CHILDREN’S ONCOLOGY GROUP

ANBL0032

PHASE III RANDOMIZED STUDY OF CHIMERIC ANTIBODY 14.18 (Ch14.18) IN HIGH RISK NEUROBLASTOMA FOLLOWING MYELOABLATIVE THERAPY AND AUTOLOGOUS STEM CELL RESCUE

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SECTION

STUDY COMMITTEE

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AGENT NSC# AND IND#'s
Isotretinoin  NSC # 329481
ch14.18  NSC #623408
GM-CSF  NSC #613795
Aldesleukin (IL-2)  NSC #373364
1.0 OBJECTIVES

1.1 The primary objective is to:
Determine if monoclonal antibody Chl4.18 + cytokines + isotretinoin (13-cis-retinoic acid, or RA) improves event free survival after myeloablative therapy and stem cell rescue as compared to RA alone, in high risk neuroblastoma patients who have achieved a pre-ASCT response of CR, VGPR, or PR.

1.2 The secondary objectives are to:

1.2.1 Determine if monoclonal antibody Chl4.18 + cytokines + isotretinoin (13-cis-retinoic acid, or RA) improves overall survival after myeloablative therapy and stem cell rescue as compared to RA alone, in high risk neuroblastoma patients who have achieved a pre-ASCT response of CR, VGPR, or PR.

1.2.2 Determine if immunotherapy + RA improves event free survival and overall survival as compared to RA alone, in the subgroup of high risk INSS Stage 4 neuroblastoma patients who have achieved a pre-ASCT response of CR, VGPR, or PR.

1.2.3 In the subgroup of neuroblastoma patients who have achieved a pre-ASCT response of CR, VGPR, or PR, determine if there is a difference between the two randomized regimens in reducing the minimal residual disease (MRD) burden as detected by the following parameters: meta-iodobenylguanidine (MIBG) scan, immunocytology (IC) of blood and bone marrow samples, RT-PCR for tyrosine hydroxylase, PGP 9.5, and MAGE-1 in blood and bone marrow.

1.2.4 Determine if change from baseline of MRD as measured by above parameters is associated with event free and overall survival.

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1.2.8 To determine a descriptive profile of human anti-chimeric antibody (HACA) during immunotherapy.

1.2.9 To compare the outcome data of the patients with persistent disease documented by biopsy (Stratum 07) to the historical data for the analogous patients from CCG-3981.

1.2.10 To determine the variability of 13-cis-retinoic-acid pharmacokinetics and relationship to pharmacogenomic parameters and determine if these levels and/or genetic variations correlate with EFS or systemic toxicity.
1.2.11 To further describe and refine the EFS and OS estimates and baseline characteristics for subjects receiving Chl4.18 + cytokines + RA, following cessation of the randomized portion of the study.

1.2.12 To further describe the safety and toxicity of Chl4.18 + cytokines + RA under the new administration guidelines implemented following cessation of the randomized portion of the study with focus on: a) number of courses delivered per subject; b) number of dose reductions or stoppage (ch14.18 and/or IL-2); and c) number of toxic deaths.

1.2.13 To determine the potential effect of ch14.18 on cardiac repolarization and to evaluate ch14.18 plasma levels.

1.2.14 To determine if the presence of naturally occurring anti-glycan antibodies correlates with allergic reactions and blood levels of ch14.18.

1.2.15 To determine if the genotype of FcR and Kir/Kir-Ligand correlate with EFS.

2.0 BACKGROUND AND RATIONALE
Neuroblastoma is the third most common malignancy of childhood. More than one half of patients have high-risk tumors and have dismal outcome despite aggressive therapy (3 year event-free survival less than 15%). A somewhat improved outcome has been obtained with autologous bone marrow transplantation after intensive chemotherapy. However, more than one half of these patients will still relapse and succumb to the tumor. This is especially true for those patients who were diagnosed to have Stage IV neuroblastoma after 1 year of age. In LCME1 study which prescribed autologous marrow transplantation, the progression free survival for 62 Stage IV patients was 40%, 20% and 13% at 2, 4 and 7 years, respectively. The Japanese Study Group reported overall survival rates of 58.8%, 34.4% and 28.9% at 2, 5, and 10 years respectively. In a POG study of 81 Stage IV patients, the EFS at 2 years was 32%. In CCG-321, the 4 year event-free survival for 67 Stage IV patients was 40% for those non-randomly assigned to autologous bone marrow transplantation, as compared to 19% for those treated with chemotherapy. In CCG3891 study of 379 patients, the 5-year EFS for patients randomly assigned to autologous stem cell transplantation was significantly higher (30% +/- 4%) than those randomly assigned to chemotherapy (19% +/- 3%) (P = .04).

2.1 Passive immunotherapy with anti-GD2 monoclonal antibodies
The major obstacle to cure these patients derives from refractory microscopic disease. A promising approach is immunotherapy targeting a tumor associated antigen, GD2, which has been shown to be uniformly expressed by neuroblastoma, some melanomas, brain tumors, small cell carcinomas of the lung, and some sarcomas. In normal human tissues, GD2 expression is restricted to neurons, skin melanocytes and peripheral pain fibers. The high expression of GD2 in neuroblastoma and restricted distribution in normal tissues makes anti-GD2 monoclonal antibodies (mabs) suitable for tumor targeted immunotherapy.

2.1.1 Immunotherapy with anti-GD2 mabs
Clinical trials with anti-tumor mabs thus far suggest that mabs specific for GD2 may be useful in passive immunotherapy of cancer. Therapeutic responses have been obtained in phase I and phase II studies of a murine IgG1 mab, 3F8, murine IgG2 mab, 14G2a, and human-mouse chimeric mab, ch14.18.
Ch14.18 was constructed by combining the variable regions of murine IgG3 b 14.18, and the constant regions of human IgG1 -κ. In a phase I clinical trial reported by Yu et al., 10 neuroblastoma patients received a total of 19 courses of ch14.18 at doses of 10 to 200 mg/m²/course. One PR and 4 mixed responses were observed in 9 evaluable patients. Another phase I trial of ch14.18 by Handgretinger et al employed 30-50 mg/m²/d x 5d/course, and 2 CR, 2 PR in 9 patients were reported.

The biological activities of the infused ch14.18 in vivo have been demonstrated by the capacity of post-infusion sera to mediate complement dependent cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC). Pharmacokinetic and immunological studies showed that ch14.18 has longer plasma half-life and less immunogenicity when compared to the murine mab 14G2a. Toxicities of ch14.18 mabs were similar in all studies. Most prevalent toxicities were pain, tachycardia, hypertension, hypotension, fever and urticaria. Most of these toxicities were dose-dependent and rarely noted at dosages of 10 mg/m² and below in children. Other toxicities include hyponatremia, hypokalemia, nausea, vomiting, diarrhea, serum sickness, and occasional changes in pupil reaction to light and accommodation. Reversible motor weakness was observed in one patient.

2.1.2 Immunotherapy with anti-GD2 mabs and cytokines
It is believed that antibody-dependent cellular cytotoxicity (ADCC) plays an important role in immunotherapy. Unfortunately, ADCC is often depressed in cancer patients. Since GM-CSF not only raises the number of leukocytes but also enhances their ch14.18 mediated ADCC, it is likely that GM-CSF may increase the therapeutic efficacy of ch14.18. Thus, Yu et al conducted a pilot trial of ch14.18 + GM-CSF in patients with recurrent/refractory neuroblastoma. Altogether, 61 courses of ch14.18 + GM-CSF were administered to 17 patients with refractory or recurrent neuroblastoma. There were 5 CR and 3 stable disease. To date, 4 patients are alive with a median follow up of 96 months (84-113 months). This study led to a phase II study, POG #9347 which accrued 32 patients with recurrent/refractory neuroblastoma. A total of 73 courses of ch14.18 + GM-CSF were administered. Therapeutic responses were demonstrated in 5 of 28 evaluable patients, with 2CR, 2 PR, 1 mixed response and 2 stable disease. The 2 PR consisted of 63% and >70% reduction, respectively, in bulky retroperitoneal tumors. The median survival for these 7 patients was 27+M, in contrast to 3M only for the 21 patients with PD. Considering that these patients had failed multiagent, multimodality therapy, including BMT in 20 patients, these findings suggest that immunotherapy with ch14.18 + GM-CSF warrants further pursuit.

Aldesleukin (IL-2) has been shown to augment lymphocyte-mediated ADCC in vitro and in vivo. In a phase I trial, CCG-0901, 33 pediatric patients received escalating doses of the murine mab 14G2a combined with fixed dose of IL-2. The MTD was determined to be 15 mg/m²/d x 5d when combined with 3 MIU/m²/d of Aldesleukin (IL-2) for 4 days each week for 3 weeks. Aldesleukin (IL-2) toxicities were similar to those toxicities seen with Aldesleukin (IL-2) alone. Two patients had a measurable clinical response (1 CR and 1 PR). Three patients had a transient decrease in neuroblastoma involvement of the marrow. A separate study has been completed in adults, combining ch14.18 (2-10 mg/m²/d) x 5 days with Aldesleukin (IL-2) (1.5MIU/m²/d for 4 days each week, for 3 weeks). Twenty-four patients were treated. Allergic reactions and pain were considered dose limiting toxicities. One patient showed a PR and one a CR.

In the above-mentioned POG 9347 trial of ch14.18 and GM-CSF, serial monitoring of ADCC showed that all responding patients had a significant increase of ch14.18 mediated neutrophil ADCC activity. Enhancement of natural killer cell activity by IL-2 has also been observed in some patients treated with the combination of ch14.18 and Aldesleukin (IL-2). However, there is a paucity of information regarding the ADCC activities of neutrophils and lymphocytes in the period immediately following autologous stem cell transplant, although NK activity was said to recover rapidly within one month after transplant. Another intriguing issue is the potential contribution of neutrophils to tumor control during Aldesleukin (IL-2) therapy. There are conflicting results showing granulocyte phenotypic and functional
activation but impaired chemotaxis. Furthermore, little is known as to the effect of Aldesleukin (IL-2) on neutrophil mediated ADCC.

2.1.3 Feasibility of giving ch14.18 and cytokines in the early post-transplant period.
To explore the feasibility of giving ch14.18 in the early post-transplant period, a phase I study of ch14.18 + GM-CSF was conducted (CCG-0935). The MTD of ch14.18 was determined to be 40 mg/m²/day x 4 days. This study was amended (CCG-0935A) to substitute Aldesleukin (IL-2) for GM-GSF in alternate cycles. After engraftment, patients were given ch14.18 at 40 mg/m² with GM-CSF (Courses 1 and 3) or 20 mg/m² with Aldesleukin (IL-2) (Course 2). However, more toxicities were observed with both GM-CSF and Aldesleukin (IL-2) in combination with ch14.18. These include pain, fever, capillary leak, O₂ requirement due to capillary leak, hypotension, mild reversible increased hepatic transaminases, and infection. There was also a transient thrombocytopenia with GM-CSF and Aldesleukin (IL-2) cycles. Subsequently, the antibody dose was changed to 25 mg/m² for both GM-CSF and Aldesleukin (IL-2) cycles and the Aldesleukin (IL-2) dose was reduced in an attempt to decrease capillary leak. Patients who had undergone high-dose chemotherapy with autologous stem cell rescue for neuroblastoma received ch14.18 with GM-CSF (Courses 1, 3 and 5) or Aldesleukin (IL-2) (Courses 2 and 4) after engraftment. Twenty-five patients were enrolled. The MTD of ch14.18 was determined to be 25 mg/m²/d for 4 days given concurrently with 4.5 x 10⁶ U/m²/d of IL-2 for 4 days. IL-2 was also given at a dose of 3 x 10⁶ U/m²/d for 4 days starting 1 week before ch14.18. Two patients experienced dose-limiting toxicity due to ch14.18 and IL-2. Common toxicities included pain, fever, nausea, emesis, diarrhea, urticaria, mild elevation of hepatic transaminases, capillary leak syndrome, and hypotension. No death attributable to toxicity of therapy occurred. No additional toxicity was seen when cis-retinoic acid (cis-RA) was given between courses of ch14.18. No patient treated at the MTD developed HACA. These findings indicated that ch14.18 in combination with IL-2 was tolerable in the early post-HDC/SCR period. cis-RA can be administered safely between courses of ch14.18 and cytokines. This study served as a pilot for regimen B of the current study.

2.1.4 Antitumor Effects of GM-CSF and Aldesleukin (IL-2) Alone
GM-CSF has been shown both in vitro and in vivo to enhance antitumor immunity through direct activation of monocytes, macrophages, dendritic cells, and antibody-dependent cellular cytotoxicity (ADCC), and indirect T cell activation via TNF, interferon and IL-1. GM-CSF might enhance functions of cells critical for immune activation against tumor cells, alone or with other cytokines or monoclonal antibodies, making it an important agent in cancer biotherapy. GM-CSF has been administered in a phase II open-label trial as a surgical adjuvant therapy in patients with advanced melanoma at very high risk of recurrence. An interim analysis of 25 patients has demonstrated a significant prolongation of disease-free survival compared to historical controls. A follow-up report showed that overall survival and disease-free survival at 2 years was significantly prolonged in 48 patients who received GM-CSF compared with matched historical controls. GM-CSF demonstrated minor activity in two phase II trials in patients with metastatic renal cell carcinoma. GM-CSF resulted in transient decrease in PSA levels in metastatic hormone refractory prostate cancer. The amplification of cell-mediated effector mechanisms against residual tumor/leukemic cells is one potential use of GM-CSF to reduce relapse rates in minimal residual disease setting. GM-CSF has been administered to 20 patients with AML undergoing autologous BMT. After a median follow-up of 24 months, the actuarial risk of relapse was 37.4% in GM-CSF-treated patients compared with 49.5% in historical controls. GM-CSF dependent ADCC was studied in 34 patients with a variety of tumors post-ABMT including three with neuroblastoma. A significant increase in monocyte-mediated ADCC following GM-CSF therapy has been documented in comparison to pretreatment values and controls. A higher rate of CR, and a trend towards better disease-free survival has been reported by others in patients with acute leukemia or Hodgkin’s/non-Hodgkin’s lymphoma post-transplant. However, no difference in overall survival has been seen after 1 year in any of these studies. To date, there is no information in the literature, regarding the antitumor efficacy of GM-CSF alone in patients with neuroblastoma post-ABMT.
Aldesleukin (IL-2) causes activation of natural killer (NK) cells, generation of lymphokine-activated killer (LAK) cells, and augments ADCC.\textsuperscript{38,60-62} Aldesleukin (IL-2) has been effective at inducing measurable antitumor responses in patients with renal cell carcinoma and melanoma.\textsuperscript{63-65} In a CCG phase II trial, Aldesleukin (IL-2) was administered to children with refractory solid tumors.\textsuperscript{66} No antitumor effects were observed in children with sarcomas or neuroblastomas, whereas one of five children with renal cell carcinoma had a complete response. Two recent prospective randomized trials in adult patients with metastatic melanoma failed to show any benefit of Aldesleukin (IL-2) administered along with interferon following standard chemotherapy.\textsuperscript{67,68} Aldesleukin (IL-2) has been administered post-autologous BMT in a variety of diseases such as AML, lymphoma, breast cancer and neuroblastoma to eradicate the residual malignant cells via immune activation.\textsuperscript{69-74} All the reports in patients with neuroblastoma were feasibility and toxicity studies in the post-ABMT setting and were not designed to answer antitumor efficacy question.\textsuperscript{72-74} Therefore, it is not possible to know the exact antitumor efficacy of Aldesleukin(IL-2) alone in neuroblastoma patients with minimal residual disease following ABMT.

\section*{2.2 Improved survival with the use of isotretinoin (13-cis-retinoic acid, or RA)}
In a recently completed randomized trial, CCG-3891, patients with high risk neuroblastoma were treated with initial chemotherapy and then randomized to receive either myeloablative therapy and purged autologous bone marrow transplantation or further intensive chemotherapy. All patients who completed cytotoxic therapy without disease progression were then randomly assigned to receive a 6 month course of isotretinoin versus observation. Isotretinoin was given as 160mg/m\textsuperscript{2}/day x 2 weeks/month x 6 months, and began approximately 3 months following ABMT. The 3 year event free survival, from the time of the second randomization was \(46\pm6\%\) for the 130 patients who receive isotretinoin, as compared to \(29\pm5\%\) for the 128 patients randomized to no further therapy (p=0.027).\textsuperscript{75} For the subgroup of Stage IV patients, the 3-year event free survival for those assigned to isotretinoin was \(40\pm6\%\), as compared to \(25\pm5\%\) percent for those assigned to no further therapy. Unfortunately the difference was not statistically significant (P=0.09). Thus, in this study, cycles of isotretinoin will be interdigitated between antibody courses 1-5. This will allow patients to receive extended antibody therapy while continuing to benefit from the proven efficacy of isotretinoin.

In CCG-3891, 37 (11.6\%) patients with biopsy proven documented active disease post-ABMT were randomly assigned to receive RA. The outcomes of these patients were uniformly poor with disease progression in all patients within 3.5 years after 2\textsuperscript{nd} randomization. The event free survival was 35\%, 20\% and 12\% at 1, 2 and 3 year, respectively.\textsuperscript{75} Thus, in this protocol, all patients with biopsy proven active disease will be non-randomly assigned to immunotherapy regimen, or other COG/NANT Phase II studies when available in the future.

\section*{2.3 Monitoring minimal residual disease}
In a recent analysis of 549 neuroblastoma patients from the European Bone Marrow Transplantation Registry, persistent bone or bone marrow disease as detected by MIBG scan has been shown to be an independent adverse risk factor that influenced EFS.\textsuperscript{76}

Preliminary results from Seeger et al showed lower event free survival for patients who had marrow that was positive by immunocytology at harvest or after 12 weeks of induction therapy. In addition, patients whose marrow remains positive for tyrosine hydroxylase (TH)\textsuperscript{77} by RT-PCR after purging, also had a significantly poorer event free survival post transplant than those that were RT-PCR negative. These results were also obtained using RT-PCR for PGP 9.5 and MAGE. A comparison of specificity and sensitivity of 3 different PCR probes: TH, PGP 9.5, and MAGE showed TH and PGP 9.5 to be the most sensitive, though all were specific.\textsuperscript{77} In this protocol, the prognostic values of detecting minimal residual disease by MIBG scan, immunocytology and RT-PCR will be investigated.
2.4 13-cis-retinoic acid Pharmacokinetics and Pharmacogenomics Study Background and Rationale
In order to better understand the pharmacokinetics and pharmacogenomics of 13-cis-retinoic acid use in children, a study will be undertaken that will require a single 5 mL blood sample to be obtained from patients on Day 14 of the first course of oral 13-cis-retinoic acid. The objectives of this study are to determine the inter-individual variability of 13-cis-retinoic acid continuous steady state (CSS) plasma levels in high-risk neuroblastoma patients and determine if these levels and/or genetic variations in retinoic acid metabolic enzymes correlates with event-free survival or systemic toxicity (CTC Grade 3/4 skin and hepatic toxicity).

Pharmacokinetics studies performed on patients in the Phase I trial of 13-cis-retinoic acid showed significant interpatient and intrapatient variability in peak serum levels (mean peak serum 13-cis-RA concentration: 7.2 ± 5.3µM; trough concentration: 4.1 ± 2.7µM). It was noted that peak serum concentrations above 10 µM were associated with a higher incidence of Grade 3 and 4 toxicities in patients. 13-cis-RA may be subject to first-pass metabolism and subsequent plasma (and tumour) concentrations will depend on the rate of metabolism to its inactive 4-oxo metabolite. Preliminary data from a pilot study in the UK (UKCCSG Study PK 2000 08) has shown that plasma concentrations of 4-oxo-13-cis-RA can accumulate to exceed those of the parent compound. A ~10-fold variation in 4-oxo-13-cis-RA peak plasma concentrations was found. It is proposed that increased levels of this metabolite in vivo may lead to a diminished efficacy of 13-cis-RA.80

A number of cytochrome P450 (CYP) enzymes have been identified as playing a role in the metabolism of 13-cis-RA.81,82 CYP2C8 is most important in terms of activity and level of expression in the liver. Genetic polymorphisms in the CYP2C8 gene have been described82 which result in a lower rate of paclitaxel metabolism and are commonly seen in a Caucasian population.83,84 Thus, genetic variation in CYP2C8 activity could underlie individual differences in 13-cis-RA metabolism and bioavailability. A further aspect of 13-cis-RA metabolism is glucuronidation, both of the parent drug and of 4-hydroxy-metabolites. This conjugation may be mediated by UGT1A1 or UGT2B7,85 both enzymes that are subject to genetic polymorphisms.86

Results from this study will provide an insight into whether modulation of 13-cis-RA dosing according to blood levels achieved and/or genotype, could be used in future studies to optimize the treatment of high-risk neuroblastoma. This study will be conducted in collaboration with the United Kingdom Children’s Cancer Study Group (UKCCSG).

2.5 Discontinuing randomization due to early efficacy results
As of January 12th 2009, 226 eligible patients were enrolled: 113 randomized to RA-only, 113 randomized to immunotherapy + RA. With median follow-up of 2.1 yrs after randomization, event free survival was significantly higher (p=0.0115) for patients randomized to immunotherapy, with 2-yr estimates of 66%±5% vs 46%±5% (83 events observed; 61% of expected information). Preliminary overall survival was also significantly higher (p=0.0223) for immunotherapy (86%±4% vs 75%±5% at 2 yrs). The interim monitoring boundary for large early benefit of immunotherapy was reached and randomization was stopped.

2.6 Rationale for revised ch14.18 administration guidelines and additional supportive care as of amendment # 9
Enrollment to ANBL0032 was suspended on January 13, 2009 because of an apparent increase in allergy-like serious adverse events that occurred after a new lot of ch14.18 (Lot# L0512003) was initially distributed to COG institutions in July 2008. As a result of these events, NCI received a partial clinical hold letter from FDA for ANBL0032 that described information that would be needed to resolve the
clinical hold. A series of investigations were rapidly initiated to investigate possible causes for the apparent increase in allergic-like events.

Nine patients developed at least one Grade ≥ 3 “Allergic reaction / hypersensitivity” event while receiving ch14.18 Lot # L0512003. Seven of the nine events occurred during either Course 2 or 4, in which ch14.18 is administered with aldesleukin.

