Clin Cancer Res* 3:1277-1288.
38. King, D.M., M.R. Albertini, H. Schalch, J.A. Hank, J. Gan, J. Surfus, D. Mahvi, J.H. Schiller, T. Warner, K. Kim, J. Eickhoff, K. Kendra, R. Reisfeld, S.D. Gillies, and P. Sondel. 2004. Phase I clinical trial of the immunocytokine EMD 273063 in melanoma patients. *J Clin Oncol* 22:4463-4473.
39. Neal, Z.C., J.C. Yang, A.L. Rakhmilevich, I.N. Buhtoiarov, H.E. Lum, M. Imboden, J.A. Hank, H.N. Lode, R.A. Reisfeld, S.D. Gillies, and P.M. Sondel. 2004. Enhanced activity of hu14.18-IL2 immunocytokine against murine NXS2 neuroblastoma when combined with interleukin 2 therapy. *Clin Cancer Res* 10:4839-4847.
40. Osenga, K.L., J.A. Hank, M.R. Albertini, J. Gan, A.G. Sternberg, J. Eickhoff, R.C. Seeger, K.K. Matthay, C.P. Reynolds, C. Twist, M. Krailo, P.C. Adamson, R.A. Reisfeld, S.D. Gillies, and P.M. Sondel. 2006. 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* 12:1750-1759.
41. Folkman, J. 1971. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 285:1182-1186.
42. Bergers, G., and L.E. Benjamin. 2003. Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 3:401-410.
43. Hicklin, D.J., and L.M. Ellis. 2005. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol* 23:1011-1027.
44. Powers, C.J., S.W. McLeskey, and A. Wellstein. 2000. Fibroblast growth factors, their receptors and signaling. *Endocr Relat Cancer* 7:165-197.
45. Varner, J.A., and D.A. Cheresh. 1996. Integrins and cancer. *Curr Opin Cell Biol* 8:724-730.
46. Taga, T., A. Suzuki, I. Gonzalez-Gomez, F.H. Gilles, M. Stins, H. Shimada, L. Barsky, K.I. Weinberg, and W.E. Laug. 2002. alpha v-Integrin antagonist EMD 121974 induces apoptosis in brain tumor cells growing on vitronectin and tenascin. *Int J Cancer* 98:690-697.
47. Brooks, P.C., R.L. Klemke, S. Schon, J.M. Lewis, M.A. Schwartz, and D.A. Cheresh. 1997. Insulin-like growth factor receptor cooperates with integrin alpha v beta 5 to promote tumor cell dissemination in vivo. *J Clin Invest* 99:1390-1398.
48. Brooks, P.C., R.A. Clark, and D.A. Cheresh. 1994. Requirement of vascular integrin alpha v beta 3 for angiogenesis. *Science* 264:569-571.
49. Pfaff, M., K. Tangemann, B. Muller, M. Gurrath, G. Muller, H. Kessler, R. Timpl, and J. Engel. 1994. Selective recognition of cyclic RGD peptides of NMR defined conformation by alpha IIb beta 3, alpha V beta 3, and alpha 5 beta 1 integrins. *J Biol Chem* 269:20233-20238.
50. Brooks, P.C., A.M. Montgomery, M. Rosenfeld, R.A. Reisfeld, T. Hu, G. Klier, and D.A. Cheresh. 1994. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 79:1157-1164.
51. Friedlander, M., P.C. Brooks, R.W. Shaffer, C.M. Kincaid, J.A. Varner, and D.A. Cheresh. 1995. Definition of two angiogenic pathways by distinct alpha v integrins. *Science* 270:1500-1502.