No deviations from standard procedures in shipping of ch14.18 to the affected sites were identified. Additionally, no deviations were noted in their pharmacies’ ch14.18 storage, preparation, and administration procedures, and there were no problems reported with storage of the agent at the sites between receipt and drug preparation. Extensive testing was performed to compare ch14.18 from Lot# L0512003 to ch14.18 from older lots, and there were no out of range observations for any of the lots tested. Testing included, but was not limited to, Size-Exclusion Chromatography (SEC), Complement Dependent Cytotoxicity, GD-2 Binding ELISA, Capillary isoelectric focusing (CIEF), endotoxin analysis, and Peptide Mapping and LCMS Analysis. Additionally, analyses of samples recovered from sites at which cases of allergy/anaphylaxis occurred revealed no differences in purity in comparison to reference samples using the same tests described above.

A comprehensive investigation of potential causes for the apparent increase in allergic-like reactions identified no obvious explanatory factors. There is evidence for a change in the manner in which adverse events were reported, possibly related to the change from reporting using CTC Version 2.0 to CTCAE Version 3.0 during the course of the study. While Grade 3/4 hypotension was reported more commonly with the older lot of ch14.18 (Lot # 182006), Grade 3/4 allergic reaction was reported more frequently with the new lot (Lot # L0512003). However, it is apparent that regardless of the lot of ch14.18 used, there is an increased toxicity rate for Courses 2 and 4 in which ch14.18 is administered with aldesleukin, compared to Courses 1, 3, and 5 in which ch14.18 is administered with GM-CSF. Modifications in the supportive care and dose modification guidelines have been incorporated into Amendment # 9 in order to enhance the safety profile for Courses 2 and 4. Careful monitoring and reporting of toxicity are also incorporated into the amendment to evaluate the impact of these changes.

2.7 Twelve-Lead ECG in Cardiac Safety Substudy
Currently, there is limited data available regarding the cardiac safety profile of ch14.18 as determined by electrocardiogram (ECG) evaluation. According to the ICH E14 guidance which was approved in October 2005, the clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs is recommended for new investigational agents. Therefore, a study evaluating the effect of ch14.18 on cardiac safety parameters including RR, PR, QRS and QT interval durations will be undertaken at a subset of qualified study centers. Specifically, ECG assessments will be conducted at five time points (Section 5.6.6) in a total of 60 subjects over the course of therapy with ch14.18. Each of the five time points correspond to a scheduled ch14.18 pharmacokinetic blood draw to allow for the correlation of potential ECG findings to actual ch14.18 blood levels. ECG data will be centrally reviewed and summarized.

2.8 Background and rationale for anti-glycan antibody studies
Administration of ch14.18 is often associated with allergic reactions, which may occur even during the first infusion in some patients. Natural occurring antibodies to non-human glycans, galactose alpha-1,3-galactose (alpha-gal) and Neu5Gc, are present in all humans. ch14.18, which is produced in rodent cells, contains alpha-gal and Neu5Gc. Levels of anti-alpha-gal have been correlated with allergic reactions to cetuximab (Erbitux), a chimeric antibody against the EGF receptor that is extensively modified with this glycans. Thus, it is possible that presence and/or levels of antibodies to these non-human glycans may correlate with allergic reactions to ch14.18, and the levels of anti-glycan antibodies may increase after repeated courses of immunotherapy, which may affect the blood levels of ch14.18 and patients’ outcome.
We plan to study clinical relevance of anti-glycan antibodies in the blood samples collected from patients enrolled on ANBL0931 and ANBL0032 prior to amendment 12.

2.9 **Rationale for genotyping FcR, KIR and KIR ligand**

In general, the anti-tumor activities of unconjugated mAbs require the contribution of either complement or Fcγ receptor (FcγR)-expressing effector cells in order to achieve tumor cell killing. However, because most tumor cells, including neuroblastoma, express increased amounts of complement-inhibiting proteins, which protect the cells against lysis by complement, antibody-dependent cell cytotoxicity (ADCC) is considered the key antitumor mechanism of therapeutic antibodies in vivo. Natural killer (NK) cells, T lymphocytes, monocytes and granulocytes are capable of mediating ADCC against antibody-coated targets via their expression of FcγR for IgG. The FcγR genes display polymorphisms that greatly influence the affinity of IgG for the Fcγ receptor. NK cells bearing the FcγRIIIa-158V/V allele mediate ADCC more effectively than those with F/F allele. Similarly, for FcγRIIA, the high-affinity H allele at 131 results in greater affinity of FcγRIIa for IgG, whereas the low-affinity R allele correlates with decreased binding. The FCGR3A and FCGR2A gene polymorphism was reported to influence the response of lymphoma to rituximab (anti-CD20). As Ch14.18 is very effective in mediating ADCC, its efficacy in neuroblastoma may be associated with FCGR3 and/or FCGR2 genotype.

Killer-Immunoglobulin-like Receptors (KIR) recognize specific HLA molecules, regulate function of human NK cells and control their self-tolerance. The interactions between KIR on donor NK cells and KIR ligands (KIR-L) on recipient tissues influence anti-tumor efficacy of allogeneic hematopoietic stem cell transplantation (HSCT). Furthermore, inherited KIR and KIR-L alleles influence the antitumor effects of autologous HSCT. Since the genes encoding for KIR and KIR-L are inherited independently, it is possible for an individual to be KIR-receptor ligand mismatched with oneself. In a COG phase II study of 38 relapsed/refractory neuroblastoma receiving *humanized* anti-GD2 linked to IL2, 7 of 24 mismatched patients experienced either complete response or improvement of their disease after immunocytokine therapy, while there was no response or comparable improvement of disease in 14 patients who were matched (p = 0.03). This data suggests that patients with KIR receptor-ligand mismatch may be associated with better clinical response to immunotherapy with anti-GD2.

3.0 **AGENT INFORMATION**

3.1 **ISOTRETINOIN** (13-cis-retinoic acid, RO-43,780, Accutane®, Amnesteem®, Claravis™, Sotret®) NSC#329481 (05/09/11)

**Source and Pharmacology**: Isotretinoin is a naturally occurring analogue of Vitamin A. Retinoids are required for the maintenance of normal cell growth, differentiation, and loss within epithelial tissues. Various retinoids have been shown to suppress or reverse epithelial carcinogenesis and to prevent the development of invasive cancers in many animal systems. Retinoids act primarily in the post-carcinogen phases of promotion and progression, which makes them more useful for chemoprevention. However in certain malignancies (notably acute promyelocytic leukemia and neuroblastoma), high-doses of retinoids can have significant anti-tumor activity. The exact mechanism of RA-induced maturation of tumor cells is not known. The mechanism by which retinoids regulate the growth and differentiation of normal and malignant cells has not been completely elucidated, but it is thought that these effects result from the ability of retinoids to modulate the transcriptional regulatory activity of a set of nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs) belonging to the super family of thyroid/steroid hormone receptors.

Isotretinoin is highly lipophilic and 99.9% bound to plasma protein (almost entirely albumin) and has a half-life of 10-20 hours. Oral absorption of isotretinoin is enhanced when given with a high fat meal increasing both the peak plasma concentration and AUC by more than double. Following oral administration of
isotretinoin, at least three metabolites have been identified in human plasma: 4-oxo-isotretinoin, retinoic acid (tretinoin), and 4-oxo-retinoic acid (4-oxo-tretinoin). Retinoic acid and 13-cis-retinoic acid are geometric isomers and show reversible inter-conversion. The administration of one isomer will give rise to the other. Isotretinoin is also irreversibly oxidized to 4-oxo-isotretinoin, which forms its geometric isomer 4-oxo-tretinoin. All of these metabolites possess retinoid activity that is in some in vitro models more than that of the parent isotretinoin. The metabolites of isotretinoin and any conjugates are ultimately excreted in the feces and urine in relatively equal amounts (total of 65-83%). In a study comparing the pharmacokinetics of isotretinoin in pediatric and adult patients there were no statistically significant differences.

**Toxicity:**

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Happens to 21-100 children out of every 100</td>
<td>Happens to 5-20 children out of every 100</td>
<td>Happens to &lt;5 children out of every 100</td>
</tr>
<tr>
<td>Immediate:</td>
<td>Within 1-2 days of receiving drug</td>
<td>Nausea and vomiting</td>
<td>Anaphylaxis, bronchospasm</td>
</tr>
<tr>
<td>Prompt:</td>
<td>Within 2-3 weeks, prior to the next course</td>
<td>Rash (L), conjunctivitis (L), Headache (L), decrease in high density lipoproteins (L), Cholesterol elevation (L), Transaminase elevation (L), Anemia (L)</td>
<td>Alopecia, appetite disturbances, Hyperglycemia, Hyper- or hypo-skin pigmentation, nail changes, eruptive xanthomas, seizures, dizziness, Pseudotumor-cerebri (papilledema, headache, Nausea, vomiting, visual disturbances), Psychiatric disorders (depression, aggressive and/or violent behaviors, suicidal ideation, suicide, dream disturbances), insomnia, Lethargy, malaise, nervousness, paresthesias, Weight loss, myelosuppression, elevated platelet counts, agranulocytosis, allergic vasculitis (L), chest pain, pancreatitis (including very rarely fatal hemorrhagic pancreatitis), hearing impairment, inflammatory bowel disease, visual disturbances (decrease in night vision, corneal opacities which resolve on d/c, photophobia, color vision disturbances, cataracts), edema, inflammation of the gums, Drying of respiratory tract with voice alteration, respiratory infections, erythema multiforme, Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN).</td>
</tr>
<tr>
<td>Delayed:</td>
<td>Any time later during therapy, excluding the above conditions</td>
<td>Skeletal hyperostosis</td>
<td>Osteoporosis, bone fractures or delayed healing, premature epiphyseal closure, Rhabdomyolysis, abnormal menses, renal disturbances (WBC in urine, proteinuria, hematuria, renal calculi), calcification of tendon and ligaments</td>
</tr>
<tr>
<td>Unknown frequency and timing:</td>
<td>Major human fetal abnormalities related to isotretinoin administration in females have been documented. There is an increased risk of spontaneous abortion. In addition, premature births have been reported. Documented external abnormalities include: skull abnormality; ear abnormalities (including anotia, microopinna, small or absent external auditory canals); eye abnormalities (including microphthalmia); facial dysmophria and cleft palate. Documented internal abnormalities include: CNS abnormalities (including cerebral abnormalities, cerebellar malformation, hydrocephalus, microcephaly, cranial nerve deficit); cardiovascular abnormalities; thymus gland abnormality; parathyroid hormone deficiency. In some cases death has occurred with certain of the abnormalities previously noted. Cases of IQ scores less than 85 with or without obvious CNS abnormalities have also been reported. It is not known whether this drug is excreted in human milk. Because of the potential for adverse effects, nursing mothers should not receive isotretinoin.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(L) Toxicity may also occur later.
**Formulation and Stability:** Isotretinoin, a retinoid, is available in 10 mg, 20 mg, 30 mg and 40 mg soft gelatin capsules for oral administration. Inactive ingredients vary depending on the manufacturer but capsule formulations may include the following inactive ingredients: beeswax, butylated hydroxyanisole, edetate disodium, hydrogenated soybean oil flakes, hydrogenated vegetable oil, soybean oil and vitamin E. Gelatin capsules may contain glycerin and parabens (methyl and propyl), with the following dye systems: iron oxide (red and black) and titanium dioxide; FD&C Red No. 3 or 7, FD&C Blue No. 1 or 2, FD&C Yellow No. 6 or 10, propylene glycol, and shellac glaze.


**Guidelines for Administration:** See Treatment and Dose Modifications sections of the protocol.

Give orally with food (ideally, high fat) or milk to enhance absorption. For children unable to swallow the capsules whole, the following options may be used:

1. Soften capsule (in warm water), bite, swallow, or suck out contents or place softened capsule in fatty food such as peanut butter and swallow. The method of softening the capsule and withdrawing contents with an oral syringe is not preferred as it is difficult to remove all of the drug from the capsule.

2. Squeeze out the entire contents of the capsule into a small medicine cup and give with fatty food, such as peanut butter. (If at all possible have the child suck on the empty capsule in hopes of getting more of the intended dose.)

3. The preferred method is to poke a hole in the capsule to allow the capsule to be chewed and embed the capsule in a food or candy enjoyed by the child.

Under no circumstances should isotretinoin be removed from the capsules for more than 1 hour prior to administering to the patient.

Note: Women of childbearing potential should wear gloves when handling isotretinoin capsules.

If the contents are withdrawn from the capsule and mixed with food or milk, administer as closely as possible to mixing to avoid oxidation or conversion by exposure to light to a more toxic and less potent product.

Isotretinoin is contraindicated in patients with paraben allergies as the capsule is preserved with the agent. Patients should be warned about enhanced photosensitivity; the use of sunscreen and avoidance of direct sunlight should be recommended. Patients should limit the intake of vitamin A.

**Supplier:** Commercially available from various manufacturers. See package insert for further information.

**SITES WITHIN THE UNITED STATES:**
Isotretinoin MUST be prescribed under the Committed to Pregnancy Prevention Program (iPLEDGE). (Physicians must complete a one-time registration with the program in order to be able to prescribe the drug. Each physician (or their office representative), dispensing pharmacy, and patient must be registered on line @ https://www.ipledgeprogram.com (or call 1-866-495-0654 to begin the registration process).

Upon registration you will receive all of the information and educational materials necessary to prescribe isotretinoin. Because of
isotretinoin’s teratogenicity and to minimize fetal exposure, isotretinoin is approved for marketing only under a special restricted distribution program approved by the Food and Drug Administration. This program is called iPLEDGE. Isotretinoin must only be prescribed by prescribers who are registered and activated with the iPLEDGE program. Isotretinoin must only be dispensed by a pharmacy registered and activated with iPLEDGE, and must only be dispensed to patients who are registered and meet all the requirements of iPLEDGE. The iPLEDGE program is a computer-based risk management system that uses verifiable, trackable links between prescriber, patient, pharmacy, and wholesaler to control prescribing, using, dispensing and distribution of isotretinoin.

OVERVIEW: PROGRAM REQUIREMENTS

The iPLEDGE program has specific requirements for prescribers, patients, and pharmacists. One of the prescriber’s main responsibilities is knowing and educating patients about these requirements. Note: The FDA has provided the following statement concerning the physician training aspects of the iPLEDGE site:

“FDA and the sponsors would like the oncology community who uses isotretinoin to enroll in iPLEDGE and to know that the "I know how to diagnose and treat the various presentations of acne," statement can be truthfully answered by oncologists who "know how to diagnose and treat" suspected cases by referring these cases to dermatologists for management. All the sponsors and FDA agree on this interpretation.”

Prescribers are responsible for registering every patient, who meets the program requirements, in the iPLEDGE program via the automated system. They are responsible for educating patients about the side effects of isotretinoin and the high risk of birth defects for female patients of childbearing potential while taking the drug. As part of this process, they are also responsible for counseling patients about the monthly steps they must follow to receive isotretinoin.

Prescribers can only write a patient’s prescription for isotretinoin once a month, and then only up to a maximum of a 30-day supply. Patients must plan for monthly appointments to receive their prescriptions. At each of these appointments, the prescriber must counsel the patient about the iPLEDGE program requirements and then confirm via the iPLEDGE automated system that this counseling occurred. They must also enter this information after the first appointment.

There are different program requirements for male patients and female patients who are not of childbearing potential and for female patients of childbearing potential. The prescriber must determine if a patient is a female patient of childbearing potential and document that she meets the specific requirements of the program. These include taking pregnancy tests (processed in a CLIA-certified laboratory) and using 2 forms of birth control consistently. Both of these requirements must be followed before, during, and after treatment, but are not necessary for patients the physician determines are not of childbearing potential. To receive monthly prescriptions, a female patient of childbearing potential must also answer questions in the iPLEDGE system about the program requirements and pregnancy prevention. She must also enter the two forms of birth control she is using. In addition to the monthly counseling information, the prescriber must also enter into the system the patient’s 2 forms of contraception and the results of the monthly pregnancy test obtained from a CLIA-certified laboratory. This information is the criteria the system uses to authorize a pharmacy to fill a prescription.

Requirements for Pharmacists

• Isotretinoin can only be obtained from pharmacies registered with and activated in the iPLEDGE program.
• Registered and activated pharmacies can obtain isotretinoin only from wholesalers registered with the iPLEDGE program.
• The dispensing pharmacist must obtain authorization and a Risk Management Authorization (RMA) number before filling and dispensing prescriptions.
• Upon receiving authorization, the dispensing pharmacist can fill a prescription for a maximum 30-day supply of isotretinoin.
• Upon authorization, the iPLEDGE system provides a Risk Management Authorization (RMA) number to the dispensing pharmacist. The pharmacist should record the RMA number directly on the prescription.
• Upon authorization, the iPLEDGE system provides a “Do Not Dispense to Patient After” date (7 days from office visit date) to the dispensing pharmacist. The pharmacist should record this date on the prescription bag sticker.

CANADIAN SITES
The iPLEDGE program for pregnancy prevention is not applicable in Canada. Canadian prescribers are responsible for monitoring and counseling patients (if they are in this age range) regarding birth control and patient’s caregivers who may handle the agent. Isotretinoin is marketed in Canada with no additional regulatory requirements.

3.2 CHIMERIC MONOCLONAL ANTIBODY 14.18
(Chimeric MoAb 14.18; human/murine anti-GD2 monoclonal antibody; chimeric anti-GD2; chimeric mAb 14.18; ch14.18) NSC #623408 IN# 4308. (11/21/12)

Source and Pharmacology:
Chimeric MoAb 14.18 is an anti-GD2 monoclonal antibody which is a chimeric construct composed of the variable region heavy and light chain genes of the murine mAb14.G2a and the human constant region genes for heavy chain IgG1 and light chain kappa. The disialoganglioside GD2 antigen has been shown to be highly enriched on tumors of neuroectodermal origin, including melanoma, glioma, osteosarcoma, and neuroblastoma. Chimeric MoAb 14.18 is prepared from culture medium containing the antibody secreted by vector transfected hybridoma cell line14.18. In the culture media, Chimeric MoAb 14.18 is purified by column chromatography. The final product is tested to ensure identity, potency, purity, and quality. Chimeric MoAb 14.18 exerts its antitumor effect by binding specifically to the disialoganglioside GD2 antigen, leading to the lysis of GD2 tumor cells through the process of antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity. By targeting the GD2 antigen on the cell surface, chimeric MoAb 14.18 may also prevent circulating malignant cell attachment to the extracellular matrix. Additionally, chimeric MoAb 14.18 mediates lysis of several melanoma and neuroblastoma cell lines in a dose dependent manner in the presence of potent mediators of chimeric MoAb 14.18-dependent cytotoxicity, such as human peripheral blood mononuclear cells and granulocytes, in particular neutrophils, especially in the presence of recombinant human granulocyte-macrophage colony-stimulating factor.

The PK profile of ch14.18 has been determined in adults with melanoma and children with neuroblastoma. Although the plasma clearance for both groups of patients follow a two-compartment model, circulating antibody is cleared from the plasma at a much faster rate in children than adults (mean t1/2 = 66.6 ± 27.4 hours in children versus 123 ± 29 hours and 181 ± 73 hours in two adult trials, respectively). Maturation of the hepatic and renal systems with age is thought to impact on drug metabolism and elimination and could account for these differences. In general, the mAb half-life following the first course of treatment was longer than the half-lives following subsequent courses in a given patient.
Comprehensive Adverse Events and Potential Risks list (CAEPR) for Chimeric MoAb 14.18 (NSC 623408)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via AdEERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' for further clarification. Frequency is provided based on 190 patients. Below is the CAEPR for chimeric MoAb 14.18.

**NOTE:** Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

<table>
<thead>
<tr>
<th>Adverse Events with Possible Relationship to Chimeric MoAb 14.18 (CTCAE 4.0 Term) [n= 190]</th>
<th>Specific Protocol Exceptions to Expedited Reporting (SPEER)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Likely (&gt;20%)</td>
<td>Less Likely (&lt;=20%)</td>
</tr>
<tr>
<td><strong>BLOOD AND LYMPHATIC SYSTEM DISORDERS</strong></td>
<td></td>
</tr>
<tr>
<td>Disseminated intravascular coagulation</td>
<td>Disseminated intravascular coagulation (Gr 2)</td>
</tr>
<tr>
<td><strong>CARDIAC DISORDERS</strong></td>
<td></td>
</tr>
<tr>
<td>Sinus tachycardia</td>
<td>Cardiac arrest</td>
</tr>
<tr>
<td><strong>GASTROINTESTINAL DISORDERS</strong></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>Abdominal pain (Gr 3)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Diarrhea (Gr 3)</td>
</tr>
<tr>
<td>Nausea</td>
<td>Nausea (Gr 2)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>Vomiting (Gr 3)</td>
</tr>
<tr>
<td><strong>GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS</strong></td>
<td></td>
</tr>
<tr>
<td>Edema limbs</td>
<td>Edema limbs (Gr 2)</td>
</tr>
<tr>
<td>Fever</td>
<td>Fever (Gr 3)</td>
</tr>
<tr>
<td>Pain</td>
<td>Pain (Gr 3)</td>
</tr>
<tr>
<td><strong>IMMUNE SYSTEM DISORDERS</strong></td>
<td></td>
</tr>
<tr>
<td>Allergic reaction</td>
<td>Allergic reaction (Gr 3)</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td></td>
</tr>
<tr>
<td>Serum sickness</td>
<td></td>
</tr>
<tr>
<td><strong>INFECTIONS AND INFESTATIONS</strong></td>
<td></td>
</tr>
<tr>
<td>Infection*</td>
<td>Infection* (Gr 3)</td>
</tr>
<tr>
<td><strong>INVESTIGATIONS</strong></td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase increased</td>
<td>Alanine aminotransferase increased (Gr 3)</td>
</tr>
<tr>
<td>Aspartate aminotransferase increased</td>
<td>Aspartate aminotransferase increased (Gr 3)</td>
</tr>
</tbody>
</table>

Version 2.4, November 14, 2012
<table>
<thead>
<tr>
<th>METABOLISM AND NUTRITION DISORDERS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Anorexia</td>
<td>Hypoalbuminemia</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Back pain</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NERVOUS SYSTEM DISORDERS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuralgia</td>
<td>Peripheral sensory neuropathy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RENAL AND URINARY DISORDERS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>Dyspnea</td>
</tr>
<tr>
<td></td>
<td>Hypoxia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SKIN AND SUBCUTANEOUS TISSUE DISORDERS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pruritus</td>
<td>Rash maculo-papular</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VASCULAR DISORDERS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary leak syndrome</td>
<td>Hypotension</td>
</tr>
</tbody>
</table>

| 1This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail. |
|---|---|
| 2Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC. Also reported on chimeric MoAb 14.18 trials but with the relationship to chimeric MoAb 14.18 still undetermined: |
| --- | --- |
| **BLOOD AND LYMPHATIC SYSTEM DISORDERS** | Anemia; Blood and lymphatic system disorders - Other (thrombotic microangiopathy [e.g., thrombotic thrombocytopenic purpura (TTP) or hemolytic uremic syndrome (HUS)]); Bone marrow hypocellular; Febrile neutropenia |
| **CARDIAC DISORDERS** | Acute coronary syndrome; Chest pain - cardiac; Left ventricular systolic dysfunction; Mobitz (type) II atrioventricular block; Myocardial infarction; Palpitations; Pericardial effusion; Sinus bradycardia; Supraventricular tachycardia; Ventricular tachycardia |
| **EAR AND LABYRINTH DISORDERS** | Ear pain; Hearing impaired |
| **ENDOCRINE DISORDERS** | Hyperthyroidism; Hypothyroidism |
| **EYE DISORDERS** | Blurred vision; Dry eye; Eye disorders - Other (cycloplegia); Eye disorders - Other (pupil dilation); Eyelid function disorder; Optic nerve disorder; Papilledema; Photophobia; Scleral disorder |
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Cheilitis; Colitis; Constipation; Dysphagia; Esophagitis; Gastrointestinal disorders - Other (pneumatosis intestinalis); Hemorrhoidal hemorrhage; Ileus; Intra-abdominal hemorrhage; Lower gastrointestinal hemorrhage; Mucositis oral; Oral pain; Rectal hemorrhage; Stomach pain

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema face; Edema trunk; Fatigue; Hypothermia; Infusion related reaction; Injection site reaction; Irritability; Localized edema; Non-cardiac chest pain; Sudden death NOS

HEPATOBILIARY DISORDERS - Hepatobiliary disorders - Other (cholestasis)

IMMUNE SYSTEM DISORDERS - Cytokine release syndrome

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fracture

INVESTIGATIONS - Activated partial thromboplastin time prolonged; Alkaline phosphatase increased; Blood bilirubin increased; Cardiac troponin I increased; Cholesterol high; Ejection fraction decreased; GGT increased; Investigations - Other (isolated glycosuria); Lipase increased; Lymphocyte count increased; Neutrophil count decreased; Urine output decreased; Weight gain; Weight loss; White blood cell decreased

METABOLISM AND NUTRITION DISORDERS - Acidosis; Dehydration; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hypertriglyceridemia; Hypocalcemia; Hypoglycemia; Hypomagnesemia; Hypoproteinemia; Hypophosphatemia

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Arthritis; Bone pain; Chest wall pain; Muscle weakness lower limb; Myalgia; Neck pain; Pain in extremity

NERVOUS SYSTEM DISORDERS - Cognitive disturbance; Depressed level of consciousness; Dysgeusia; Dysphasia; Encephalopathy; Headache; Hydrocephalus; Meningismus; Movements involuntary; Myelitis; Nystagmus; Oculomotor nerve disorder; Paresthesia; Peripheral motor neuropathy; Seizure; Somnolence; Tremor

PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Delirium; Hallucinations; Insomnia; Personality change; Restlessness

RENAL AND URINARY DISORDERS - Acute kidney injury; Chronic kidney disease; Hematuria; Renal and urinary disorders - Other (acute renal insufficiency); Renal and urinary disorders - Other (urethritis); Renal hemorrhage; Urinary retention

REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Hematosalpinx; Ovarian hemorrhage; Pelvic pain; Penile pain; Prostatic hemorrhage; Spermatic cord hemorrhage; Testicular hemorrhage; Uterine hemorrhage; Vaginal hemorrhage

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Apnea; Atelectasis; Bronchospasm; Laryngeal edema; Laryngopharyngeal dysesthesia; Pharyngolaryngeal pain; Pleural effusion; Pneumonitis; Pulmonary edema; Respiratory, thoracic and mediastinal disorders - Other (tachypnea); Wheezing

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Dry skin; Erythema multiforme; Hyperhidrosis

VASCULAR DISORDERS - Flushing

Note: Chimeric MoAb 14.18 in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

Formulation and Stability: Chimeric MoAb 14.18 is provided as sterile solution in single-dose vials containing 25 mg/5 mL (5 mg/mL) in phosphate buffered saline. Intact vials should be stored in the refrigerator (2 ºC to 8 ºC). Stability studies of the intact vials are ongoing.

Guidelines for Administration: (See Treatment and Dose Modifications and/or Supportive Care sections of the protocol)

Preparation Instructions: Particulate matter was found in vials from lots 32038 and 182006 during stability testing post-vial filling. The lots met all other release specifications, including identity and potency. Tests were conducted with a Millex GV 25 mm (0.2 micron) filter to remove the particulates. Post filtration studies demonstrated minimal
loss of potency. The dose must be filtered with a 0.2 micron low protein binding filter prior to addition to the 0.9% sodium chloride.

Additional product testing has revealed that a significant volume may remain in the filter-needle apparatus when injecting the dose through the filter into the infusion bag. The following methodology must be used to ensure consistent delivery of the full dose into the infusion bag:

Withdraw the required dose of chimeric MoAb 14.18 from the vial(s) through a 0.2 micron low protein binding syringe filter. Replace the filter/needle assembly with a new needle and inject the exact volume for dose into a 100 mL bag of 0.9% sodium chloride. The stability and compatibility of the antibody with closed system safety devices has not been tested. A small number of sites have reported the formation of a precipitate in solutions prepared and administered using these systems, but a definitive link has not been established.

Chimeric MoAb 14.18 is stable at room temperature for at least 24 hours; however, the final dosage form should be prepared immediately prior to administration as there is a maximum infusion time of 20 hours. The minimum infusion time for the antibody infusion is 10 hours.

Lot number information will be collected on the NCI Drug Accountability Record Form (DARF) (see the Agent Accountability section below).

**Preparation Note:**
Vials (empty and partial) used to prepare each chimeric 14.18 infusion should be retained in the pharmacy and held at 2-8ºC in quarantine for one week. The vials should be segregated and labeled with a unique identifier and date of infusion for later reference. If, after one week, the patient has not experienced a Grade 4 serious adverse event (SAE), the vials may be discarded per standard institutional policy. In the event of a Grade 4 SAE related to an acute infusion reaction or allergic reaction, please contact the Biopharmaceutical Development Program (BDP) at 301-846-5369 for further instructions on where/how to ship the material for possible testing. Please make sure that an AdEER report is filed for such an event and provide the AdEERs number when BDP is notified. Shipping instructions and pre-paid shippers will be provided for shipment to the appropriate facility. No shipment should occur until a discussion has taken place with the BDP.

**Patient Care Implications:** Pain is the most common adverse effect of chimeric MoAb 14.18. It is predominately neuropathic and manifests as abdominal cramps or back and extremity pain. Prophylactic administration of morphine and lidocaine by continuous infusion is required before and during the infusions of chimeric MoAb 14.18.

Acute allergic or infusion reactions are common and may include hypotension, urticaria, hypoxia, and dyspnea. Premedication with antihistamines and acetaminophen are required for chimeric MoAb 14.18 administration.

Human anti-mouse antibodies (HAMA) may block the effectiveness of therapy by prematurely clearing the treatment antibody and limiting further immunotherapy. HAMA responses may also be associated with immune-complex related adverse events such as serum sickness and anaphylaxis. HAMA responses were detected in over 50% of the patients tested. Although, no increase in allergic reactions has been observed in patients treated with chimeric MoAb 14.18 in the presence of HAMA, immune complex formation may have induced serum sickness in some patients.

**Supplier:** Investigational agent supplied by the NCI
Obtaining the Agent

THERE IS A LIMITED SUPPLY OF ANTIBODY AND SUPPLIES SHOULD ONLY BE ORDERED IF A PATIENT IS EITHER RECEIVING TREATMENT OR IS BEING EVALUATED FOR ENROLLMENT INTO THE STUDY.

Agent Ordering

NCI supplied agent may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (fdf). If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees must submit agent requests through the PMB Online Agent Order Processing (OAOP) application <https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx>. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account <https://eapps-ctep.nci.nih.gov/iam/> and the maintenance of an “active” account status and a “current” password. For questions about drug orders, transfers, returns, or accountability, call (301) 496-5725 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

Agent Accountability

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the CTEP home page at http://ctep.cancer.gov/protocolDevelopment/default.htm#agents_drugs for the Procedures for Drug Accountability and Storage and http://ctep.cancer.gov/forms/default.htm to obtain a copy of the DARF and Clinical Drug Request form.)

Agent Returns

Investigators/Designees must return unused DCTD supplied investigational agent to the NCI clinical repository as soon as possible when: the agent is no longer required because the study is completed or discontinued and the agent cannot be transferred to another DCTD sponsored protocol; the agent is outdated or the agent is damaged or unfit for use. Regulations require that all agents received from the DCTD, NCI be returned to the DCTD, NCI for accountability and disposition. Return only unused vials/bottles. Do NOT return opened or partially used vials/bottles unless specifically requested otherwise in the protocol. See the CTEP web site for Policy and Guidelines for Investigational agent Returns at: http://ctep.cancer.gov/protocolDevelopment/default.htm#agents_drugs. The appropriate forms may be obtained at: http://ctep.cancer.gov/forms/docs/return_form.pdf.

3.3 SARGRAMOSTIM (Granulocyte Macrophage Colony Stimulating Factor, rhu GM-CSF, rGM-CSF, GM-CSF, Leukine®) NSC #613795 (02/28/12)

Source and Pharmacology:

Sargramostim (recombinant human GM-CSF) is a glycoprotein produced in yeast (S. cerevisiae) by recombinant DNA technology. rGM-CSF is a hematopoietic growth factor which supports survival, clonal expansion, and differentiation of hematopoietic progenitor cells. rGM-CSF induces partially committed
progenitor cells to divide and differentiate in the granulocyte-macrophage pathways. rGM-CSF stimulates the production of monocytes, granulocytes, erythrocytes, and sometimes, megakaryocytes in the bone marrow. It also induces mature neutrophil and monocytes to increase phagocytosis, superoxide generation, ADCC, tumoricidal killing and cytokine production (IL-1 and tumor necrosis factor). Recombinant human GM-CSF is a glycoprotein of 127 amino acids characterized by three primary molecular masses of 15500, 16800, and 19500 daltons. The amino acid sequence differs from the natural sequence by a substitution of leucine at position 23 and the CHO moiety may be different from the native protein. After subcutaneous administration of sargramostim, peak levels were obtained in 1-4 hours and were detectable at therapeutic levels for 12-16 hours post injection. The elimination t½ ranges from 1.5-2.7 hours after SubQ or IV administration.

### Toxicity:

<table>
<thead>
<tr>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Happens to 21-100 children out of every 100</td>
<td>Happens to 5-20 children out of every 100</td>
<td>Happens to &lt; 5 children out of every 100</td>
</tr>
<tr>
<td><strong>Immediate:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 1-2 days of receiving drug</td>
<td>Headache, malaise, fatigue, rash, pruritis, bone pain, myalgia, arthralgia, fever, chills</td>
<td>Abdominal pain, weakness, anorexia, nausea, local injection reactions</td>
</tr>
<tr>
<td><strong>Prompt:</strong></td>
<td></td>
<td>In high doses: capillary leak syndrome: (pleural effusion, peripheral edema, ascites, weight gain, hypotension), pneumonitis, peripheral edema, elevation of creatinine, bilirubin and hepatic enzymes in patients with pre-existing renal or hepatic dysfunction</td>
</tr>
<tr>
<td>Within 2-3 weeks, prior to the next course</td>
<td>Weight gain</td>
<td></td>
</tr>
<tr>
<td><strong>Delayed:</strong></td>
<td>Thrombocytopenia</td>
<td></td>
</tr>
<tr>
<td>Any time later during therapy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Unknown Frequency and Timing:** Fetal and teratogenic toxicities: It is not known whether sargramostim can cause fetal harm or affect reproduction capacity when administered to a pregnant woman. It is unknown whether the drug is excreted in breast milk.

### Formulation and Stability:

Sargramostim is available as a lyophilized sterile, white, preservative free powder with 250 mcg (1.4 million International Units) per vial and as a sterile, preserved injectable solution in a 500 mcg/mL (2.8 million International Units/mL) 1 mL vial. The sargramostim reconstituted lyophilized vial contains 40 mg/mL mannitol, USP; 10 mg/mL sucrose, NF; and 1.2 mg/mL tromethamine, USP, as excipients. The liquid formulation also contains 1.1% benzyl alcohol (11 mg/mL). Store refrigerated at 2-8°C (36-46°F). Do not freeze or shake.

**Dosage form, manufacturer, and lot number** information will be collected on the NCI Drug Accountability Record Form (DARF) or an alternate institutional form to enable assessments for infusion-related reactions (see the Agent Accountability section below).

### Guidelines for Administration:

See [Treatment](#) and [Dose Modifications](#) and [Supportive Care](#) sections of the protocol.

Reconstitute lyophilized powder for injection with 1 mL Sterile Water for Injection or 1 mL Bacteriostatic Water for Injection. Use Sterile Water for Injection without benzyl alcohol for neonates and infants < 2 years of age or patients with hypersensitivity to benzyl alcohol. During reconstitution, direct the diluent at the side of the vial and gently swirl the contents to avoid foaming during dissolution. Avoid excessive or vigorous agitation; do not shake. Reconstituted solutions prepared with Bacteriostatic
Water for Injection (0.9% benzyl alcohol) or the liquid preserved solution which has been punctured may be stored for up to 20 days at 2°-8°C (36°-46°F) prior to use. Discard reconstituted solution after 20 days. Reconstituted solutions prepared with Sterile Water for Injection (without preservative) should be administered as soon as possible and within 6 hours following reconstitution.

Use sargramostim for subcutaneous injection without further dilution. Perform dilution for IV infusion in 0.9% Sodium Chloride Injection. If the final concentration is < 10 mcg/mL, add albumin (human) at a final concentration of 0.1% to the saline prior to addition of sargramostim to prevent adsorption to the components of the drug delivery system. For a final concentration of 0.1% albumin (human), add 1 mg albumin (human) per 1 mL 0.9% Sodium Chloride Injection (e.g., use 1 mL 5% Albumin [Human] in 50 mL 0.9% Sodium Chloride Injection). Intravenous dilutions are stable for up to 48 hours at room temperature or refrigerated but should be used within 6 hours due to microbiological concerns. Do not use an in-line membrane filter for IV infusion.

Supplier:
Commercially available from various manufacturers. See package insert for more detailed information. Only sargramostim (yeast-derived recombinant human GM-CSF) will be used in this study. The Escherichia coli-derived product (molgramostim) will not be used.

Agent Accountability
The investigator, or a responsible party designated by the investigator, must maintain a careful record of the dosage form, manufacturer, and lot numbers of drug dispensed to study patients of all commercial supplies of sargramostim using the NCI Drug Accountability Record Form (DARF) or an alternate institutional form capturing the same information. Access http://ctep.cancer.gov/forms/default.htm to obtain a copy of the DARF. This will enable assessments of infusion-related reactions.

3.4 Aldesleukin, Proleukin®, (IL-2, recombinant human Interleukin 2, IL-2, Interleukin-2
Proleukin®) NSC # 373364 (02/10/10)

Source and Pharmacology: Aldesleukin for injection, a human recombinant interleukin-2 product, is a highly purified protein with a molecular weight of approximately 15,300 daltons, Aldesleukin a lymphokine, is produced by recombinant DNA technology using a genetically engineered E. coli strain containing an analog of the human interleukin-2 gene. In vitro studies performed on human cell lines demonstrate the immunoregulatory properties of Aldesleukin, including: a) enhancement of lymphocyte mitogenesis and stimulation of long-term growth of human interleukin-2 dependent cell lines; b) enhancement of lymphocyte cytotoxicity; c) induction of killer cell (lymphokine-activated (LAK) and natural (NK) activity; and d) induction of interferon-gamma production. The in vivo administration of Aldesleukin in animals and humans produces multiple immunological effects in a dose dependent manner. These effects include activation of cellular immunity with profound lymphocytosis, eosinophilia, and thrombocytopenia, and the production of cytokines including tumor necrosis factor, IL-1 and gamma interferon. In vivo experiments in murine tumor models have shown inhibition of tumor growth. The exact mechanism by which Aldesleukin mediates its antitumor activity in animals and humans is unknown. The drug is rapidly distributed into extravascular space following IV administration. After completing an infusion, approximately 30% of the dose can be found in plasma. Following a 5 minute infusion, the distribution and elimination half-life is 13 and 85 minutes, respectively. Aldesleukin is eliminated by metabolism in the kidneys through both glomerular filtration and peritubular extraction with little or no active protein excreted in the urine. Greater than 80% of the amount of Aldesleukin distributed to plasma, cleared from the circulation and presented to the kidney is metabolized to amino acids in the cells lining the proximal convoluted tubules. In humans, the mean clearance rate in cancer patients is 268 mL/min. Clearance is preserved in patients with rising serum creatinine values.
### Toxicity of aldesleukin (IL-2):

<table>
<thead>
<tr>
<th>Frequency and Timing</th>
<th>Common</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Happens to 21-100 subjects out of every 100</td>
</tr>
<tr>
<td>Immediate:</td>
<td></td>
</tr>
<tr>
<td>Within 1-2 days of receiving drug</td>
<td>Fever, chills, malaise, fatigue, flu-like syndrome, diarrhea, rash, pruritus, hypotension, peripheral edema, oliguria</td>
</tr>
<tr>
<td>Prompt:</td>
<td></td>
</tr>
<tr>
<td>Within 2-3 weeks, prior to next course</td>
<td>Asthenia, anorexia, eosinophilia, leucocytosis, anemia, thrombocytopenia, elevated bilirubin, elevated creatinine, ↑SGOT</td>
</tr>
<tr>
<td>Delayed:</td>
<td></td>
</tr>
<tr>
<td>Any time later during therapy, excluding the above conditions</td>
<td>Aldesleukin has been shown to have embryolethal effects in rats when given in doses at 27 to 36 times the human dose (scaled by body weight). Significant maternal toxicities were observed in pregnant rats administered IV doses 2.1 to 36 times higher than the human dose during the critical period of organogenesis. No evidence of teratogenicity was observed other than that attributed to maternal toxicity. It is not known whether this drug is excreted in human milk.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occasional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Happens to 5-20 subjects out of every 100</td>
</tr>
<tr>
<td>Capillary leak syndrome, tachycardia, arrhythmia, acidosis with compensatory alkalosis, vomiting, nausea, hypo-magnesemia, hypocalcemia, hypophosphatemia, dizziness, cough, rhinitis, insomnia, confusion, somnolence, anxiety, lymphopenia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Happens to &lt;5 subjects out of every 100</td>
</tr>
<tr>
<td>Hypersensitivity reactions, stupor, coma, apnea, dyspnea, hypotension (Grade 4), seizures, myocardial infarction, angina, sudden death, myocarditis, headache, paranoid reaction, psychosis,</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prompt</th>
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</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Asthenia, anorexia, eosinophilia, leucocytosis, anemia, thrombocytopenia, elevated bilirubin, elevated creatinine, ↑SGOT</td>
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<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
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<td>Exacerbation of auto-immune disease, thyroid disorders</td>
</tr>
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<tbody>
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</tr>
</tbody>
</table>
Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
Interleukin-2 (NSC 373364)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with **bold** and *italicized* text. This subset of AEs (ASAEL) contains events that are considered ‘expected’ for expedited reporting purposes only. Refer to the ‘CTEP, NCI Guidelines: Adverse Event Reporting Requirements’ [http://ctep.info.nih.gov/protocolDevelopment/default.htm#adverse_events_aeers](http://ctep.info.nih.gov/protocolDevelopment/default.htm#adverse_events_aeers) for further clarification. The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information. Below is the CAEPR for interleukin-2.