52. Mitjans, F., T. Meyer, C. Fittschen, S. Goodman, A. Jonczyk, J.F. Marshall, G. Reyes, and J. Piulats. 2000. In vivo therapy of malignant melanoma by means of antagonists of alphav integrins. *Int J Cancer* 87:716-723.
53. MacDonald, T.J., T. Taga, H. Shimada, P. Tabrizi, B.V. Zlokovic, D.A. Cheresh, and W.E. Laug. 2001. Preferential susceptibility of brain tumors to the antiangiogenic effects of an alpha(v) integrin antagonist. *Neurosurgery* 48:151-157.
54. Burke, P.A., S.J. DeNardo, L.A. Miers, K.R. Lamborn, S. Matzku, and G.L. DeNardo. 2002. Cilengitide targeting of alpha(v)beta(3) integrin receptor synergizes with radioimmunotherapy to increase efficacy and apoptosis in breast cancer xenografts. *Cancer Res* 62:4263-4272.
55. Lode, H.N., T. Moehler, R. Xiang, A. Jonczyk, S.D. Gillies, D.A. Cheresh, and R.A. Reisfeld. 1999. Synergy between an antiangiogenic integrin alphav antagonist and an antibody-cytokine fusion protein eradicates spontaneous tumor metastases. *Proc Natl Acad Sci U S A* 96:1591-1596.
56. Eskens, F.A., H. Dumez, R. Hoekstra, A. Perschl, C. Brindley, S. Bottcher, W. Wynendaele, J. Drevs, J. Verweij, and A.T. van Oosterom. 2003. Phase I and pharmacokinetic study of continuous twice weekly intravenous administration of Cilengitide (EMD 121974), a novel inhibitor of the integrins alphavbeta3 and alphavbeta5 in patients with advanced solid tumours. *Eur J Cancer* 39:917-926.
57. Raguse, J.D., H.J. Gath, J. Bier, H. Riess, and H. Oettle. 2004. Cilengitide (EMD 121974) arrests the growth of a heavily pretreated highly vascularised head and neck tumour. *Oral Oncol* 40:228-230.
58. Albertini, M.R., J.A. Hank, K.M. Kim, H. Schalch, J.H. Schiller, D. Mahvi, J. Gan, R. Reisfeld, S.D. Gillies, D. Wayne, and P.M. Sondel. Opened 2005. Phase II trial of hu14.18-IL2 (EMD 273063) in subjects with advanced melanoma. In UWCCC protocol CO-04601, NCI protocol 6304.
59. Frisch, S.M., K. Vuori, E. Ruoslahti, and P.Y. Chan-Hui. 1996. Control of adhesion-dependent cell survival by focal adhesion kinase. *J Cell Biol* 134:793-799.
60. Kurenova, E., L.H. Xu, X. Yang, A.S. Baldwin, Jr., R.J. Craven, S.K. Hanks, Z.G. Liu, and W.G. Cance. 2004. Focal adhesion kinase suppresses apoptosis by binding to the death domain of receptor-interacting protein. *Mol Cell Biol* 24:4361-4371.
61. Aixinjueluo, W., K. Furukawa, Q. Zhang, K. Hamamura, N. Tokuda, S. Yoshida, R. Ueda, and K. Furukawa. 2005. Mechanisms for the apoptosis of small cell lung cancer cells induced by anti-GD2 monoclonal antibodies: roles of anoikis. *J Biol Chem* 280:29828-29836.
62. Eliceiri, B.P., X.S. Puente, J.D. Hood, D.G. Stupack, D.D. Schlaepfer, X.Z. Huang, D. Sheppard, and D.A. Cheresh. 2002. Src-mediated coupling of focal adhesion kinase to integrin alpha(v)beta5 in vascular endothelial growth factor signaling. *J Cell Biol* 157:149-160.
63. Castells, M.C., L.B. Klickstein, K. Hassani, J.A. Cumplido, M.E. Lacouture, K.F. Austen, and H.R. Katz. 2001. gp49B1-alpha(v)beta3 interaction inhibits antigen-induced mast cell activation. *Nat Immunol* 2:436-442.
64. Wang, L.L., I.K. Mehta, P.A. LeBlanc, and W.M. Yokoyama. 1997. Mouse natural killer cells express gp49B1, a structural homologue of human killer inhibitory receptors. *J Immunol* 158:13-17.
65. Gu, X., A. Laouar, J. Wan, M. Daheshia, J. Lieberman, W.M. Yokoyama, H.R. Katz, and N. Manjunath. 2003. The gp49B1 inhibitory receptor regulates the IFN-gamma responses of T cells and NK cells. *J Immunol* 170:4095-4101.
66. Zhou, J.S., D.S. Friend, A.M. Feldweg, M. Daheshia, L. Li, K.F. Austen, and H.R. Katz. 2003. Prevention of lipopolysaccharide-induced microangiopathy by gp49B1: evidence for an important role for gp49B1 expression on neutrophils. *J Exp Med* 198:1243-1251.
67. Zhou, J.S., D.S. Friend, D.M. Lee, L. Li, K.F. Austen, and H.R. Katz. 2005. gp49B1 deficiency is associated with increases in cytokine and chemokine production and severity of proliferative synovitis induced by anti-type II collagen mAb. *Eur J Immunol* 35:1530-1538.
68. Kagari, T., H. Doi, and T. Shimozato. 2002. The importance of IL-1 beta and TNF-alpha, and the noninvolvement of IL-6, in the development of monoclonal antibody-induced arthritis. *J Immunol* 169:1459-1466.