<table>
<thead>
<tr>
<th>Adverse Events with Possible Relationship to Interleukin-2 (CTCAE 4.0 Term)</th>
<th>EXPECTED AEs FOR ADEERS REPORTING</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BLOOD AND LYMPHATIC SYSTEM DISORDERS</strong></td>
<td>Agent Specific Adverse Event List (ASAEL)</td>
</tr>
<tr>
<td></td>
<td><strong>Expected</strong></td>
</tr>
<tr>
<td>Anemia</td>
<td>Anemia</td>
</tr>
<tr>
<td><strong>CARDIAC DISORDERS</strong></td>
<td></td>
</tr>
<tr>
<td>Conduction disorder</td>
<td>Conduction disorder</td>
</tr>
<tr>
<td>Left ventricular systolic dysfunction</td>
<td><em>Left ventricular systolic dysfunction</em></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td></td>
</tr>
<tr>
<td>Myocarditis</td>
<td>Myocarditis</td>
</tr>
<tr>
<td>Palpitations</td>
<td>Palpitations</td>
</tr>
<tr>
<td>Sinus tachycardia</td>
<td>Sinus tachycardia</td>
</tr>
<tr>
<td>Supraventricular tachycardia</td>
<td>Supraventricular tachycardia</td>
</tr>
<tr>
<td>Ventricular arrhythmia</td>
<td>Ventricular arrhythmia</td>
</tr>
<tr>
<td><strong>EAR AND LABYRINTH DISORDERS</strong></td>
<td></td>
</tr>
<tr>
<td>Hearing impaired</td>
<td>Hearing impaired</td>
</tr>
<tr>
<td><strong>EYE DISORDERS</strong></td>
<td></td>
</tr>
<tr>
<td>Blurred vision</td>
<td>Blurred vision</td>
</tr>
<tr>
<td><strong>GASTROINTESTINAL DISORDERS</strong></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>Dry mouth</td>
</tr>
<tr>
<td>Gastrointestinal disorders - Other (Perforation, GI – Select)</td>
<td>Gastrointestinal disorders - Other (small bowel fistula)</td>
</tr>
<tr>
<td>Gastrointestinal disorders - Other (small bowel fistula)</td>
<td></td>
</tr>
<tr>
<td>Mucositis oral</td>
<td>Mucositis oral</td>
</tr>
<tr>
<td>Nausea</td>
<td>Nausea</td>
</tr>
<tr>
<td>Vomiting</td>
<td>Vomiting</td>
</tr>
<tr>
<td><strong>GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS</strong></td>
<td></td>
</tr>
<tr>
<td>Chills</td>
<td>Chills</td>
</tr>
<tr>
<td>Edema limbs</td>
<td>Edema limbs</td>
</tr>
<tr>
<td>Adverse Events with Possible Relationship to Interleukin-2 (CTCAE 4.0 Term)</td>
<td>EXPECTED AEs FOR ADEERS REPORTING</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Fatigue</strong></td>
<td><strong>Fatigue</strong></td>
</tr>
<tr>
<td><strong>Fever</strong></td>
<td><strong>Fever</strong></td>
</tr>
<tr>
<td>IMMUNE SYSTEM DISORDERS</td>
<td>IMMUNE SYSTEM DISORDERS</td>
</tr>
<tr>
<td>Allergic reaction</td>
<td>Allergic reaction</td>
</tr>
<tr>
<td>Autoimmune disorder</td>
<td>Autoimmune disorder</td>
</tr>
<tr>
<td>INFECTIONS AND INFESTATIONS</td>
<td>INFECTIONS AND INFESTATIONS</td>
</tr>
<tr>
<td>Infections and Infestations – Other (Infection – Select)</td>
<td>Infections and Infestations – Other (Infection – Select)</td>
</tr>
<tr>
<td>INVESTIGATIONS</td>
<td>INVESTIGATIONS</td>
</tr>
<tr>
<td>Activated partial thromboplastin time prolonged</td>
<td>Activated partial thromboplastin time prolonged</td>
</tr>
<tr>
<td>Alanine aminotransferase increased</td>
<td>Alanine aminotransferase increased</td>
</tr>
<tr>
<td>Alkaline phosphatase increased</td>
<td>Alkaline phosphatase increased</td>
</tr>
<tr>
<td>Aspartate aminotransferase increased</td>
<td>Aspartate aminotransferase increased</td>
</tr>
<tr>
<td>Blood bilirubin increased</td>
<td>Blood bilirubin increased</td>
</tr>
<tr>
<td>Creatinine increased</td>
<td>Creatinine increased</td>
</tr>
<tr>
<td>GGT increased</td>
<td>GGT increased</td>
</tr>
<tr>
<td>INR increased</td>
<td>INR increased</td>
</tr>
<tr>
<td>Platelet count decreased</td>
<td>Platelet count decreased</td>
</tr>
<tr>
<td>Weight gain</td>
<td>Weight gain</td>
</tr>
<tr>
<td>White blood cell decreased</td>
<td>White blood cell decreased</td>
</tr>
<tr>
<td>METABOLISM AND NUTRITION DISORDERS</td>
<td>METABOLISM AND NUTRITION DISORDERS</td>
</tr>
<tr>
<td>Acidosis</td>
<td>Acidosis</td>
</tr>
<tr>
<td>Anorexia</td>
<td>Hyperuricemia</td>
</tr>
<tr>
<td>Hyperuricemia</td>
<td>Hypocalcemia</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>Hypoglycemia</td>
</tr>
<tr>
<td>Hypomagnesemia</td>
<td>Hyponatremia</td>
</tr>
<tr>
<td>Hypotension</td>
<td>MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>Arthralgia</td>
</tr>
<tr>
<td>Myalgia</td>
<td>Myalgia</td>
</tr>
<tr>
<td>Myositis</td>
<td>Myositis</td>
</tr>
<tr>
<td>NERVOUS SYSTEM DISORDERS</td>
<td>NERVOUS SYSTEM DISORDERS</td>
</tr>
<tr>
<td>Depressed level of consciousness</td>
<td>Depressed level of consciousness</td>
</tr>
<tr>
<td>Dizziness</td>
<td>Dizziness</td>
</tr>
<tr>
<td>Headache</td>
<td>Headache</td>
</tr>
<tr>
<td>Memory impairment</td>
<td>Memory impairment</td>
</tr>
<tr>
<td>Nervous system disorders - Other (sleep disturbances)</td>
<td>Nervous system disorders - Other (sleep disturbances)</td>
</tr>
<tr>
<td>Peripheral sensory neuropathy</td>
<td>Peripheral sensory neuropathy</td>
</tr>
<tr>
<td>Seizure</td>
<td>Seizure</td>
</tr>
<tr>
<td>PSYCHIATRIC DISORDERS</td>
<td>PSYCHIATRIC DISORDERS</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>
### Adverse Events with Possible Relationship to Interleukin-2 (CTCAE 4.0 Term)

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>EXPECTED AEs FOR ADEERS REPORTING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety</td>
<td></td>
</tr>
<tr>
<td>Confusion</td>
<td>Confusion</td>
</tr>
<tr>
<td>Personality change</td>
<td>Personality change</td>
</tr>
<tr>
<td>Psychosis</td>
<td>Psychosis</td>
</tr>
</tbody>
</table>

| Renal and Urinary Disorders                        |                                   |
|----------------------------------------------------|                                   |
| Acute kidney injury                                |                                   |

| Respiratory, Thoracic and Mediastinal Disorders    |                                   |
|----------------------------------------------------|                                   |
| Adult respiratory distress syndrome                | Adult respiratory distress syndrome|
| Allergic rhinitis                                  | Allergic rhinitis                 |
| Apnea                                              |                                    |
| Cough                                              |                                    |
| Dyspnea                                            | Dyspnea                           |

| Skin and Subcutaneous Tissue Disorders             |                                   |
|----------------------------------------------------|                                   |
| Alopecia                                           | Alopecia                          |
| Pruritus                                           | Pruritus                           |
| Rash maculo-papular                                | Rash maculo-papular               |

| Vascular Disorders                                 |                                   |
|----------------------------------------------------|                                   |
| Capillary leak syndrome                            | Capillary leak syndrome           |
| Hypotension                                        | Hypotension                        |
| Peripheral ischemia                                |                                    |
| Thromboembolic event                               |                                    |
| Vascular disorders - Other (vasodilation)          |                                    |

1 This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

Also reported on interleukin-2 trials but with the relationship to interleukin-2 still undetermined:
- **Cardiac Disorders** - Mobitz type I; Pericardial effusion
- **Eye Disorders** - Eye disorders - Other (mydriasis)
- **Gastrointestinal Disorders** - Gastrointestinal disorders – Other (Hemorrhage, GI – Select); Pancreatitis
- **General Disorders and Administration Site Conditions** - General disorders and administration site conditions - Other (phospholipid syndrome); Hypothermia; Multi-organ failure
- **Infections and Infestations** – Infections and infestations – Other (Infection documented clinically or microbiologically with Grade 3 or 4 neutrophils [ANC <1.0 x 10^9/L]) – Select
- **Musculoskeletal and Connective Tissue Disorders** - Soft tissue necrosis lower limb
- **Nervous System Disorders** - Encephalopathy; Syncope
- **Psychiatric Disorders** - Agitation
- **Renal and Urinary Disorders** - Renal and urinary disorders - Other (acute tubular necrosis)
- **Respiratory, Thoracic and Mediastinal Disorders** - Bronchopulmonary hemorrhage; Bronchospasm; Hypoxia; Pneumothorax; Pulmonary edema
- **Vascular Disorders** - Phlebitis
**Note:** Interleukin-2 in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

**Formulation and Stability:** Aldesleukin is supplied as a sterile, white to off-white, lyophilized cake in single-use vials. Each vial contains 22 million international units (MIU). When reconstituted with 1.2 mL Sterile Water for Injection, USP, each mL contains 18 million IU (1.1 mg) Aldesleukin, 50 mg mannitol, and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The manufacturing process for Aldesleukin involves fermentation in a defined medium containing tetracycline hydrochloride. The presence of the antibiotic is not detectable in the final product. Aldesleukin contains no preservatives. Store refrigerated at 2° to 8°C (36° to 46°F).

Reconstitute the 22 MIU vial with 1.2 mL of sterile water **without preservative** for Injection, USP, to achieve a final concentration of 18 MIU/mL or 1.1mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. After reconstitution and dilution, store in a refrigerator at 2° to 8°C (36° to 46°F). Do not freeze. Administer aldesleukin within 48 hours of reconstitution. The solution should be brought to room temperature prior to infusion in the patient.

**Guidelines for Administration:** See [Treatment](#) and [Dose Modification](#) sections of the protocol.

Reconstituted aldesleukin should be further diluted as instructed below. **Use only Dextrose 5%**.

Due to the low doses of aldesleukin used in this protocol, a final volume must be chosen that assures the final concentration is within a stable range (see chart below). Most doses will need to be delivered on a low volume ambulatory pump such as the CADD pump. The instructions are separated depending on whether the dose will be delivered via conventional pump in intravenous bag (e.g.: Viaflex™) for larger patients, or via a CADD-like pump.

**Final concentrations have been converted to MIU from micrograms to decrease the risk of calculation errors.** Avoid final concentrations in the range of 1.13-1.6 MIU/mL where the stability is variable.

<table>
<thead>
<tr>
<th>Final Concentration</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.016-1.12 MIU/mL</td>
<td>Stable with the addition of 0.1% albumin as below</td>
</tr>
<tr>
<td>1.61 – 9 MIU/mL</td>
<td>Does not require addition of albumin</td>
</tr>
</tbody>
</table>

**Viaflex™ Bag Instructions:**

When preparing the product, the following order should be used:

1. Determine the final concentration/final volume to be used (see above). The following are standard volumes that achieve an even rate for a continuous 24 hour infusion.

<table>
<thead>
<tr>
<th>Final Volume</th>
<th>12 mLs</th>
<th>24 mLs</th>
<th>48 mLs</th>
<th>72 mLs</th>
<th>96 mLs</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hour supply</td>
<td>0.5 mL/hr</td>
<td>1 mL/hr</td>
<td>2 mL/hr</td>
<td>3 mL/hr</td>
<td>4 mL/hr</td>
</tr>
<tr>
<td>48 hour supply</td>
<td>0.25 mL/hr</td>
<td>0.5 mL/hr</td>
<td>1 mL/hr</td>
<td>1.5 mL/hr</td>
<td>2 mL/hr</td>
</tr>
</tbody>
</table>

2. Determine amount of 5% dextrose needed and place in empty intravenous bag (e.g.: Viaflex™).
3. Add albumin to achieve final concentration of 0.1% as necessary (either 5% or 25% albumin may be used).

<table>
<thead>
<tr>
<th>Final Volume</th>
<th>12 mLs</th>
<th>24 mLs</th>
<th>48 mLs</th>
<th>72 mLs</th>
<th>96 mLs</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% albumin</td>
<td>0.25 mL</td>
<td>0.5 mL</td>
<td>1 mL</td>
<td>1.5 mL</td>
<td>2 mL</td>
</tr>
<tr>
<td>25% albumin</td>
<td>NA</td>
<td>0.1 mL</td>
<td>0.2 mL</td>
<td>0.3 mL</td>
<td>0.4 mL</td>
</tr>
</tbody>
</table>

4. Add aldesleukin last.

**CADD Cassette Instructions:**
When preparing the product, the following order should be used:
1. Determine the final concentration/final volume to be used (see above). For CADD pump administration, the entire 4 days of dosing can be prepared as a single cassette. CADD pumps are programmed as rate per day (e.g. final volume of 96 mLs to run at 24 mLs/day x 4 days).

<table>
<thead>
<tr>
<th>Final Volume</th>
<th>12 mLs</th>
<th>24 mLs</th>
<th>48 mLs</th>
<th>72 mLs</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 hour supply</td>
<td>3 mLs/day</td>
<td>6 mLs/day</td>
<td>12 mLs/day</td>
<td>18 mLs/day</td>
</tr>
<tr>
<td>96 mLs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Determine amount of 5% dextrose needed and place in CADD cassette.
3. Add albumin to achieve final concentration of 0.1% as necessary (either 5% or 25% albumin can be used).

<table>
<thead>
<tr>
<th>Final Volume</th>
<th>12 mLs</th>
<th>24 mLs</th>
<th>48 mLs</th>
<th>72 mLs</th>
<th>96 mLs</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% albumin</td>
<td>0.25 mL</td>
<td>0.5 mL</td>
<td>1 mL</td>
<td>1.5 mL</td>
<td>2 mL</td>
</tr>
<tr>
<td>25% albumin</td>
<td>NA</td>
<td>0.1 mL</td>
<td>0.2 mL</td>
<td>0.3 mL</td>
<td>0.4 mL</td>
</tr>
</tbody>
</table>

5. Add aldesleukin last.
6. Remove any air from the cassette as necessary.

Bring the final preparation to room temperature prior to administration. In-line filters should not be used when administering aldesleukin.

Lot number information will be collected on the NCI Drug Accountability Record Form (DARF) (see the **Agent Accountability** section below).

**Stability:** The above dilutions are stable for 6 days at room temperature in a CADD cassette pump. The above dilutions are stable in intravenous bags (e.g.: Viaflex™) for 48 hours at room temperature or refrigerated.

**Supplier:** Commercially available. See package insert for further information.

**Agent Accountability**
The investigator, or a responsible party designated by the investigator, must maintain a careful record of the lot numbers of drug dispensed to study patients of all commercial supplies of aldesleukin using the NCI Drug Accountability Record Form (DARF). Access [http://ctep.cancer.gov/forms/default.htm](http://ctep.cancer.gov/forms/default.htm) to obtain a copy of the DARF.

### 4.0 ELIGIBILITY REQUIREMENTS

**Important note:** The eligibility criteria listed below are interpreted literally and cannot be waived (per COG policy posted 5/11/01). All clinical and laboratory data required for determining eligibility of a patient enrolled on this trial must be available in the patient's medical/research record which will serve as the source document for verification at the time of audit.

The following criteria are required for all newly enrolled patients of this study.

Enrollment on A3973, ANBL0532 or ANBL00B1 is no longer an eligibility requirement for ANBL0032.
4.1 All patients must be diagnosed with neuroblastoma, and categorized as high risk at the time of diagnosis and must be \( \leq 30.99 \) years of age at diagnosis. Exception: patients who are initially diagnosed as non-high-risk neuroblastoma, but later converted (and/or relapsed) to high risk neuroblastoma are also eligible.

4.2 All patients must have completed therapy including intensive induction followed by ASCT and radiotherapy to be eligible for ANBL0032. Radiotherapy may be waived for patients who either have small adrenal masses which are completely resected up front, or who never have an identifiable primary tumor. Please notify Study Chairs. Examples of such therapies include:

- Following treatment per A3973 protocol
- Following treatment per POG 9341/9342 protocol
- Following treatment per CCG3891
- Following treatment on NANT 2001-02
- Enrollment on or following treatment per ANBL02P1
- Enrollment on or following treatment per ANBL07P1

Tandem transplant patients are eligible:

1) Following treatment on or per ANBL0532;
2) Following treatment per POG 9640;
3) Following treatment per COG ANBL00P1; or,
4) Following treatment per CHP 594/DFCI 34-DAT.

4.3 No more than 12 months from the date of starting the first induction chemotherapy after diagnosis to the date of ASCT except for the rare occasions as noted below. For tandem ASCT patients, this will be the date of the FIRST stem cell infusion. Exception: For those who are initially diagnosed as non-high risk neuroblastoma, but later converted (and/or relapsed) to high risk neuroblastoma, the 12 months restriction should start from the date of induction therapy for high risk neuroblastoma (not from the initial induction therapy for non-high risk disease), to the date of ASCT.

4.4 At pre-ASCT evaluation patients must meet the International Neuroblastoma Response Criteria (INRC) for CR, VGPR, or PR for primary site, soft tissue metastases and bone metastases. Patients who meet those criteria must also meet the protocol specified criteria for bone marrow response as outlined below:

- \( \leq 10\% \) tumor (of total nucleated cellular content) seen on any specimen from a bilateral bone marrow aspirate/biopsy.
- Patient who have no tumor seen on the prior bone marrow, and then have \( \leq 10\% \) tumor on any of the bilateral marrow aspirate/biopsy specimens done at pre-ASCT and/or pre-enrollment evaluation will also be eligible (note that per INRC this would have been defined as “overall” response of PD).

4.5 Pre-enrollment tumor survey:

Prior to enrollment on ANBL0032, a determination of mandatory disease staging must be performed (tumor imaging studies including CT or MRI, MIBG scan, bone marrow aspiration & biopsy). This disease assessment is required for eligibility and should be done preferably within 2 weeks, but must be done within a maximum of 4 weeks before enrollment.

- For those with residual disease before radiotherapy, re-evaluation of irradiated residual tumors is preferably performed at the earliest 5 days after completing radiotherapy. Patients with residual
disease are eligible. Biopsy is not required. Patients who have biopsy proven residual disease after ASCT will be enrolled on Stratum 07.

- Patients must not have progressive disease at the time of study enrollment except for protocol specified bone marrow response as defined in Section 4.4. These patients will be enrolled on Stratum 07.

4.6 **Patients must be enrolled before treatment begins. The date protocol therapy is projected to start must be no later than ten (10) calendar days after the date of study enrollment.**

Patients should be enrolled preferably between Day 56 and Day 85 after PBSC infusion (day from 2nd stem cell infusion for tandem transplant). Patients must be enrolled no later than Day 100 after PBSC infusion. Enrollment must occur after completion of radiotherapy, and after completion of tumor assessment post-ASCT and radiotherapy. Informed consent should be obtained within 3 weeks pre-ASCT up to the time of registration.

All clinical and laboratory studies for organ functions to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated in Section 4.9 below.

4.7 Patients must not have received prior anti-GD2 antibody therapy

4.8 Patients must have a Lansky or Karnofsky Performance Scale score of $\geq 50\%$ (see Appendix I) and patients must have a life expectancy of $\geq 2$ months.

4.9 Patients must have adequate organ functions at the time of registration:

**Hematological:**
Total absolute phagocyte count (APC = neutrophils + monocytes) is at least 1000/µL

**Renal-**
Adequate Renal Function Defined As:
- Creatinine clearance or radioisotope GFR $\geq 70$ mL/min/1.73 m$^2$ or
- A serum creatinine based on age/gender as follows:

<table>
<thead>
<tr>
<th>Age</th>
<th>Maximum Serum Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>1 month to &lt; 6 months</td>
<td>0.4</td>
</tr>
<tr>
<td>6 months to &lt; 1 year</td>
<td>0.5</td>
</tr>
<tr>
<td>1 to &lt; 2 years</td>
<td>0.6</td>
</tr>
<tr>
<td>2 to &lt; 6 years</td>
<td>0.8</td>
</tr>
<tr>
<td>6 to &lt; 10 years</td>
<td>1</td>
</tr>
<tr>
<td>10 to &lt; 13 years</td>
<td>1.2</td>
</tr>
<tr>
<td>13 to &lt; 16 years</td>
<td>1.5</td>
</tr>
<tr>
<td>$\geq 16$ years</td>
<td>1.7</td>
</tr>
</tbody>
</table>

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

**Hepatic-** total bilirubin $\leq 1.5$ x normal, and SGPT (ALT) $\leq 5$ x normal. Veno-occlusive disease, if present, should be stable or improving.
Cardiac - shortening fraction of $\geq 30\%$ by echocardiogram, or if shortening fraction abnormal, ejection fraction of $\geq 55\%$ by gated radionuclide study or echocardiogram.

Note: The echocardiogram or gated radionuclide study must be performed within 4 weeks prior to enrollment.

Pulmonary - FEV$_1$ and FVC $> 60\%$ of predicted by pulmonary function test. For children who are unable to do PFTs, no evidence of dyspnea at rest and no exercise intolerance should be documented.

Note: The pulmonary function test must be performed within 4 weeks prior to enrollment.

Central nervous system - Patients with seizure disorder may be enrolled if on anticonvulsants and well-controlled. CNS toxicity $<$ Grade 2.

4.10
Written informed consent in accordance with institutional and FDA guidelines must be obtained from parent or legal guardian.

4.11
Females of childbearing potential must have a negative pregnancy test. Patients of childbearing potential must agree to use an effective birth control method. Female patients who are lactating must agree to stop breast-feeding.

5.0 REQUIRED OBSERVATIONS

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

5.1 Observations Prior to Starting Protocol Therapy

Optional tests:

5.1.1 Minimal Residual Disease (MRD) Evaluations of Blood and Bone Marrow (for those patients who consent to optional draw).

MRD assessment will be performed in Dr. Seeger’s laboratory at the following time points:

1) **MRD #1**: done preferably within 2 weeks but maximum 4 weeks before enrollment. An aliquot of bone-marrow samples (3-5 mL heparinized marrow from each site) obtained for tumor survey (see below Section 5.1.3), or separate marrow sample (if sufficient marrow not available), along with blood sample (10 mL heparinized peripheral blood) should be obtained prior to starting therapy and should be shipped to Dr. Seeger’s laboratory as MRD#1.

2) **MRD #2**: within 2 weeks of the completion of protocol therapy. Bone marrow and blood samples should be obtained within 2 weeks of completing the last course of ANBL0032 therapy and shipped to Dr. Seeger’s laboratory as MRD#2.
In addition, an optional MRD assessment will be performed when a bone marrow assessment is performed during follow-up after completion of protocol therapy and at relapse, when applicable (see Section 5.4).

For sending BM and blood specimens to Dr. Seeger prior to study enrollment, please print out the ANBL0032 Specimen shipping form, complete it and send with the specimen. Please note that consent must be obtained prior to sending the specimen for MRD #1. For MRD #2, complete the ANBL0032 Specimen shipping form online and send a copy with the specimen. See Section 5.6.1 for specimen acquisition and shipping procedures.

5.1.2 FcR and KIR/KIR-L genotyping:
Blood samples will be performed for all consenting patients in this study within 1 week before starting the first GM-CSF injection. For further details on sample collection and shipping, refer to Section 5.6.2.

Required tests:

5.1.3 Mandatory Disease Staging
- Tumor measurement (CT and/or MRI, bone scan if tumor is not MIBG avid)
- MIBG scan*
- Bone marrow aspirates / biopsy from two sites (biopsy may be omitted if negative prior to ASCT)
*See MIBG scan guidelines in Appendix II.

5.1.4 Physical exam and blood/urine laboratory tests
Physical Exam (including weight, height, vital signs, and performance status [see Appendix I]), CBC and differential with APC, chemistry survey (electrolytes, BUN, creatinine, albumin, AST, ALT, total bilirubin, LDH), urinary VMA, HVA, Urinalysis, Thyroxine and TSH within 1 week before starting protocol therapy.

5.1.5 ch14.18 PK and HACA
Ch14.18 PK and HACA analysis will be done ONLY for patients participating in the cardiac safety sub-study (details of which are provided in Section 5.6.6). Samples will be collected before starting immunotherapy and at subsequent time-points that coincide with the ECG monitoring studies. For further details on sample collection and shipping, refer to Section 5.6.3.
### 5.2 Evaluation during Isotretinoin (13-Cis-Retinoic Acid, or RA) Therapy (Regimen A): Closed to accrual as of amendment #9.