69. Arm, J.P., C. Nwankwo, and K.F. Austen. 1997. Molecular identification of a novel family of human Ig superfamily members that possess immunoreceptor tyrosine-based inhibition motifs and homology to the mouse gp49B1 inhibitory receptor. *J Immunol* 159:2342-2349.
70. Borges, L., M.L. Hsu, N. Fanger, M. Kubin, and D. Cosman. 1997. A family of human lymphoid and myeloid Ig-like receptors, some of which bind to MHC class I molecules. *J Immunol* 159:5192-5196.
71. Chang, C.C., R. Ciubotariu, J.S. Manavalan, J. Yuan, A.I. Colovai, F. Piazza, S. Lederman, M. Colonna, R. Cortesini, R. Dalla-Favera, and N. Suciu-Foca. 2002. Tolerization of dendritic cells by T(S) cells: the crucial role of inhibitory receptors ILT3 and ILT4. *Nat Immunol* 3:237-243.
72. Cella, M., C. Dohring, J. Samaridis, M. Dessing, M. Brockhaus, A. Lanzavecchia, and M. Colonna. 1997. A novel inhibitory receptor (ILT3) expressed on monocytes, macrophages, and dendritic cells involved in antigen processing. *J Exp Med* 185:1743-1751.
73. Zou, W. 2005. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer* 5:263-274.
74. Gillies, S.D., Y. Lan, K.M. Lo, M. Super, and J. Wesolowski. 1999. Improving the efficacy of antibody-interleukin 2 fusion proteins by reducing their interaction with Fc receptors. *Cancer Res* 59:2159-2166.
75. Chen, X., R. Park, A.H. Shahinian, J.R. Bading, and P.S. Conti. 2004. Pharmacokinetics and tumor retention of 125I-labeled RGD peptide are improved by PEGylation. *Nucl Med Biol* 31:11-19.
76. Morton, D.L., R. Essner, and C.M. Balch. 2003. Surgical excision of distant metastases. In Cutaneous Melanoma. D.L. Balch, A.N. Houghton, A.J. Sober, and S. Soong, editors. Quality Medical Publishing, St. Louis, MO. 547-572.
77. Ollila, D.W., E.C. Hsueh, S.L. Stern, and D.L. Morton. 1999. Metastasectomy for recurrent stage IV melanoma. *J Surg Oncol* 71:209-213.
78. Hsueh, E.C., R. Essner, L.J. Foshag, D.W. Ollila, G. Gammon, S.J. O'Day, P.D. Boasberg, S.L. Stern, X. Ye, and D.L. Morton. 2002. Prolonged survival after complete resection of disseminated melanoma and active immunotherapy with a therapeutic cancer vaccine. *J Clin Oncol* 20:4549-4554.
79. Balch, C.M., A.C. Buzaid, S.J. Soong, M.B. Atkins, N. Cascinelli, D.G. Coit, I.D. Fleming, J.E. Gershenwald, A. Houghton, Jr., J.M. Kirkwood, K.M. McMasters, M.F. Mihm, D.L. Morton, D.S. Reintgen, M.I. Ross, A. Sober, J.A. Thompson, and J.F. Thompson. 2001. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J Clin Oncol* 19:3635-3648.
80. Balch, C.M., S.J. Soong, J.E. Gershenwald, J.F. Thompson, D.S. Reintgen, N. Cascinelli, M. Urist, K.M. McMasters, M.I. Ross, J.M. Kirkwood, M.B. Atkins, J.A. Thompson, D.G. Coit, D. Byrd, R. Desmond, Y. Zhang, P.Y. Liu, G.H. Lyman, and A. Morabito. 2001. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol* 19:3622-3634.
81. Pidhorecky, I., R.J. Lee, G. Proulx, D.R. Kollmorgen, C. Jia, D.L. Driscoll, W.G. Kraybill, and J.F. Gibbs. 2001. Risk factors for nodal recurrence after lymphadenectomy for melanoma. *Ann Surg Oncol* 8:109-115.
82. Baum, C.L., and C.J. Arpey. 2005. Normal cutaneous wound healing: clinical correlation with cellular and molecular events. *Dermatol Surg* 31:674-686; discussion 686.
83. van der Bilt, J.D., and I.H. Borel Rinkes. 2004. Surgery and angiogenesis. *Biochim Biophys Acta* 1654:95-104.
84. Gilbert, M. Opened 2005. Phase II study of Cilengitide in operative patients with recurrent or progressive glioblastoma multiforme. In North American Brain Tumor Consortium Study NABTC-0302.
85. Presta, L.G., H. Chen, S.J. O'Connor, V. Chisholm, Y.G. Meng, L. Krummen, M. Winkler, and N. Ferrara. 1997. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res* 57:4593-4599.

86. Yang, J.C., L. Haworth, R.M. Sherry, P. Hwu, D.J. Schwartzentruber, S.L. Topalian, S.M. Steinberg, H.X. Chen, and S.A. Rosenberg. 2003. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 349:427-434.
87. Miller, K.D., L.I. Chap, F.A. Holmes, M.A. Cobleigh, P.K. Marcom, L. Fehrenbacher, M. Dickler, B.A. Overmoyer, J.D. Reimann, A.P. Sing, V. Langmuir, and H.S. Rugo. 2005. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. *J Clin Oncol* 23:792-799.
88. Hurwitz, H., L. Fehrenbacher, W. Novotny, T. Cartwright, J. Hainsworth, W. Heim, J. Berlin, A. Baron, S. Griffing, E. Holmgren, N. Ferrara, G. Fyfe, B. Rogers, R. Ross, and F. Kabbinavar. 2004. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 350:2335-2342.
89. Scappaticci, F.A., L. Fehrenbacher, T. Cartwright, J.D. Hainsworth, W. Heim, J. Berlin, F. Kabbinavar, W. Novotny, S. Sarkar, and H. Hurwitz. 2005. Surgical wound healing complications in metastatic colorectal cancer patients treated with bevacizumab. *J Surg Oncol* 91:173-180.
90. Tanaka, H., H. Taniguchi, T. Mugitani, Y. Koishi, M. Masuyama, H. Koyama, M. Hoshima, and T. Takahashi. 1996. Angiogenesis inhibitor TNP-470 prevents implanted liver metastases after partial hepatectomy in an experimental model without impairing wound healing. *Br J Surg* 83:1444-1447.
91. te Velde, E.A., E.E. Voest, J.M. van Gorp, A. Verheem, J. Hagendoorn, M.F. Gebbink, and I.H. Borel Rinkes. 2002. Adverse effects of the antiangiogenic agent angiostatin on the healing of experimental colonic anastomoses. *Ann Surg Oncol* 9:303-309.
92. Mall, J.W., W. Schwenk, A.W. Philipp, R. Buttemeyer, and C. Pollmann. 2003. Intraperitoneal administration of the angiogenesis inhibitor thalidomide does not impair anastomotic healing following large bowel resection in a rabbit model. *World J Surg* 27:1119-1123.
93. Reitan, S.K., and K. Hannestad. 2002. Immunoglobulin heavy chain constant regions regulate immunity and tolerance to idiotypes of antibody variable regions. *Proc Natl Acad Sci U S A* 99:7588-7593.
94. Weng, W.K., D. Czerwinski, J. Timmerman, F.J. Hsu, and R. Levy. 2004. Clinical outcome of lymphoma patients after idiotype vaccination is correlated with humoral immune response and immunoglobulin G Fc receptor genotype. *J Clin Oncol* 22:4717-4724.
95. Wong, J.H., K.A. Skinner, K.A. Kim, L.J. Foshag, and D.L. Morton. 1993. The role of surgery in the treatment of nonregionally recurrent melanoma. *Surgery* 113:389-394.
96. Wornom, I.L., 3rd, J.W. Smith, S.J. Soong, R. McElvein, M.M. Urist, and C.M. Balch. 1986. Surgery as palliative treatment for distant metastases of melanoma. *Ann Surg* 204:181-185.

APPENDIX 1: Eastern Cooperative Oncology Group (ECOG) Performance Status

Eastern Cooperative Oncology Group (ECOG) Performance Status[1]

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (e.g., light house work, office work)
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

[1] Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-55.