<table>
<thead>
<tr>
<th>Days post-ASCT</th>
<th>Prior to RA</th>
<th>66 (Monday)</th>
<th>On Day 14 of cycle #1 of Cis RA</th>
<th>94</th>
<th>122</th>
<th>150</th>
<th>178</th>
<th>206</th>
<th>234</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start 1\textsuperscript{st} cycle of RA</td>
<td>Start 2\textsuperscript{nd} cycle of RA</td>
<td>Start 3\textsuperscript{rd} cycle of RA</td>
<td>Start 4\textsuperscript{th} cycle of RA</td>
<td>Start 5\textsuperscript{th} cycle of RA</td>
<td>Start 6\textsuperscript{th} cycle of RA</td>
<td>2 weeks after last dose of RA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE\textsuperscript{1}</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBC/Diff\textsuperscript{2}</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemistry, urinary VMA, HVA\textsuperscript{3}</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yu’s Lab ADCC\textsuperscript{4}</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow aspirates/ biopsies (2 sites)\textsuperscript{4}</td>
<td>X\textsuperscript{**}</td>
<td>X\textsuperscript{**} (optional)</td>
<td>X\textsuperscript{*}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor survey\textsuperscript{5}</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIBG scan</td>
<td>X\textsuperscript{*}</td>
<td>X (optional)</td>
<td>X\textsuperscript{*}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-cis retinoic acid pharmacokinetics\textsuperscript{6}</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1} MIBG, bone marrow and blood mandatory for MRD assessment.

\textsuperscript{2} Bone marrow and blood are optional for Day 150 and are only done for patient care. The bone marrow aspirates and biopsies are not for submission to Seeger’s lab.

\textsuperscript{3} Physical Exam (including weight, height, vital signs, and performance status [see Appendix I]) before each cycle.

\textsuperscript{4} Chemistry survey (electrolytes, calcium, BUN, creatinine, triglycerides, AST, ALT, albumin, total bilirubin, LDH) and urinary VMA and HVA before each cycle.

\textsuperscript{5} Yu’s lab: 15 mL blood in preservative-free heparin or green-top tube obtained before starting 1\textsuperscript{st} and 4\textsuperscript{th} courses of RA and 2 weeks after the last dose of RA (to be drawn Monday through Thursday, avoid Friday). Freshly obtained samples should be sent at room temperature by overnight carrier. See Section 5.6.2.

\textsuperscript{6} Only the MRD studies in the two time points marked mandatory have to be sent to Dr. Seeger’s lab. 3-5 mL heparinized marrow from each site plus 10 mL heparinized peripheral blood needs to be submitted. (Also see Section 5.6.1). For MRD #1 assessment prior to enrollment, please print the ANBL0032 Specimen Shipping Form, complete it and send with specimen. Please note that consent must be obtained prior to sending the specimen for MRD #1.

\textsuperscript{1} Before starting fourth cycle of RA Therapy and after six cycles of Cis-RA:

- Tumor measurement by pertinent imaging studies (CT, MRI, bone scan if tumor is not MIBG avid, etc).

\textsuperscript{5} For patients consenting to pharmacokinetics study, send 5 mL blood in sodium heparin to be drawn 4 hours after administration of the 13-cis retinoic acid dose on Day 14 of cycle #1. (See section 5.7 for details of specimen submission.)
5.3 **Evaluations During Immunotherapy (Regimen B)**

5.3.1 **During the 1st, 3rd and 5th Courses (Ch14.18 + GM-CSF):** (in case of Grade 4 hypersensitivity reaction at any time, please see footnote below***)

**Courses #1, 3, 5:** Day 0 is designated as the day of 1st dose of GM-CSF for the 1st course of immunotherapy (see immunotherapy calendar in Section 6.1). Please note: laboratory tests used to determine eligibility may be counted as the Prior to Immunotherapy or Day -1 studies if the tests were performed within 1 week prior to starting protocol therapy.

<table>
<thead>
<tr>
<th>Days post GM-CSF for Courses 1, 3, 5</th>
<th>Prior to Immunotherapy</th>
<th>1st day of ch14.18 GM-CSF</th>
<th>2nd day of ch14.18 GM-CSF</th>
<th>3rd day of ch14.18 GM-CSF</th>
<th>4th day of ch14.18 GM-CSF</th>
<th>GM-CSF Start RA</th>
<th>GM-CSF ends, continue RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1, 55, 111 (Thursday)</td>
<td>The day before GM-CSF</td>
<td>1st day of ch14.18 GM-CSF</td>
<td>2nd day of ch14.18 GM-CSF</td>
<td>3rd day of ch14.18 GM-CSF</td>
<td>4th day of ch14.18 GM-CSF</td>
<td>GM-CSF Start RA</td>
<td>GM-CSF ends, continue RA</td>
</tr>
<tr>
<td>3, 59, 115</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4, 60, 116</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5, 61, 117</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6, 62, 118</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 (Courses 3 and 5 only) or 11 (Course 1), 66, 122 (Monday)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13, 69, 125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 (Monday, 1st course only)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| PE†                                  | X                      | X                         | X                         | X                         | X                         |                       |                          |
| CBC/Diff with APC                    | X                      | X                         |                           |                           |                           |                       |                          |
| BUN, creatinine, AST, ALT, albumin, total bili | X                     |                           |                           |                           |                           |                       |                          |
| Electrolytes                         | X                      | X’ (Day -1 only)          | X’ (Day 6 only)           |                           |                           |                       |                          |
| LDH                                  | X                      |                           |                           |                           |                           |                       |                          |
| Calcium, triglycerides               |                        |                           |                           |                           |                           |                       | X                        |
| Urine VMA, HVA                       | X                      |                           |                           |                           |                           |                       |                          |
| Urinalysis                           | X                      |                           |                           |                           |                           |                       |                          |
| Thyroxine, TSH                       | X                      |                           |                           |                           |                           |                       |                          |
| ch14.18 PK and HACA²                 | X*                     |                           |                           |                           |                           |                       | Optional                 |
| Tumor Survey                         | X*                     |                           |                           |                           |                           |                       |                          |
| MIBG scan, Bone marrow aspirates/ biopsies (2 sites), and MRD testing³ | X*                     |                           |                           |                           |                           |                       |                          |
| 13-cis retinoic acid pharmacokinetics⁴ |                        |                           |                           |                           |                           |                       | X                        |
| ECG Assessments                      | X’ (Day -1 only)       |                           |                           | X’ (Day 6 only)           |                           |                       |                          |
| FcR, KIR/KIR-L genotyping            | X⁰                     |                           |                           |                           |                           |                       |                          |

* Days as designated in Immunotherapy Calendars

† Tumor survey, MIBG, bone marrow for mandatory disease staging prior to enrollment. Tumor measurement by pertinent imaging studies (CT, MRI, bone scan if tumor is not MIBG avid). Repeat MIBG later in therapy only if positive at diagnosis.

1 Physical Exam (including weight, height, vital signs, and performance status [see Appendix I]). Note: performance status and height are only required at the start of each course.

2 ch14.18 PK and HACA (ONLY for patients participating in the optional cardiac safety sub-study): 5 mL blood in preservative-free heparin or green-top tube for immunotherapy Course #1: Day -1 (1 day before starting 1st dose of GM-CSF, on a Thursday) and Day 6 (at the end of the 4th daily infusion of ch14.18). Freshly obtained samples should be sent at room temperature by overnight carrier.

3 MRD testing (Optional): 3-5 mL heparinized marrow from each site plus 10 mL heparinized peripheral blood to Dr. Seeger's lab (also see Section 5.6.1). For MRD #1 assessment prior to study enrollment, please use the ANBL0032 Specimen Shipping Form, complete it and send with the specimen. Please note that consent must be obtained prior to sending the specimen for MRD #1.

4 cisRA pharmacokinetics (Optional): Send 5 mL blood in sodium heparin to be drawn 4 hours after administration of the 13-cis retinoic acid dose on Day 14 of cis-RA administration in Course #1 to Reynolds’ lab. (See Section 5.6.5 for details of specimen submission.)

⁰ Only for patients participating in the optional cardiac safety sub-study, 12-lead ECGs will be obtained at Course #1: Day -1 (1 day before starting 1st dose of GM-CSF, on a Thursday) and Day 6 (at the end of the 4th daily infusion of ch14.18).
5. FcR and Kir genotyping (optional): 7 mL heparinized blood on Day -1 of Course 1 only; note: may be obtained within 1 week before starting the first GM-CSF injection.
6. Include evaluation of phosphorous and magnesium on Day-1 and Days 3-6 of Courses 1, 3 and 5. Note: Electrolytes including phosphorous and magnesium will also be evaluated on Day 7 of Courses 1, 3 and 5.

** In case of Grade 4 hypersensitivity reactions at any time, please order the following lab tests and send the results to Dr. Alice Yu by email (aliceyu@ucsd.edu) and also include the results in AdEERS: Complement levels (C3, C4, CH50 and c1q binding); IgE level, blood levels of tryptase and histamine (to be done locally); In addition, please draw 5 mL heparinized blood and send to the bioanalytical laboratory (see address information in Section 5.6.3) for HACA determination. The leftover ch14.18 vials should be shipped for inspection per instruction in Section 5.6.4.

Note: Hypersensitivity reaction will be referred as ‘allergic reaction and anaphylaxis’ per CTCAE v4.0 followed from October 1st, 2011.

5.3.2 During the 2nd and 4th Courses (Aldesleukin (IL-2) + Ch 14.18): (in case of Grade 4 hypersensitivity reaction at any time, please see footnote below**)

<table>
<thead>
<tr>
<th>Courses #2 and 4:</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Days post GM-CSF for Courses 2 and 4</td>
<td>24, 80 (Monday)</td>
<td>27, 83</td>
<td>31, 87 (Monday)</td>
<td>32, 88</td>
<td>33, 89</td>
<td>34, 90 (Thursday)</td>
<td>38, 94 (Monday)</td>
</tr>
<tr>
<td>1st day of the 1st week IL-2</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th day of the 1st week IL-2</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day of ch14.18 +IL-2</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd day of ch14.18 +IL-2</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd day of ch14.18 +IL-2</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th day of ch14.18 +IL-2</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start RA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Physical Exam (including weight, height, vital signs, and performance status [see Appendix I]). PE is required on Days 1, 2 & 3 only when IL-2 is given in an in-patient setting. Note: performance status and height are only required at the start of each course.

2. ch14.18 PK and HACA (ONLY for patients participating in the optional cardiac safety sub-study): 5 mL blood in preservative-free heparin or green-top tube obtained for the 4th course of immunotherapy, on the first day of the first 4-day/week Interleukin-2 therapy (Day 80, before starting aldesleukin (IL-2) infusion, on a Monday) and at the end of the 4th daily infusion of ch14.18 (Day 90). Freshly obtained samples should be sent at room temperature by overnight carrier.

3. Only prior to 4th course of immunotherapy.

4. Only for patients participating in the optional cardiac safety sub-study, 12-lead ECGs will be obtained for the 4th course of immunotherapy, on the first day of the first 4-day/week Interleukin-2 therapy (Day 80, before starting aldesleukin (IL-2) infusion, on a Monday) and at the end of the 4th daily infusion of ch14.18 (Day 90).

5. Include evaluation of phosphorous and magnesium on the days of ch14.18 infusion. Note: Electrolytes including phosphorous and magnesium will also be evaluated on the day after the last ch14.18 infusion of Courses 2 and 4.

** In case of Grade 4 hypersensitivity reactions at any time, please order the following lab tests and send the results to Dr. Alice Yu by email (aliceyu@ucsd.edu) and also include the results in AdEERS: Complement levels (C3, C4, CH50 and c1q binding); IgE level, blood levels of tryptase and histamine (to be done locally); In addition, please draw 5 mL heparinized blood and send to the bioanalytical laboratory (see address information in Section 5.6.3) for HACA determination. The leftover ch14.18 vials should be shipped for inspection per instruction in Section 5.6.4.

Note: Hypersensitivity reaction will be referred as ‘allergic reaction and anaphylaxis’ per CTCAE v4.0 followed from October 1st, 2011.
5.3.3 During and after completion of 6th course of RA:

<table>
<thead>
<tr>
<th>Days</th>
<th>During Course 6 (therapy starts on Day 14 of the course)</th>
<th>Within 1 week prior to administration</th>
<th>Within 2 weeks after completion of 6th course of RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE¹</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>CBC/Diff with APC</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>BUN, creatinine, AST, ALT, albumin, total bili</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Electrolytes</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Calcium, triglycerides</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Urine VMA, HVA</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Urinalysis</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Thyroxine, TSH</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>ch14.18 PK and HACA²</td>
<td>X²</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Tumor Survey</td>
<td>X*</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>MIBG scan³, Bone marrow aspires/ biopsies (2 sites) and MRD testing¹</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>ECG Assessments²</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

* Tumor survey, MIBG, bone marrow aspirates/biopsies for mandatory disease staging within 2 weeks of completion of the 6th course of RA. Tumor measurement by pertinent imaging studies (CT, MRI, bone scan if tumor is not MIBG avid)

¹ Physical Exam (including weight, height, vital signs, and performance status [see Appendix I])

² ch14.18 PK and HACA (ONLY for patients participating in the optional cardiac safety sub-study): 5 mL blood in preservative-free heparin or green-top tube. Freshly obtained samples should be sent at room temperature by overnight carrier (see Section 5.6.3).

³ MRD testing (Optional): collection of 3-5 mL heparinized marrow from each site plus 10 mL heparinized peripheral blood shipped to Dr. Seeger's lab (also see Section 5.6.1). Please use the ANBL0032 Specimen Shipping Form, complete it and send with the specimen.

⁴ MIBG scans performed only if the tumor is MIBG avid at diagnosis.

⁵ Only for patients participating in the optional cardiac safety sub-study, 12-lead ECGs will be obtained within two weeks from study Day 163 (i.e. within 2 weeks from completion of 6th course of RA).
5.4 **Evaluations During Follow-Up after completion of Isotretinoin (13-Cis-Retinoic Acid) therapy and Immunotherapy:**

For patients receiving cis-retinoic acid ± immunotherapy, begin follow-up studies counting time 0 as date of disease evaluation after completion of last course of isotretinoin.

<table>
<thead>
<tr>
<th>Observation</th>
<th>3 Months</th>
<th>6 Months</th>
<th>9 Months</th>
<th>1 Year</th>
<th>1.5 Years</th>
<th>2 Years</th>
<th>2.5 Years</th>
<th>3 Years</th>
<th>3.5 Years</th>
<th>4 Years</th>
<th>4.5 Years</th>
<th>5 Years</th>
<th>Annually After 5 Years</th>
<th>At Relapse</th>
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<tbody>
<tr>
<td>Physical Exam%</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
<td>X (Asp &amp; BX)</td>
<td></td>
</tr>
<tr>
<td>Height, Weight%</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X&amp;</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X&amp;</td>
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<td>CBC with differential and platelets%</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
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<td>X (Asp &amp; BX)</td>
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</tr>
<tr>
<td>MUGA or ECHO, ECG, Pulmonary function tests</td>
<td>X+</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X (Asp &amp; BX)</td>
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<tr>
<td>Tumor Imaging$</td>
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<td>MIBG# (See Appendix II)</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Urine Catecholamines*</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>TSH, T4</td>
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<tr>
<td>HRQOL Questionnaire</td>
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<td></td>
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</tr>
<tr>
<td>Chemistry**</td>
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<td>X</td>
<td>X⊙</td>
<td>X</td>
<td>X⊙ (optional)</td>
<td></td>
</tr>
</tbody>
</table>

% For post-transplant patients perform physical exam and CBC monthly for one year after transplant.

* Perform if positive at diagnosis.

# Bone marrow evaluations, MIBG scans and bone scans every 3 months only if positive at completion of isotretinoin acid therapy. Repeat until at least three consecutive (immunocytologically) negative bone marrows or MIBG scans are documented. If BMA done it is optional to send 5 mL to Dr. Seeger's Lab for MRD analysis.

$ Perform as scheduled, then as clinically indicated.

+ If abnormal, repeat at 2 years and as needed. If child is < 5 years when treated as directed, an additional test should be performed when child becomes age 5.

## Send 5 mL sample of bone marrow aspirate to Dr. Seeger's Lab for MRD analysis – label “relapse”. This is an optional test.

& Perform sitting height and standing height, if child is ≥ 5 years.

^ Perform if child is ≥ 5 years. If child is < 5 years, delay evaluation until child becomes 5 years of age. If results are abnormal, follow-up with an endocrinologist is recommended and evaluations should be repeated one year later.

† Only patients enrolled on study A3973 will be required to complete the HRQOL questionnaire. Perform if child is ≥ 5 years. If child is < 5 years, delay giving questionnaire until child = 5 years of age. Evaluate HRQOL at 1 and 5 years off-therapy.

⊙ Evaluate yearly until 5 years after completion of therapy.

** Chemistry survey (electrolytes, calcium, BUN, creatinine, albumin, AST, ALT, total bilirubin, LDH) as indicated for good patient care.
5.5  **Health-Related Quality of Life (HRQOL) (part of A3973 protocol)**

5.5.1  Children who are at least 1 year off therapy in remission and at least 5 years of age are eligible for HRQOL evaluation. Institutions will be contacted by a study chair designee for the address and telephone number of the family. The family will then be contacted to request permission to complete the HRQOL questionnaire, including a parental/family coping survey followed by the Child Health Questionnaire when the child is at least 5 years of age. Only patients enrolled on the A3973 protocol will be required to complete the HRQOL questionnaire.

5.5.2  Late effects will be measured in children who are at least 1 year off therapy and at least 5 years of age. Pulmonary functions tests, cardiac function tests (EKG, MUGA or ECHO), thyroid function tests (T4, TSH), standing height, sitting height, and weight will be performed as scheduled in table 5.4. Recording of any second malignant neoplasms will also be done.

5.6  **Processing/Handling of Specimen Required for Special Studies**

5.6.1  **Minimal Residual Disease (MRD) Bone Marrow/Heparinized Blood Samples (Dr. Seeger's Lab.)**  
Bone marrow and blood sample collection for minimal residual disease assessment (optional test that requires patient’s consent):

3-5 mL from each posterior iliac crest in preservative-free heparin (100 units of heparin/mL of marrow), and 10 mL blood in preservative-free heparin (100 units of heparin/mL of blood). Freshly-obtained samples should be sent at room temperature by overnight carrier in sterile containers (e.g., tubes or syringes). EACH batch of specimens should be sent with a completed ANBL0032 Specimen Shipping form unless the first specimen happens to be collected prior to ANBL0032 enrollment. For sending pre-enrollment specimens (MRD #1) to Dr. Seeger, please print out the ANBL0032 specimen shipping form, complete it and send a paper copy with the specimen. Please note that consent must be obtained prior to sending specimens for MRD assessments.

**Shipping instructions and address:**

Samples will only be received Monday through Friday.

- EACH VIAL should be labeled with:
  1) Patient's COG registration number
  2) Specimen type - bone marrow vs. blood
  3) Date and time bone marrow/blood was drawn
  4) Course and day of therapy bone marrow/blood was drawn

Bone marrow samples should be sent at room temperature via Federal Express Priority Overnight (refer to [https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf](https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf)) to:

Robert C. Seeger, M.D. (Contact person:- Peter Wakamatsu)  
Children’s Hospital of Los Angeles  
Division of Hematology/Oncology, Mailstop #57  
4650 Sunset Blvd.  
Los Angeles, CA 90027  
Phone  (323) 361-5630  
FAX  (323) 361-3889
5.6.2 FcR and Kir genotyping (Dr. Yu's Lab) (optional test that requires patient’s consent):

Sample collection:
7 mL blood in preservative-free heparin (100 units/mL of blood) or green-top tube should be collected within 1 week before starting the first GM-CSF injection (on Monday through Thursday, to avoid Friday shipment) for FcR and Kir/Kir-L genotyping. Freshly-obtained samples should be sent at room temperature by overnight carrier in sterile containers (e.g., tubes or syringes). If it is impossible to avoid sample collection on a Friday, those samples may be kept at 4°C and shipped overnight on Monday with prior notification to lab (see contact details below).

Shipping instructions and address:
Samples will only be received Monday through Friday. Please notify by fax, phone or e-mail (yulab@ucsd.edu) and provide air bill number before shipping.

EACH VIAL should be labeled with:
1) Patient's COG registration number
2) Date and time blood was drawn
3) Course and day of therapy blood was drawn
4) AND EACH specimen should be sent with a completed ANBL0032 Specimen Shipping form.

Samples should be sent at room temperature via Federal Express Priority Overnight (refer to https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf) to:
Alice Yu, M.D., Ph.D. (Attention Lou Bridgeman)
UCSD Medical Center
Clinical Teaching Facility, B-114
212 W. Dickinson Street
San Diego, CA 92103-8447
Phone (858)-246-0027 (Office), (619)-543-2438 (Lab)
FAX (858)-246-0019 (Office), (619)-543-5413 (Lab)
E-mail: yulab@ucsd.edu

5.6.3 ch14.18 PK and HACA:
The ch14.18 PK and HACA tests are required ONLY for those patients participating in the cardiac safety sub-study.
These tests will be done at the following time-points (corresponding to the ECG monitoring tests mentioned in Section 5.6.6):
- Baseline prior to GM-CSF (DAY -1), {Cycle 1}
- Day 6 at the end of the ch14.18 infusion (C_{max}), {Cycle 1}
- Day 80 prior to IL-2 (trough), {Cycle 4}
- Day 90 at the end of the ch14.18 infusion (C_{max}), {Cycle 4}
- Study End: within 2 weeks of Day 163

Sample collection: 5 mL blood in preservative-free heparin or green-top tube should be collected for ch14.18 PK and HACA assays (100 units of heparin/mL of blood). Freshly-obtained samples should be sent at room temperature by overnight carrier in sterile containers (e.g., tubes or syringes). For those samples obtained from regimen B patients, plasma will be processed by the bioanalytical laboratory and analyzed for HACA and ch14.18 levels.
Shipping instructions and address:
When possible, samples should be shipped by FedEx priority overnight for central laboratory receipt Monday-Friday. If this is not possible, samples may be shipped overnight on Friday for central laboratory receipt on Saturday with 48 hours notification. For all sample shipments, please notify BRT by fax, phone or e-mail (tburleson@brt-labs.com) and provide the air bill number before shipping.

EACH VIAL should be labeled with:
1) Patient's COG registration number
2) Date and time blood was drawn
3) Course and day of therapy blood was drawn

AND EACH specimen should be sent with a completed ANBL0032 Specimen Shipping form.

Samples should be sent at room temperature via Federal Express Priority Overnight (4437-6232-9) to:
Burleson Research Technologies
120 First Flight Lane
Morrisville, NC 27560
Phone: (919) 719-2500
Fax: (919) 719-2505

5.6.4 Instructions for saving and shipping left-over ch14.18 vial in case of serious hypersensitivity reactions (‘allergic reactions and anaphylaxis’ per CTCAE v 4.0 followed from October 1st, 2011):
Vials (empty and partial) used to prepare each chimeric 14.18 infusion should be retained in the pharmacy and held at 2-8°C in quarantine for one week. The vials should be segregated and labeled with a unique identifier and date of infusion for later reference. If, after one week, the patient has not experienced a Grade 4 serious adverse event (SAE), the vials may be discarded per standard institutional policy. In the event of a Grade 4 SAE please contact the Biopharmaceutical Development Program (BDP) at 301-846-5369 for further instructions on where/how to ship the material for possible testing. Shipping instructions and pre-paid shippers will be provided for shipment to the appropriate facility. No shipment should occur until a discussion has taken place with the BDP.

5.6.5 13 Cis Retinoic Acid Pharmacokinetics and Pharmacogenomics (optional test that requires patient’s consent):
Sample Collection:
ALL SAMPLES MUST BE PROTECTED FROM LIGHT AT ALL TIMES BY WRAPPING IN FOIL IMMEDIATELY.
One 5mL blood sample will be obtained 4 hours after administration of 13-cis-RA on Day 14 of Course 1 of 13-cis-RA treatment. Unseparated blood and plasma must be protected from light at all times (i.e. wrapped in aluminium foil). The blood will be collected in a heparin tube and immediately chilled in an ice-water bath and plasma will be separated by low speed centrifugation (1500-2000 rpm for 15 minutes at 4°C). The plasma will be transferred to a polypropylene tube, immediately frozen and stored at – 20 °C wrapped in foil for pharmacokinetic drug analysis. The cell pellet (RBC and WBC) will be frozen and stored at –80 °C for the pharmacogenomic studies. Each tube must be labeled with patient identifier, study number, date and time the sample was drawn. Complete the relevant PK fields on the ANBL0032 Specimen Shipping Form RDE and ship with the samples. Data will also be collected to indicate whether 13-cis-RA was taken by capsule or out of capsule (i.e., capsules snipped and contents mixed with fatty food). Do not label the tubes or ANBL0032 Specimen Shipping form with the patient’s name.

Shipment of Samples
Send samples ON DRY ICE WRAPPED IN FOIL, (with a copy of the ANBL0032 Specimen Shipping Form) via Federal Express, Priority Overnight (refer
to [https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf](https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf), Monday through Thursday **WITHIN ONE MONTH OF OBTAINING SAMPLE** to:

C Patrick Reynolds, MD PhD  
Texas Tech UHSC – Amarillo,  
School of Medicine Cancer Center  
3601 4th Street  
Lubbock, Texas- 79430-6450  
**Phone:** (806) 743-1558  
**Fax:**  (806) 743-2691  
**Email:** Patrick.Reynolds@TTUHSC.edu  
**Lab phone:** (806) 743-2707  
**Contact in lab:** Tito Woodburn  
**Email:** tito.woodburn@ttuhsc.edu

Batch shipping of several patients is encouraged to decrease costs. Each batch of specimens should be sent with a completed ANBL0032 Specimen Shipping form. TTUHSC will collect and batch the samples, for PK analysis.

**Description of Assay**

Plasma concentrations of 13-cis-RA, ATRA and the metabolite 4-oxo-13-cis-RA, will be determined by HPLC analysis by Dr. Min Kang at Texas Tech University Health Sciences Center School of Medicine. DNA obtained from this sample will be genotyped for metabolizing enzymes thought to be involved in the metabolism of 13-cis-RA, such as CYP2C8 and CYP3A7, using PCR methodology.

5.6.6 **Twelve-Lead ECG in Cardiac Safety Sub-study**

This study will be performed at selected sites (listed in Section 11.2) and patient participation is optional.

**Cardiac sub-study eligibility criteria:**

For patients participating in the ECG cohort, the following criteria must be met for inclusion in the ECG analysis population.

- Patients must not have clinically significant cardiac disease (NYHA - class > 2) including unstable angina, acute myocardial infarction within six months of Day 1, congestive heart failure, and arrhythmia requiring therapy, with the exception of extrasystoles or minor conduction abnormalities.
- The baseline ECG QTcF must be ≤ 480 ms. If the screening QTcF is > 480 ms, this may be repeated once and if the QTcF is ≤ 480 ms, the patient will be included in the ECG cohort.

**ECG Monitoring time-points:**

Twelve lead ECGs will be obtained digitally using an ECG machine capable of recording 10 seconds of digital data, for all twelve leads, and the capability of transmitting that data to a central ECG laboratory. ECGs to be used in the analysis will be selected by predetermined time points as detailed below and will be read centrally using a high-resolution manual on-screen caliper semiautomatic method with annotations. In this trial, twelve-lead ECGs will be assessed at a subset of qualified study centers to allow for the collection of ECG data on a total of 60 evaluable subjects. Triplicate twelve-lead ECGs will be recorded after at least 5 minutes of rest in the semi-recumbent position at the following five time points:

- Baseline prior to GM-CSF (DAY -1), {Cycle 1}
- Day 6 at the end of the ch14.18 infusion (C_{max}), {Cycle 1}
- Day 80 prior to IL-2 (trough), {Cycle 4}
- Day 90 at the end of the ch14.18 infusion (C_{max}), {Cycle 4}
- Study End: within 2 weeks of Day 163
Each of these time points correspond to a scheduled pharmacokinetic blood draw for the assessment of ch14.18 blood levels. This scheduling will allow for the approximate correlation of ch14.18 blood levels to potential ECG effects.

**ECG Methodology: Central Laboratory:**
ECGs will be recorded at the sites on each trial patient participating in the cardiac safety sub-study and sent to ERT (Philadelphia, PA, USA), a central ECG laboratory, for a treatment-blinded high-resolution measurement of the cardiac intervals and morphological assessment by a central cardiologist blinded to the study treatment. In addition to the paper ECG printout provided at the time of ECG assessment, study sites will have online access to ECG results and central review commentary within 72 hours. Sites participating in the ECG cohort will be directly notified of critical ECG findings as assessed by ERT within approximately 72 hours of assessment.

Digital ECGs will be transmitted to the central ECG laboratory and processed via its validated data management system, EXPERT. Interval duration measurements will be collected using computer assisted caliper placements on three consecutive beats. Trained analysts will then review all ECGs for correct lead and beat placement and will adjudicate the pre-placed algorithm calipers as necessary using the proprietary validated electronic caliper system applied on a computer screen. A cardiologist will then verify the interval durations and perform the morphology analysis, noting any T-U wave complex that is compatible with an effect on cardiac repolarization.

The ECG analysis will be conducted in Lead II, or in Lead V5 if Lead II is not analyzable. If V5 is not analyzable then Lead V2 will be used, followed by the most appropriate lead if necessary. ECG readers will be blinded to subject identifiers, treatment and visit.

On-screen measurements of the RR, PR, QRS, and QT interval durations will be performed and variables for QTcF, QTcB and heart rate (HR) will be obtained by the following processes:

**Interval measurements will be obtained by the following method:**
- Three (3) RR mean RR Interval is reported
- Three (3) PR mean PR Interval is reported
- Three (3) QRS mean QRS Width is reported
- Three (3) QT mean QT Interval is reported

The following calculations will be made from the interval measurements:

**Three (3) QTcF measurements- QTc correction by the Fridericia’s formula**
- QTcF1 = QT1/√RR1
- QTcF2 = QT2/√RR2
- QTcF3 = QT3/√RR3
- Mean QTcF = (QTcF1 + QTcF2 + QTcF3)/3

**Three (3) QTcB measurements- QTc correction by the Bazett’s formula**
- QTcB1 = QT1/√RR1
- QTcB2 = QT2/√RR2
- QTcB3 = QT3/√RR3
- Mean QTcB = (QTcB1 + QTcB2 + QTcB3)/3

**Three (3) Heart Rate measurements**
- HR1 = 60 / RR1
• HR2 = 60 / RR2
• HR3 = 60 / RR3
• Mean HR = (HR1 + HR2 + HR3)/3

Each fiducial point (onset of P wave, onset of Q wave, offset of S wave, and offset of T wave) will be electronically marked. The original ECG waveform and such annotations will be saved separately in XML format for independent review.

6.0 TREATMENT PLAN

6.1 Overall Treatment Outline

Please note:
As of amendment 9, accrual to regimen A (isotretinoin alone) is closed and all newly enrolled eligible patients will receive treatment per regimen B only (ch14.18 + cytokines in addition to isotretinoin). In the first cohort of randomized ANBL0032 patients, Regimen B has been proven to be effective, based on superior EFS and OS as compared to those patients randomized to regimen A.

Eligibility requirement for cross-over patients impacted by Amendment 9:
ANBL0032 Patients who were previously randomized to regimen A (isotretinoin alone) have the option of crossing over to regimen B (ch14.18 + cytokines in addition to isotretinoin) provided that:
1) The patient has not experienced disease progression since enrollment onto ANBL0032
2) The patient has NOT received any further anti-neuroblastoma therapy following completion of cis-RA therapy.
3) The patient meets all eligibility requirements noted in Sections 4.7 through 4.11.

A new informed consent was required to be signed by all existing patients, prior to initiation of further therapy after amendment #9 activation. It should be noted that previously ANBL0032 patients were required to be enrolled within the first 100 days following stem cell infusion based on pre-clinical data suggesting that this type of immunotherapy would be most effective in the MRD setting. Thus, no data exists that documents the benefit of starting immunotherapy after 110 days, and available information suggests that the chance for benefit of immunotherapy given after 1-2 years is very low. In addition, regimen B has significant toxicities which can be potentially life threatening, and available data from this study suggests that toxicities of this immunostimulatory regimen may be greater at later times following stem cell transplant.

Patients will be stratified by pre-ASCT CR versus VGPR versus PR and by purging vs. non-purging of the stem cells for ASCT. All patients will receive isotretinoin (13-cis-retinoic acid, or RA) at 80 mg/m²/dose twice a day for 14 days every 28 days, for 6 courses. In addition, patients will receive 5 courses of ch14.18 + cytokines, with ch14.18 + GM-CSF administered in Courses 1, 3, and 5, and ch14.18 + aldesleukin (IL-2) given in Courses 2 and 4. The intervals between antibody administrations are 28 days for all courses.

Patients with persistent active disease documented by biopsy after the post ASCT / radiotherapy evaluation are eligible to enroll onto study under stratum 07. Biopsy is suggested but not required for persistent soft tissue density by CT, MRI, bone scan, or MIBG scan. Findings of MIBG scan can be used...
to guide biopsy but not as a criterion for proven active disease. Those who do not undergo biopsy will be enrolled and stratified based upon INRC disease response. The criteria for active disease in the bone marrow is based on morphological examination, regardless of the results of immunocytology and PCR analysis, since one of the study objectives of this protocol is to assess the value of the latter two parameters in detecting MRD.

**Enrollment**

After completion of ASCT, local radiation, and tumor assessment ASCT, the institution must again contact the C.O.G. on-line registration system to have the patient enrolled. Questions that the institution should be prepared to answer are:

a) During the ASCT, did the patient receive purged or unpurged stem cells?

b) What was the clinical assessment of the patient's response prior to ASCT?

c) What was the clinical assessment of the patient’s response after ASCT and radiotherapy? If not CR, did patient undergo biopsy? If biopsied, was there evidence of active residual disease? If active disease based on biopsy, enroll on Stratum 07.

d) What was the date of stem cell infusion (for tandem transplant, please enter 2nd infusion date)?

Crossover patients are already enrolled on ANBL0032. Hence, re-enrollment of crossover patients is unnecessary and should NOT be attempted. A new Case Report Form (CRF) has been added which should be completed for crossover patients.

Patients enrolled after amendment #9 activation will NOT be allowed registration beyond Day 100 after ASCT.

This treatment regimen (ch14.18 plus GM-CSF/IL2 plus isotretinoin) has been proven to be effective in the context of starting therapy within the first 110 days following stem cell infusion (within 10 days of study enrollment). In addition, this regimen has significant toxicities which can be potentially life threatening and may be worsened at later times following ASCT (greater immune reconstitution may predispose to more stimulatory activation of immune mediated toxicity). Thus, since there is no evidence that this regimen is effective if initiated later than 110 days after ASCT, and it has significant associated toxicities, possibly worse with time, eligible patients (considered after amendment # 9 activation) must be able to enter this study within 100 days of ASCT.
**Overall Schema**

[Diagram showing the overall schema of the protocol]

**FOOTNOTE**

NBL – Neuroblastoma  
ASCT – Autologous stem cell transplant  
XRT – Radiation therapy  
MRD – Minimal residual disease  
IC – Informed consent  

*Optional MRD assessment:* Bone marrow for RT-PCR to Seeger’s lab.

**Study therapy: Regimen B**

- **Courses #1,3,5:** ch14.18+ GM-CSF+ isotretinoin  
- **Courses #2,4:** ch14.18 + IL-2 + isotretinoin  
- **Course #6:** Isotretinoin ONLY

Optional *MRD#2 – End of 6th course of isotretinoin.
Prior to amendment 9:

**Prior to Amendment # 9:** In the rare cases with persistent tumor post ASCT/ radiotherapy (time point #1), biopsy is required. Those with biopsy proven active disease will be non-randomly assigned to regimen B (Isotretinoin + immunotherapy). Those who do not undergo biopsy will be randomized as all other patients.

MRD assessment:
Bone marrow for immunocytology & RT-PCR, MIBG scan and other radiographical studies.
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<th>WED</th>
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Day 0 is Day 56 post-PBSC infusion. Day in bold parenthesis refers to post-PBSC infusion day.

Ch14.18 at 25 mg/m²/day x 4 for all 5 courses

GM-CSF at 250 mcg/m² for 14 days; Aldesleukin (IL-2) at 3 M IU/m²/day for first week, 4.5 M IU/m²/day for 2nd week

RA: Isotretinoin (13-cis-retinoic acid) (80mg/m²/dose bid) x 14 days every 28 days for a total of 6 cycles.

MRD assessment: Bone marrow for immunocytology & RT-PCR, MIBG scan, CT/MRI and bone scan. To be done at 2 time points:
(1) Up to 4 weeks before study enrollment
(2) Within 2 weeks at the completion of protocol therapy (after the last dose of RA)

@ FcR and Kir/Kir-L genotyping: 7 mL blood in preservative-free heparin or green-top tube obtained within 1 week before starting the first GM-CSF injection (on a Monday through Thursday, to avoid Friday shipment). Freshly obtained samples should be sent at room temperature by overnight carrier to Dr. Yu’s lab.

$: Blood samples for ch14.18 PK and HACA analysis (for patients participating in the cardiac safety sub-study): 5 mL blood in preservative-free heparin or green-top tube obtained for the 1st and 4th courses of immunotherapy and within two weeks of study Day 163 (after the last dose of RA). Freshly obtained samples should be sent at room temperature by overnight carrier. ECG monitoring (as detailed in Section 5.6.5) must also be done at each of these time-points.

**cisRA pharmacokinetics: 5 mL blood sample will be obtained from patients on Day 14 of the first course of oral 13 cis retinoic acid, to be shipped to Dr. Reynolds’ lab.

6.2 Isotretinoin (13-Cis-Retinoic) Therapy Post-Autologous Stem Cell Transplant for both regimen A and B (please note regimen A is closed to accrual as of amendment #9)

All patients will receive 6 cycles of isotretinoin (13-cis-retinoic acid) therapy beginning Day 56-85 after ASCT when liver function < Grade 2 toxicity, adequate renal function, ≤ Grade 1 proteinuria or hematuria, calcium, uric acid and triglycerides ≤ 2 x normal, and at least 7 days since completing radiation therapy. Isotretinoin (13-cis-retinoic acid, or RA) is given at 160 mg/m²/day (5.33 mg/kg/day) PO divided into two equal doses for 14 days, followed by 14 day rest for total of six cycles. Patients ≤ 12 kg will be given 5.33 mg/kg/day divided BID. (Round up to the nearest 10 mg.) Capsules come as 10, 20, 30 and 40 mg sizes, and can be emptied into a high fat food such as ice cream or peanut butter to administer. Capsules can be chewed (best absorbed if chewed with high fat food) or teach child to swallow entire capsules, which is feasible and to be encouraged. Under no circumstances should Isotretinoin (13-cis-retinoic acid) be removed from the capsules for more than 1 hour prior to administering to the patient. The first dose of isotretinoin (13-cis-retinoic acid) will fall on Day 11 of immunotherapy. There will be no concomitant administration of cis-retinoic acid and aldesleukin (IL-2) except for the first course of isotretinoin, in which the last dose of isotretinoin will overlap with first dose of aldesleukin. However, isotretinoin (13-cis-retinoic acid,) will overlap with GM-CSF for 3 days in Course 1 and for 4 days each in Courses 3 and 5 of immunotherapy.
6.3 **Regimen B: immunotherapy**  
See guidelines for management of toxicities in Appendix III. Before starting immunotherapy, please obtain a copy of management recommendations and algorithms for anaphylaxis and hypotension available in Appendix III and the training module on the COG website. It is recommended that these be reviewed, printed and available on the inpatient unit to facilitate treatment decisions should these symptoms occur.

### Schema for the administration of 5 courses of ch14.18 and cytokines

<table>
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<th>Course 1</th>
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Ch14.18 treatment will be administered every 28 days at 25 mg/m²/day x 4 days for all 5 courses GM-CSF at 250 micrograms/m²/day for 14 days; Aldesleukin (IL-2) at 3 MIU/m²/day for first week, 4.5 MIU/m²/day for second week.

**Immunosuppressive drugs, myelosuppressive chemotherapy, and chronic steroid therapy must not be given after study entry. Steroids may be used for severe allergic reactions not responding to other measures. The use of steroids at any time during immunotherapy requires clear justification and documentation as they may interfere with the anti-tumor activity of ch14.18.**

The use of IVIG post-ASCT is discouraged. If necessary, its use should be limited to the first 100 days post-ASCT, because IVIG may interfere with antibody (ch14.18) dependent cellular cytotoxicity. For patients randomized to immunotherapy, IVIG should not be given within 2 weeks of starting ch14.18 treatment and 1 week after completing ch14.18 therapy.

**PRIOR TO EACH COURSE OF IMMUNOTHERAPY, a patient must have:**

- a. ALT \( \leq 5 \times \) normal, total bilirubin \( < 1.5 \times \) normal  
- b. Skin toxicity no greater than Grade 1  
- c. No evidence of serious infection, or infection under control with no active disease and negative blood culture  
- d. Serum creatinine \( < 1.5 \text{ mg/dL} \)  
- e. Platelet count \( \geq 20,000/\mu L \) (maybe transfused). Patients with a history of neuroblastoma metastatic to the central nervous system should receive platelet transfusion support to maintain platelet count \( \geq 50,000 \).

If patient meets criteria for discontinuation of immunotherapy, treatment should be continued with isotretinoin.

6.3.1 **Immunotherapy program:**

6.3.1.1 **Ch14.18 Monoclonal Antibody**  
Patients should receive an IV bolus of normal saline (10 mL/kg) over one hour just prior to the ch14.18 infusion.

Ch14.18 will be administered at 25 mg/m²/day x 4 days. Each dose of ch14.18 should be infused IV over approximately 10 hours, starting at 1.25 mg/m²/hr x 0.5 hr, then increasing to 2.5 mg/m²/hr for the remainder of the dose, if tolerated. The infusion duration may be extended up to 20 hours for anticipated toxicities (pain, fever, tachycardia, tachypnea, hypotension), not responding to other supportive measures, and the duration used should be recorded. In the setting of dose reductions described below, the infusion must be no longer than 20 hours even if the full dose of ch14.18 antibody has not been delivered. Cytokine and antibody administration should not be given beyond the specified schedule regardless of whether doses were modified or held per guidelines below.
6.3.1.2 Premedication and Supportive Care for the prevention of anticipated toxicities associated with ch14.18:

Neuropathic pain and allergic reactions with angioedema are commonly seen in patients receiving monoclonal antibody. The following premedications will be utilized to avoid these problems:

a. Hydroxyzine (0.5-1 mg/kg; max dose 50 mg) OR diphenhydramine (0.5-1 mg/kg; max dose 50 mg) IV over 10-15 minutes to start 20 minutes prior to ch14.18 infusion and continue these agents, as tolerated every 4-6 hrs until end of ch14.18 infusion (doses may be skipped at discretion of treating physician if child is over sedated)

b. Acetaminophen (10 mg/kg; max dose 650 mg) PO given 20 minutes prior to ch14.18 infusion; may be given q4 hrs prn fever. Administer acetaminophen scheduled every 4-6 hours during IL2 plus ch14.18 courses. May administer ibuprofen (5-10 mg/kg/dose, not to exceed every 6 hours) between acetaminophen doses for control of persistent fever provided that there is no ongoing bleeding problem and the platelet count is >50,000/μL, and no renal dysfunction. If renal dysfunction occurs or bleeding occurs, stop ibuprofen.

c. Meperidine 0.25-0.5 mg/kg IV (max dose 50 mg) every 2hr PRN chills.

d. Give a morphine sulfate loading dose of 50 mcg/kg immediately prior to ch14.18 administration and then continue with morphine sulfate drip with an infusion rate of 20-50 micrograms/kg/hr to continue for two hours after completion of the ch14.18 infusion. Additional doses of morphine can be given during the ch14.18 infusion to treat neuropathic pain followed by an increase in the morphine sulfate infusion rate, but patients should be monitored closely for hypotension and respiratory depression. If patients cannot tolerate morphine (e.g., itching), fentanyl or hydromorphone can be substituted for morphine.

e. Alternatively, lidocaine infusion may be used in conjunction with IV bolus of morphine on prn basis. The administration guidelines for lidocaine infusion are shown below:

   Administration of lidocaine:
   1. Give lidocaine IV bolus at 2 mg/kg in 50 mL NS over 30 min prior to the start of Mab Ch14.18 infusion.
   2. At the beginning of Mab Ch14.18 infusion, start IV lidocaine infusion at 1 mg/kg/hr and continue until two hours after the completion of Ch14.18 infusion.
   3. May give Morphine IV bolus 25-50 microgram/kg every 2h prn pain.
   4. Patient should be monitored closely for sedation scale, EKG (rate and rhythm), HR, BP, RR, oxygen saturation (pulse oximeter) and pain score. If patient develops dizziness, perioral numbness, tinnitus attributable to lidocaine, then stop lidocaine infusion.

g. Gabapentin may also be used in conjugation with IV morphine. Start gabapentin at time of morphine loading dose, dose gabapentin at 10 mg/kg/day and titrate up to 30-60 mg/kg/day (maximum dose: 3,600 mg/day) depending on the clinical response.

6.3.1.3 Treatment of Hypersensitivity Reactions during Immunotherapy

Note: Coughing may herald the onset of bronchospasm and if occurs, patient should be closely monitored.

- Grade 1 or 2 hypersensitivity reactions: Mild symptoms (e.g., localized urticaria, rigors):
  i. Decrease the rate of ch14.18 infusion to 50% until recovery from symptoms, remain at bedside and monitor subject; may complete infusion at planned rate when symptoms resolve, if tolerated. If not, use the slower rate.
  ii. Hydroxyzine or diphenhydramine should be administered every 4-6hr at the discretion of the treating physician (see Section 6.3.1.2).
  iii. Meperidine may be used for rigors.

- Grade 3 or 4 hypersensitivity reactions: For severe symptoms (any reaction such as bronchospasm, hypersensitivity-related edema/angioedema, or anaphylactic shock):
i. **IMMEDIATELY discontinue ch14.18 infusion and discontinue IV GM-CSF or IL-2 infusion.**

ii. Give epinephrine, diphenhydramine and corticosteroids, bronchodilator, O₂, fluid or other medical measures as needed. Refer to the management guidelines and algorithms in Appendix III and the training module at the COG website that should be available on treatment unit (see Section 6.3).

iii. For angioedema that doesn’t affect the airway or mild bronchospasm without any other symptoms, if the symptoms and signs resolve rapidly with above measures, then ch14.18 can be resumed at half the rate with very close observation. Cytokine should not be resumed until the following day. If symptomatic angioedema or asymptomatic bronchospasm recurs when the ch14.18 is restarted, discontinue immunotherapy for that day and if symptoms/signs resolve completely that day, resume the next day with additional pre-medication of hydrocortisone 1 mg/kg IV. For this re-challenge, the infusion should be given in an ICU setting.

   a. For **Courses 1, 3, and 5**, give GM-CSF at 50% dose starting the next day and thru the last dose of ch14.18. If tolerated, may give GM-CSF at full dose after completing the 4th dose of ch14.18. It is **strongly recommended** that GM-CSF be given SQ if the patient has significant hypotension with IV GM-CSF.

   b. For **Courses 2 and 4**, if ch14.18 infusion is tolerated, resume IL-2 infusion at 50% of the dose starting the next morning and continue this dose for the remainder of the 4 day course.

   c. If angioedema recurs with the addition of cytokine (GM-CSF or IL-2), discontinue cytokine and ch14.18. If resolves by the following day, restart ch14.18 at the rate tolerated, but do not restart cytokine.

   d. ch14.18 antibody infusion rate should be maintained at the tolerable infusion rate for all subsequent courses with that specific cytokine.

iv. If the patient has anaphylaxis or symptomatic bronchospasm, immediately stop any ch14.18, IL2 and GM-CSF treatment.

   See section immediately below (6.3.1.4) on hypotension and hypotension algorithm from Appendix III and training module on the COG website. Keep patient in position with lower extremities elevated (Trendelburg position), if possible. Patients should be monitored as in-patient for at least 24 hours AND until the symptoms have resolved. Refer to Section 6.4.3.3 for off therapy criteria.

v. If Grade 4 hypersensitivity reaction occurs, please order the following lab tests and send the results to Dr. Alice Yu by email (aliceyu@ucsd.edu) and also include the results in AdEERS: Complement levels (C3, C4, CH50 and c1q binding); IgE level, blood levels of tryptase and histamine (to be done locally); 5 mL heparinized blood to be sent to the bioanalytical laboratory (see address information in Section 5.6.3) for HACA determination.

**Note:** Hypersensitivity reaction will be referred as ‘Allergic reaction and anaphylaxis’ per CTCAE v 4.0, starting October 1st, 2011.

**Please note:** Send the vials of ch14.18 used for the course in which the reaction occurred to Biological Resources Branch, NCI (see instruction and address information in Section 5.6.4)
6.3.1.4 Treatment of Hypotension during Ch14.18

Hypotension associated with anaphylaxis usually begins very soon after exposure to the allergen and may be accompanied by urticaria, angioedema, or wheezing. Up to 10% of patients can present with hypotension alone. Hypotension can also occur as a side effect of ch14.18 and of the cytokines. Hypotension can be exacerbated by fever, narcotics, antihistamines, and vascular leak syndrome. The combination of hypotension and fever can be side effects of ch14.18 and of the cytokines and do not necessarily imply a hypersensitivity reaction. Refer to Appendix III.

If hypotension is associated with anaphylaxis, also see Section 6.3.1.3.

**If significant hypotension occurs, treat as follows:**

[significant hypotension is defined as symptomatic and/or systolic blood pressure < 80 mm/hg for age > 12 years old), < 70 (age 1-12 years old), <65 for infant OR a decrease that is more than 15% below baseline]

i. HOLD ch14.18 infusion. Keep patient in position with lower extremities elevated (Trendelburg position), if possible.

ii. If patient is receiving IL-2 or IV GM-CSF, HOLD cytokine administration.

iii. Give normal saline 10mL/kg over 30 minutes

iv. Decrease narcotic dose if possible

v. **If significant hypotension persists, give another fluid bolus as any of the following:**
   a. Normal saline 10mL/kg over 30 minutes
   b. Normal saline 3-5 mL/kg with albumin (25%) 1 gm/kg over 30-60 min (recommended as an alternative to NS alone if albumin =< 3.0)
   c. PRBC 10 mL/kg over 2-3 hrs (can be in place of or in addition to the above and is recommended if Hgb < 10)
   d. It is suggested that when the second bolus is given, arrangement should be made to transfer the patient to an ICU setting where pressors can be given if needed.

vi. **If significant hypotension resolves**, may resume ch14.18 infusion at 1.25 mg/m²/hr.
   - If blood pressure then remains stable for 2 hrs with ch14.18 at 1.25 mg/m²/hr, may resume cytokine. If significant hypotension occurs again when cytokine is restarted, HOLD cytokine and see instructions by course below.
   - If BP is stable after 2 hrs following starting cytokine, may increase ch14.18 to 2.5 mg/m²/hr as tolerated to complete the prescribed full dose. If blood pressure is not stable with ch14.18 at 2.5 mg/m²/hr, stop ch14.18 infusion and cytokine and restart ch14.18 at 1.25 mg/m²/hr once blood pressure is stable. If blood pressure remains stable at this slower ch14.18 infusion rate, may resume cytokine the following morning. If significant hypotension occurs again when cytokine is restarted, HOLD cytokine and see instructions by course below.

For the following days of antibody infusion for this cycle, use the rate that the patient tolerated without hypotension.

a. For Courses 1, 3, and 5, if ch14.18 infusion is tolerated without GM-CSF, give GM-CSF at 50% dose starting the next day and thru the last dose of ch14.18. If tolerated, may give GM-CSF at full dose after completing the 4th dose of ch14.18. It is strongly recommended that GM-CSF be given SQ if the patient has significant hypotension with IV.
b. For Courses 2 and 4, if ch14.18 infusion is tolerated without the IL2, resume IL-2 infusion at 50% of the dose starting the next morning and continue this dose for the remainder of the 4 day course.

c. ch14.18 antibody infusion rate should be maintained at the tolerable infusion rate for all subsequent courses with that specific cytokine.

d. If significant hypotension recurs when cytokine (GM-CSF or IL-2) is restarted, discontinue cytokine.

Dose modification instructions for subsequent courses:

a. If patient tolerated 50% cytokine dose, subsequent course of ch14.18 + cytokine should start at 50% of cytokine dose and same ch14.18 rate. If tolerated, may increase cytokine to full dose the next day.

b. If GM-CSF is not tolerated with ch14.18 and has to be discontinued, subsequent planned ch14.18 + GM-CSF courses should be substituted by ch14.18 alone.

c. If aldesleukin (IL-2) is not tolerated with ch14.18 and has to be discontinued, subsequent planned ch14.18 + aldesleukin (IL-2) courses should be substituted by ch14.18 + GM-CSF immunotherapy.

vii. If hypotension is severe at any time (eg. Poor perfusion, acidemia, impairment of organ function) or after the administration of 3 boluses (including the pre-antibody infusion bolus), may use pressors in addition to fluid resuscitation. Adrenergic pressors, rather than dopamine, are recommended.

a. If responsive and blood pressure is stable off pressors for at least 6 hours, may resume ch14.18 infusion the following morning at 50% of the last rate.

b. See section vi above for subsequent dosing guidelines.

6.3.1.5 Treatment of Acute Vascular Leak during Ch14.18

If Grade 3 vascular leak syndrome (respiratory compromise or fluid support required for hypotension) occurs, treat as follows:

i. HOLD ch14.18 infusion.

ii. If patient is receiving IL-2 or IV GM-CSF, HOLD infusion.

iii. Provide appropriate supportive care depending on manifestations (eg. oxygen, IV fluids)

iv. Furosemide 0.5-1 mg/kg IV q 6-12h prn symptomatic pulmonary vascular congestion, tense ascites or significant weight gain (i.e. >10% of baseline), if blood pressure allows. Furosemide is best administered after antibody and morphine are completed (if possible) for blood pressure considerations. If poor response to furosemide alone, add metolazone 0.2-0.4 mg/kg/day (maximum: 10 mg/dose) PO prior to IV furosemide. Albumin may be required prior to furosemide if albumin < 3.0. If albumin and furosemide are given together, close attention to volume status to prevent hypotension and prerenal azotemia is important.

v. If symptomatic acute vascular leak resolves, may resume ch14.18 infusion at 50% rate. If ch14.18 is tolerated, may resume cytokine at 50% dose starting the next day and thru the last dose of ch14.18.

Dose modification instructions for subsequent courses:

a. If patient tolerated 50% cytokine dose, subsequent course of ch14.18 + cytokine should start at 50% dose of cytokine and same ch14.18 rate. If tolerated, may increase cytokine to full dose the next day.
b. If aldesleukin (IL-2) is not tolerated with ch14.18 and has to be discontinued, subsequent planned ch14.18 + aldesleukin (IL-2) courses should be substituted by ch14.18+ GM-CSF immunotherapy.

c. If GM-CSF is not tolerated with ch14.18 and has to be discontinued, subsequent planned ch14.18 + GM-CSF courses should be substituted by ch14.18 alone.

vi. If pressor support or mechanical ventilation is required (Grade IV vascular leak syndrome), discontinue immunotherapy for that course. If occurred during IL2 Course #2, patient may continue on therapy but substitute GM-CSF for IL2 in Course #4. If occurred during GM-CSF course then patient may continue on therapy but with ch14.18 alone, no further cytokine therapy will be delivered in all subsequent course. This does not apply to hypotension alone without acute vascular leak requiring pressor, which should be managed per Section 6.3.1.4.

Note: Acute vascular leak syndrome will be referred as ‘Capillary leak syndrome’ per CTCAE v 4.0 followed starting October 1st, 2011.

6.3.1.6 Other possible toxicities of ch14.18 and recommended treatment
Ch14.18 may cause fever, tachycardia, nausea, emesis, hypotension, hypokalemia and hypoalbuminemia. These toxicities are usually mild and transient, and easily managed with supportive care. Occasionally, ch14.18 may cause impaired accommodation, dilated pupils with sluggish light reflex +/- photophobia and taste changes which may last for a few weeks to months. Rarely, motor weakness has been reported. The severity and duration of each toxicity should be carefully recorded. Please see sections 6.4.3.2 and 6.4.3.3 for dose modification instructions.

6.3.2 Immunotherapy program for Courses 1, 3 and 5

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*For Course#1 only, cis RA should start from Day 11.

It is suggested that Day 0 of Courses 1, 3 and 5 be on a Friday; Day 0 of Courses 2 and 4 be on a Monday, and should fall on Day 24 and Day 80 respectively, from the start of immunotherapy (see Immunotherapy Calendar in Section 6.1). If Monday is a holiday, may delay the start of ch14.18 to Tuesday, but resume Monday start for subsequent courses. For patients participating in the cardiac safety sub-study blood will be drawn during Course 1 for ch14.18 PK and HACA assays (before starting GM-CSF and at the end of the 4th dose of ch14.18). For Course 4, for patients participating in the cardiac safety sub-study, blood will be drawn for ch14.18 PK and HACA assays on the first day of the first 4-day/week aldesleukin (IL-2) therapy (Day 80, before starting aldesleukin (IL-2) infusion, on a Monday) and at the end of the 4th daily infusion of ch14.18 (Day 90).

For Course 1 of immunotherapy, a two week course of isotretinoin (13-cis-retinoic acid) will start on Day 11, preferably on a Tuesday (i.e. overlapping with the last 3 days of GM-CSF and first day of IL2 in Course 2) so as to avoid day 14 blood drawing for cis-RA PK on a Sunday. For Courses 3 and 5 of immunotherapy, a two week course of isotretinoin (13-cis-retinoic acid) will start on Day 10 (i.e. overlapping with the last 4 days of GM-CSF). An additional cycle of cis-retinoic acid (80 mg/m²/dose BID x 14 days, every 28 days) will follow Course 5, for a total of six cycles.
Ch14.18 will be administered at 25 mg/m²/day x 4 days. See Section 6.3.1.1 above for administration guidelines.

6.3.2.1 GM-CSF
250 micrograms/m²/d subcutaneous injection (strongly recommended) or IV as a 2 hour infusion daily from Day 0 through 13 (daily with the infusion of ch14.18 and for 3 days before and 7 days afterward). GM-CSF should be administered daily prior to ch14.18, around 8:00 - 9:00 AM. GM-CSF dose will be held if the total white cell count is > 50,000/µL. This is not a toxicity of GM-CSF but rather a possible outcome related to its use. The GM-CSF will be held until the total white cell count is less than 20,000/µL and then GM-CSF will be resumed at 50% dose for the remainder of that course. Full dose GM-CSF will be used for subsequent GM-CSF courses.

If criteria for holding GM-CSF are met (e.g., see Sections 6.4.1 and 6.3.1.5), then ch14.18 should be administered daily without GM-CSF

6.3.2.2 Pre-medication Regimen for Ch14.18 and Management of Potential Toxicities:
See sections 6.3.1.2 through 6.3.1.6 and Appendix III.

6.3.2.3 Guidelines for administration of ch14.18 +GM-CSF (immunotherapy Courses 1, 3 & 5)

1. LABORATORY:
   Day-1
   Urinalysis

   Day -1 AND Day 6:
   CBC/diff/plts, ALT/AST, total bili, albumin, BUN, Cr, electrolytes including phosphorus, magnesium.

   Day 3
   CBC/diff/plts.

   Day 3 THRU Day 7:
   Electrolytes including phosphorus and magnesium.

   SPECIAL LABS: (omit this for Course 3)
   For patients participating in the cardiac safety sub-study: Day -1: (prior to 1st dose of GMCSF); and Day 6: (at the end of the 4th infusion of ch14.18); 12 lead ECG to be done, and 5 mL blood in GTT tube (preservative-free heparin) to Central Lab with appropriate specimen transmittal slip. Label and ship by Fed. Ex. to the bioanalytical laboratory as described in Section 5.6.3.

   Optional for all patients: on Day -1 of Course 1 only, may be obtained within 1 week before starting the first GM-CSF injection:
   7 mL blood in GTT tube (preservative-free heparin) to Dr. Yu’s Lab with appropriate specimen transmittal slip for FcR and KIR genotyping. Label and ship by Fed-Ex to Dr. Yu’s laboratory as described in Section 5.6.3.

   Day 14: 5 mL blood in heparin to be drawn 4 hours after administration of the 13-cis retinoic acid dose (Course #1 only) for 13-cis-RA pharmacokinetic and Pharmacogenomics study. (See Section 5.6.5 for details of specimen preparation and submission).

2. AVAILABLE ON CALL:
   A. Epinephrine (1:10,000) 0.01 mg/kg or 0.1 mL/kg: IVP (max 5 mL) every 3-5 minutes.
B. Hydrocortisone 2mg/kg (max 100 mg) IVP. Only use it for life-threatening reactions (hypotension, bronchospasm, angioedema involving the airway) not responsive to other measures.

3. **GM-CSF & ANTIBODY ADMINISTRATION SCHEDULE**

   **Day 3 thru Day 6:**
   GM-CSF (250 micrograms/m²/day) SubQ (preferred) or IV in NS over 2 hours days 3, 4, 5, 6.
   Follow GM-CSF with NS bolus (10 mL/kg) over 1 hour days 3, 4, 5, 6.
   Immediately after NS bolus begin, Chimeric Antibody ch14.18 (25 mg/m²/day) IV in 100 mL NS over 10 hours on days 3, 4, 5, 6: See Section 6.3.1.1 for administration guidelines.
   Begin antibody infusion one hour after completion of GM-CSF infusion every day.

   **Day 7 thru Day 13**
   GM-CSF (250 micrograms/m²/day) SubQ (preferred), or IV in NS over 2 hours.
   Hold GM-CSF dose if total WBC count is > 50,000/µL. Resume GM-CSF at half dose when total WBC count is < 20,000/µL for remainder of current course.

   If criteria for holding GM-CSF are met (e.g., see Sections 6.4.1 and 6.3.1.5), then ch14.18 should be administered daily without GM-CSF.

6. **MONITORING:**
   - Vital Signs every 4hr. During ch14.18 infusion VS every 15 minutes x 4 then every 1hr until completion of antibody infusion, if stable after 1 hour.
   - Strict I & O every 4 hours, Dipstick urine for heme and SG every void, BID weights during days of ch14.18 infusion
   - Call Hematology/Oncology MD for:
     A. Systolic B/P >150 or < 70 (<65 for infant; < 80 for patients > 12 years of age); HR > 160 or < 60; Temp ≥ 38.5; Respiratory rate >40.
     B. Neuropathic pain requiring an increase in narcotic infusion rate;
     C. Urticaria, bronchospasm, peripheral/sensory neurotoxicity, new coughs.

7. **ADMINISTRATION OF ISOTRETINOIN (13-CIS-RETINOIC ACID, OR RA)**

   **Day 10 thru Day 23**
   On Day 10 (Courses 3, 5); Day 11 (Course 1): CBC/diff/plts, ALT/AST, total bili, albumin, BUN, Cr., electrolytes, calcium, triglycerides, urinalysis.

   **Isotretinoin (13-cis-retinoic acid, or RA) (160mg/m²/day or 5.33mg/kg/day if ≤ 12kg)**
   PO divided into 2 doses daily x 14 days.
   Round up to the nearest 10 mg. Capsules come as 10, 20, 30 and 40 mg sizes, and can be emptied into a high fat food such as ice cream or peanut butter to administer. Capsules can be chewed (best absorbed if chewed with high fat food) or teach child to swallow entire capsules, which is feasible and to be encouraged. Under no circumstances should Isotretinoin (13-cis-retinoic acid) be removed from the capsules for more than 1 hour prior to administering to the patient.
6.3.3 Immunotherapy program for Courses 2 and 4

**Treatment Schema For Courses With Aldesleukin (IL-2)**

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4-6</th>
<th>7</th>
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<th>9</th>
<th>10</th>
<th>11-13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18-27</th>
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<tbody>
<tr>
<td>IL-2</td>
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It is suggested that Day 0 of Courses 2 and 4 be on a Monday. Ch14.18 treatment will be administered every 28 days. Day 0 of Course 2 and 4 will be on Day 24 of Course 1 and 3, respectively. (See Immunotherapy Calendar in **Section 6.1**.)

The 2nd course of isotretinoin (13-cis-retinoic acid) will start on Day 14 of Course 2 of immunotherapy. For Course 4, cis-retinoic acid will also start on Day 14.

**6.3.3.1 Aldesleukin (Interleukin-2, IL-2)**

Aldesleukin 3 MIU/m²/day will be given by continuous infusion (using a CADD or similar infusion pump) for 4 days during the first week of each course given on Days 0 – 3. The first week of therapy is usually very well tolerated and typically given as an outpatient. During the second week of each course, Aldesleukin (IL-2) 4.5 MIU/m²/day for 4 days will be given on Days 7 - 10 (with the infusion of ch14.18).

Aldesleukin (IL-2) should begin first around 8:00 - 9:00 AM. Aldesleukin (IL-2) will be continuously infused IV over 96 hours through the catheter via an ambulatory infusion pump in D5W (may contain 0.1% human serum albumin if needed) total volume dependent upon the pump. The Aldesleukin (IL-2) may be prepared every 24-48 hours when the patient is in the hospital. Aldesleukin (IL-2) should be given into a dedicated line. If the patient has only a single lumen catheter, the patient will require a peripheral IV during week 2 (days 7-10) for the ch14.18 infusion (to avoid co-administration of Aldesleukin (IL-2) and ch14.18 in the same line).

To facilitate correct Aldesleukin (IL-2) dose calculation, the following sample calculations will be provided as a guide:

<table>
<thead>
<tr>
<th>Body Surface Area (B.S.A)</th>
<th>0.5 m²</th>
<th>1 m²</th>
<th>1.5 m²</th>
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<tbody>
<tr>
<td><strong>Dose</strong></td>
<td>Amount of Aldesleukin (IL-2)(mL)</td>
<td>Dose</td>
<td>Amount of Aldesleukin (IL-2)(mL)</td>
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<tr>
<td>Week 1 dose: 3 MIU/m²/day for 4 days</td>
<td>1.5 MIU/24 hours</td>
<td>0.08 mL/24 hours</td>
<td>3 MIU/24 hours</td>
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<tr>
<td>Total dose over 96 hours</td>
<td>6 MIU/96 hours</td>
<td>0.33 mL/96 hours</td>
<td>12 MIU/96 hours</td>
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<td>Week 2 dose: 4.5 MIU/m²/day for 4 days</td>
<td>2.25 MIU/24 hours</td>
<td>0.13 mL/24 hours</td>
<td>4.5 MIU/24 hours</td>
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<tr>
<td>Total dose over 96 hours</td>
<td>9 MIU/96 hours</td>
<td>0.5 mL/96 hours</td>
<td>18 MIU/96 hours</td>
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Aldesleukin (IL-2) is provided as single-use vials containing 22 million IU Aldesleukin (IL-2). Aldesleukin (IL-2) should be reconstituted with 1.2 mL sterile water, providing a solution of 18 million IU/mL.

* For each sample dose the actual volume (in mL) of the stock solution of Aldesleukin (IL-2) (18 million IU/mL) needed to achieve that dose is provided.
6.3.3.2 ch14.18 Monoclonal Antibody
Ch14.18 will be administered at 25 mg/m$^2$/day x 4 days. See Section 6.3.1.1 above for administration guidelines and Sections 6.3.1.2 through 6.3.1.6 for pre-medications and dose modifications.

6.3.3.3 Supportive care for Aldesleukin (IL-2) when given alone
To decrease Aldesleukin (IL-2) toxicity, all patients should receive the following medications on days of Aldesleukin (IL-2) therapy.

a. Acetaminophen
   10 mg/kg PO (max dose 650 mg/dose) every 4 h prn fever. May administer ibuprofen (5-10 mg/kg/dose, not to exceed every 6 hours) between acetaminophen doses for control of persistent fever provided that there is no ongoing bleeding problem and the platelet count is >50,000/μL, and no renal dysfunction. If renal dysfunction occurs or bleeding occurs, stop ibuprofen.

b. Hydroxyzine/Diphenhydramine
   Hydroxyzine (0.5-1 mg/kg; max dose 50 mg) or diphenhydramine (0.5-1 mg/kg; max dose 50 mg) PO/IV over 10 – 15 minutes every 4-6 h prn allergic reactions or nausea.

c. Antiemetics
   Antiemetics may be used as needed. Dexamethasone and other corticosteroids MAY NOT BE GIVEN.

6.3.3.4 Possible toxicities of Aldesleukin (IL-2) (alone and during aldesleukin (IL-2 + ch14.18 infusion)
and recommended treatment

Anaphylactoid reaction to contrast materials:
Please note that radiographic contrast materials used in imaging studies for tumor measurement have been associated with an increased risk of anaphylactic reactions if used during aldesleukin (IL-2). Therefore, contrast should be avoided during the aldesleukin (IL-2) infusion and for at least 1 week after the completion of aldesleukin (IL-2).

Cardiovascular Side Effects
High-dose aldesleukin (IL-2) induces a vascular leak syndrome and "sepsis-like" physiology, i.e., decreased systemic vascular resistance, increased cardiac output and some degree of peripheral or pulmonary interstitial edema and/or ascites. The dose used in this study is not anticipated to cause significant capillary leak syndrome or hypotension. Cardiovascular toxicity may be additive when given with ch14.18.

Hypotension
Mild hypotension with systolic blood pressure 10-15% below baseline is common in patients receiving aldesleukin (IL-2). If patient is receiving IL-2 and ch14.18, see guidelines in Section 6.3.1.4. If patient is receiving IL-2 alone:
   a. Asymptomatic patients should be monitored closely.
   b. Symptomatic patients and/or those with systolic blood pressure < 70 (65 for infant, < 80 for patients > 12 years old) mm/hg or a decrease that is more than 15% below baseline should be treated immediately with:
      i. Stop aldesleukin (IL-2).
      ii. Give a fluid bolus of NS 10 mL/kg. Fluid bolus may be repeated once as needed.
      iii. If responsive, may resume aldesleukin (IL-2) infusion at 50% dose 6 hrs after resolution of hypotension. If tolerated continue aldesleukin (IL-2) at 50% dose for the remainder of the 4 day course.
iv. If unresponsive, may use IV pressors, preferably adrenergic pressors rather than dopamine.

Arrhythmias
- **Sinus Tachycardia**: This is common, especially during fever.
- **Supraventricular Tachycardia**: Occurs in < 10% of adult patients with high-dose aldesleukin (IL-2) and is an indication to discontinue aldesleukin (IL-2) until SVT resolves. If patient is hemodynamically unstable, this should be treated with Digoxin (NOT Verapamil which may result in severe hypotension). SVT has not been seen in CCG studies of aldesleukin (IL-2).

Renal Side Effects
Patients may develop oliguria, peripheral edema, and elevated creatinine (1.5-2.0 x baseline). This is due to decreased renal perfusion and intrinsic renal effects of aldesleukin (IL-2). This would be unusual at the doses used for this study. The common manifestations of aldesleukin (IL-2) induced renal dysfunction are:
- Weight gain of 1-10% of baseline body weight.
- Metabolic acidosis (total CO₂ < 15-20 meq/L).
- Lowering of K⁺, Mg++, Phos.

Oliguria and renal dysfunction is generally quickly reversible after stopping aldesleukin (IL-2). If renal dysfunction occurs, these guidelines should be followed:
- Maintain HCT > 35% with RBC transfusion.
- If creatinine is > 2 x baseline, discontinue aldesleukin (IL-2).

Hematologic Side Effects
Aldesleukin (IL-2) therapy causes predictable hematologic effects as follows:
- **Lymphopenia** on Days 2-5, and 9-12, followed by "rebound" lymphocytosis (as high as 20-50,000/mm³) which is maximal 24 hours after stopping induction of Interleukin-2.
- **Anemia** due to decreased production and increased RBC destruction. Patients may require RBC transfusions.
- **Neutrophilia and Eosinophilia** due to secondary cytokine release of GM-CSF, IL-5 and IL-3.
- **Thrombocytopenia** with lowest platelet counts following aldesleukin (IL-2), probably both due to decreased production and increased peripheral destruction. Most patients require more frequent platelet transfusions during this period.
- Neither the aldesleukin (IL-2) nor the ch14.18 will be discontinued for these transient reversible hematological side effects. If the patient has significant clinical bleeding despite platelet transfusions, the aldesleukin (IL-2) will be discontinued for that course, and PT/PTT should be checked.

Infectious Complications
- Fevers are common with aldesleukin (IL-2) therapy at higher doses. Fevers are usually NOT seen during the first week of aldesleukin (IL-2) therapy at 3 MIU/m²/day,
- Aldesleukin (IL-2) causes a reversible defect in neutrophil function and patients are at an increased risk (incidence 10-25%) of gram-positive (rarely gram-negative) bacterial infections. Prophylactic antibiotics may be administered at the discretion of the responsible physician. Prophylactic antibiotics should be considered for a patient who has had bacteremia during a previous course. In addition:
c. For fever > 101°F (38.5°C) start antibiotics empirically and perform blood cultures at least once per day.

d. Any fevers that persist > 24 hours after Interleukin-2 is completed should strongly suggest infection and NOT be ascribed to Interleukin-2 until infection has been ruled out. Thus, broad-spectrum parenteral antibiotics are indicated.

Gastrointestinal Complications
a. Nausea, vomiting and diarrhea are common during treatment with aldesleukin (IL-2) at higher doses. GI symptoms are usually NOT seen during the first week of aldesleukin (IL-2) therapy at 3 MIU/m²/day. Most patients have a limited oral intake during this time. These symptoms usually improve quickly once aldesleukin (IL-2) is discontinued.

b. A cholestatic picture with elevated bilirubin and alkaline phosphatase may occur.

c. Drugs which are hepatically metabolized may need temporary dose reductions.

CNS Complications
a. Neurologic abnormalities such as lethargy, mild confusion, subtle personality changes and memory disturbance occurs in 15-25% of adult patients during high-dose aldesleukin (IL-2) therapy. These symptoms most often result from an accumulation of narcotics, sedatives or antiemetics due to transient hepatic dysfunction. Patients who exhibit such symptoms should have psychoactive drugs reduced or discontinued. Symptoms which remain may be due to aldesleukin (IL-2).

b. More serious neurologic abnormalities such as hyper-somnolence, severe confusion and rarely seizures or coma are seen in < 10% of adult patients. In such patients, aldesleukin (IL-2) and other potentially psychoactive drugs should be discontinued immediately.

6.3.3.5 Guidelines for administration of ch14.18 + aldesleukin (IL-2) (week 2 of Immunotherapy Courses 2 & 4)

1. **LABORATORY:**
   - **Day 7 and Day 10**
     - CBC/diff/plts, ALT/AST, total bilirubin, albumin, BUN, Cr., electrolytes including phosphorus, magnesium.
   - **Day 7 Thru Day 11**
     - Electrolytes including phosphorus and magnesium.
   - **Day -1 (for Course 4 only):**
     - Urine VMA/HVA
   - **Day -1 (for Courses 2 and 4):**
     - Urinalysis

**SPECIAL LABS for Course 4 only:**
For patients participating in the cardiac safety sub-study: Day 0 (prior to 1st dose of Interleukin-2) and Day 10 (at the end of the 4th infusion of ch14.18):
12 lead ECG to be done and 5 mL blood in GTT tube (preservative-free heparin) to Central Lab with appropriate specimen transmittal slip. Label and ship by Fed-Ex to the bioanalytical laboratory as described in Section 5.6.3.
2. AVAILABLE AT THE BEDSIDE DURING CH14.18 INFUSION:
   A. Epinephrine (1:10,000) 0.01mg/kg or 0.1 mL/kg: IVP (max 5 mL) q3-5 minutes.
   B. Hydrocortisone 2 mg/kg (max 100 mg) IVP. Only use it for life-threatening reactions (hypotension, bronchospasm, angioedema involving the airway) not responsive to other measures.

3. PREMEDICATIONS AND SUPPORTIVE CARE: Refer to Section 6.3.1.2.

4. PAIN MANAGEMENT: Refer to Section 6.3.1.2

5. Aldesleukin (IL-2) & ch14.18 ADMINISTRATION SCHEDULE
   DAY 7 thru Day 10
   DAY 7 at 9 am: Begin Aldesleukin (IL-2) (4.5 MIU/m^2/day) IV continuous infusion over 24 hours in D5W with 0.1% human serum albumin as needed for a total infusion time of 96 hours (4 days).
   Day 7 thru Day 10:
   10 am: NS bolus (10 mL/kg) over 1 hour days 7, 8, 9, 10
   11 am: Chimeric Antibody ch14.18 (25 mg/m^2/day) mg IV in 100 mL NS per Section 6.3.1.1.

6. MONITORING:
   A. During the first hour of starting aldesleukin (IL-2) infusion on Day 7 and ch14.18 infusion on Days 7-10 do VS every 1hr during ch14.18 infusion, and continue only if clinically indicated.
   B. Strict I & O every 4 hours, Dipstick urine for heme and SG every void, BID weights.
   C. Call Hematology/Oncology MD for:
      - Systolic B/P >150 or < 80, HR > 160 or < 60; Temp ≥ 38.5;
      - Respiratory rate >40;
      - Neuropathic pain requiring an increase in narcotic infusion rate;
      - Urticaria, bronchospasm, peripheral/sensory neurotoxicity, new coughs.

6.4 Dose Modifications for Toxicity

6.4.1 GM-CSF (Please see sections 6.4.3.2 and 6.4.3.3 for dose modification instructions specific for GM-CSF + ch14.18)

If GM-CSF related toxicities are observed during the GM-CSF alone or during ch14.18 infusion, the dose of GM-CSF must be reduced by 50% or, if necessary, discontinued while maintaining the dose of ch14.18. The following toxicities attributed to GM-CSF would require dose modifications:

- Persistent high (> 39.5°C) fever with no obvious source of infection and negative blood cultures while on GM-CSF without ch14.18; decrease dose by 50% or stop it if fever persists with reduced dose.

GM-CSF dose will be held if the total white cell count is > 50,000/µL. This is not a toxicity of GM-CSF but rather an expected outcome related to its use. The GM-CSF will be held until the total white cell count is less than 20,000/µL and then GM-CSF will be resumed at half dose for the remainder of that course. Full dose GM-CSF will be used for subsequent GM-CSF courses.

Significant hypersensitivity reactions at the GM-CSF injection site have been seen occasionally during later courses. GM-CSF should be held whenever this occurs.
6.4.2 Dose modifications for Aldesleukin (IL-2) (Please see sections 6.4.3.2 and 6.4.3.3 for dose modification instructions specific for aldesleukin (IL-2) + ch14.18)

See sections 6.3.1.3 through 6.3.1.5 for modifications due to hypotension, hypersensitivity and acute vascular leak syndrome.

For toxicities other than hypotension, hypersensitivity and acute vascular leak syndrome and those exclusions in Section 6.4.3.1: Treatment with aldesleukin (IL-2) should be continued unless Grade 3 toxicity occurs at which time aldesleukin (IL-2) should be discontinued until a return to baseline pre-therapy values or Grade 1 toxicity. Aldesleukin can then be resumed at 50% dose. If tolerated, subsequent aldesleukin dose will remain at 50%. In the case of Grade 4 toxicity from aldesleukin (IL-2), aldesleukin (IL-2) should be discontinued. If this occurs or if Grade 3 or Grade 4 toxicity occurs after resumption at 50% of the initial dose, aldesleukin (IL-2) will be discontinued and the subject will be allowed to use GM-CSF + ch14.18 instead of aldesleukin (IL-2) + ch14.18 for subsequent courses of immunotherapy.

6.4.3 Dose modifications for ch14.18 + aldesleukin (IL-2) or GM-CSF

6.4.3.1 Expected toxicities that do not require ch14.18 dose modification.

The following expected toxicities will NOT require dose modification provided that these toxicities are judged to be tolerable by the responsible clinician, as well as the patient and family.

a. Grade 4 pain (requires intravenous narcotics).
b. Grade 3 nausea and vomiting and diarrhea.
c. Grade 3 fever.
d. Grade 3 skin toxicity that remains stable and tolerable, or improves with treatment (e.g., IV diphenhydramine) within 24 hrs.
e. Grade 3 electrolytes (especially hyponatremia ≤124 mmol/L in the absence of CNS symptoms and sequelae) that improve with treatment within 24 hrs.
f. Grade 3 hepatic toxicity that returns to Grade 1 prior to the time for next ch14.18 treatment course.
g. Grade 3 neurotoxicity with subjective findings (e.g., tingling, hot or cold hands, etc.)
h. Grade 4 hematologic toxicity, which improves to at least Grade 2 or baseline pretherapy values within one week of completing Interleukin-2 infusion.
i. Grade 3 performance (Karnofsky 30 - <50%, see Appendix I)
j. Impaired visual accommodation, correctable with eye glasses.
k. Altered taste.

6.4.3.2 Dose modification of ch14.18 + cytokine (IL-2 or GM-CSF)

For any of the following toxicities requiring dose modifications, please notify the study chair immediately.

a. Significant hypotension - see Section 6.3.1.4.
b. Grade 3 vascular leak syndrome (respiratory compromise or fluid support required) see Section 6.3.15.
c. Hypersensitivity reaction - see Section 6.3.1.3.
d. Grade 3 infection during infusion of ch14.18 + cytokine: abort the immunotherapy course. Missed doses will not be replaced. May proceed to the subsequent planned immunotherapy course only when infection resolves or under control (asymptomatic and negative blood culture). However, if the Grade 3 infection occurs after ≥ 2 days of cytokine administration only, then start the administration of ch14.18 + cytokine after the infection resolves or is...
under control. If Grade 3 infection occurs during the first 2 days of cytokine administration only, then start from the beginning of cytokine alone when infection is under control.

e. Dilated pupils with sluggish light reflex +/- photophobia: abort the immunotherapy course. If papillary abnormalities remain stable or improve before next immunotherapy course is due, give 50% ch14.18 dose and full dose cytokine. If 50% ch14.18 is tolerated without worsening of ocular toxicity, give full dose ch14.18 in subsequent courses.25 If visual toxicity worsens, patient should discontinue all immunotherapy.

6.4.3.3 Criteria for stopping ch14.18 + cytokines:
Patients should be taken off immunotherapy if the following toxicities occur (please note that patients should remain ON STUDY, and treated with isotretinoin). For any of these toxicities, please notify study chairs immediately.

a. Anaphylaxis or symptomatic bronchospasm as specified in Section 6.3.1.3.
b. Grade 3 serum sickness: patient will be taken off immunotherapy
c. Grade 4 severe, unrelenting neuropathic pain unresponsive to continuous infusion of narcotics and other adjuvant measures including lidocaine infusions.
d. Neurotoxicity: 1) Grade 3 sensory changes interfering with daily activities >2 weeks after completing ch14.18 therapy; 2) Objective motor weakness; 3) Grade 3 vision toxicity (i.e., subtotal vision loss per toxicity scale).
e. Grade 4 hyponatremia (<120 mmol/L) despite appropriate fluid management.
f. Grade 4 Skin Toxicity.
g. Grade 4 QTc
h. Grade 4 ventricular arrhythmia

6.4.3.4 Cardiac Sub-study Dose Reduction and/or therapy discontinuation Criteria

a. QTc \( \geq 501 \) ms CTCAE Grade 3 with QTcF confirmed by the central lab (per Section 5.6.6). Patient should be hospitalized or monitored by the investigator with hourly ECGs until QTcF < 500 ms. Electrolytes and clinical factors should be evaluated. In patients with a confirmed QTcF > 500 ms, ch14.18 administration will be discontinued until the QTcF prolongation returns to baseline. Per Investigator judgment, ch14.18 dosing can resume in the next treatment cycle if the QTcF has returned near its baseline value.

b. QTc > 500 ms or > 60 ms change from baseline and Torsades de pointes or polymorphic ventricular tachycardia (CTCAE Grade 4 toxicity including life threatening signs or symptoms of serious arrhythmia) should result in discontinuation of protocol therapy.

6.4.4 Dose Modifications for isotretinoin (13-cis-retinoic acid, or RA)

6.4.4.1 Prior to each course a patient must have:

a. ALT \( \leq 5 \times \) normal, total bilirubin \( < 1.5 \times \) normal
b. Skin toxicity no greater than Grade 1
c. Serum triglycerides < 500 mg/dL
d. \( \leq \) Grade 1 hematuria and/or proteinuria on urinalysis
e. Serum creatinine < 1.5 mg/dL
f. Serum calcium < 11.6 mg/dL

For new onset hematuria of any grade, or increase in hematuria from baseline, check creatinine and BP. For stable \( \leq \) Grade 1 hematuria without ↑ creatinine and BP, one may start cis-RA but with close monitoring of hematuria, creatinine and BP. If associated with ↑ creatinine or hypertension, hold cis-RA until resolved.
6.4.4.2 Dose modification criteria

a. Decrease dose to 125 mg/m²/day or 4 mg/kg/day if child weighs ≤ 12 kg for subsequent cycles for the occurrence of any Grade 3 or 4 toxicities EXCLUDING: Grade 3 or 4 hematologic, Grade 3 hepatic, Grade 3 nausea, Grade 3 vomiting, or Grade 3 fever. If the same Grade 3 or 4 toxicity recurs at 125 mg/m², then decrease dose to 100 mg/m²/day or 3.33 mg/kg/day if child weighs ≤ 12 kg. An additional course of 100 mg/m²/day should be given before discontinuing the drug.

b. It has been reported (rarely) that some patients treated with isotretinoin (13-cis-retinoic acid, or RA) develop new areas of abnormal uptake on bone scan, likely due to increased bone resorption. If such changes occur during retinoic acid phase in absence of other evidence of tumor recurrence by MIBG scan, etc., notify the Study Chair before reporting as disease progression.

c. If criteria to begin next course are not met by the date course is due to begin, delay course for one week. If criteria still not met, hold therapy until criteria are met, and reduce dose to 125 mg/m²/day or 4 mg/kg/day if child weighs ≤ 12 kg. An additional dose reduction to 100 mg/m²/day (3.33 mg/kg/day if child weighs ≤ 12 kg) should occur if criteria are not met within one week after due date for subsequent courses.

d. If serum creatinine increases by > 50% in any course of therapy, creatinine clearance or GFR should be done prior to starting next course, and a urinalysis. If creatinine clearance and/or GFR are < 50 mL/min/1.73 m², decrease dose by 50%, and monitor serum creatinine 2 times per week along with urinalysis. If patient develops worsening hematuria, and/or proteinuria, hypertension, and/or further increase in creatinine, hold drug until these parameters return to baseline levels.

e. If patient develops > Grade 1 hematuria, proteinuria, and/or hypertension during any course of therapy, hold medication until these symptoms resolve or are under control and notify Study Chair.

f. For localized cheilitis, apply topical vitamin E to lips for subsequent cycles. If this does not control symptoms sufficiently to allow sufficient oral intake, then decrease dose to 125 mg/m²/day or 4 mg/kg/day if child weighs ≤ 12 kg.

g. If serum triglycerides are > 500 mg/dL when next course is due, start patient on medical therapy for serum triglyceride reduction and begin at the same dosage. If serum triglycerides are < 500 mg/dL by the time subsequent course is due, then continue at same dosage. If triglycerides are still > 500 mg/dL after one course on medical therapy, then reduce dose to 125 mg/m²/day or 4 mg/kg/day if child weighs ≤ 12 kg for subsequent courses.

Note: Hypersensitivity will be referred as ‘allergic reaction and anaphylaxis and Vascular leak syndrome as ‘Capillary leak syndrome’ per CTCAE v 4.0 followed starting October 1st, 2011.

6.5 Concomitant Therapy

6.5.1 No other anti-cancer therapy will be allowed.

6.5.2 Immunosuppressive drugs (corticosteroids use other than for acute allergic reactions to immunotherapy, cyclosporine, etc.) and pentoxifylline are prohibited during study.

6.5.3 Corticosteroid therapy should be used only for life-threatening conditions (i.e. treatment of increased intracranial pressure in patients with CNS tumors, life-threatening allergic reactions) unresponsive to
other measures. Corticosteroids will impair the immune activation that is a critical part of the protocol therapy. **The use of steroids at any time during immunotherapy requires clear justification and documentation. Should it occur, the investigator should discuss with study coordinators before proceeding with further protocol therapy.**

6.5.4
The use of IVIG post-ASCT is discouraged. If necessary, its use should be limited to the first 100 days post-ASCT (or Day 51 of immunotherapy calendar) because IVIG may interfere with antibody (ch14.18) dependent cellular cytotoxicity. For patients randomized to immunotherapy, IVIG should not be given within 2 weeks of starting ch14.18 treatment and 1 week after completing ch14.18 therapy; i.e. if necessary, it may be given on Days 14-17 and 42-45 of immunotherapy calendar.

6.5.5
Cytokines or growth factors (G-CSF, Interferon, etc.) not included in the Treatment Plan are prohibited during the study.

6.5.6
Radiographic contrast materials have been associated with an increased risk of anaphylactic reactions if used during aldesleukin (IL-2) therefore, contrast should be avoided during the Interleukin-2 infusion and for at least 1 week after the completion of aldesleukin (IL-2).

6.5.7
Appropriate antibiotics, blood products, antiemetics, fluids, electrolytes and general supportive care are to be used as necessary.

7.0 EVALUATION CRITERIA
7.1 Common Terminology Criteria for Adverse Events (CTCAE)
This study will utilize the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. The descriptions and grading scales found in the revised CTCAE version 4.0 will be utilized for reporting beginning October 1st, 2011. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0, which can be downloaded from the CTEP web site (http://ctep.cancer.gov).

7.2 Response Criteria for Patients with Solid Tumors
This study will use the International Neuroblastoma Staging System (INSS) Response Evaluation Criteria. This will allow direct comparison with legacy COG studies as well as Phase 3 trials performed abroad.

7.3 International Staging System93
Stage 1: Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative microscopically (nodes attached to and removed with the primary tumor may be positive).

Stage 2A: Localized tumor with incomplete gross excision; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically.

Stage 2B: Localized tumor with or without complete gross excision; with ipsilateral nonadherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes must be negative microscopically.

Stage 3: Unresectable unilateral tumor infiltrating across the midline with or without regional lymph node involvement; or, localized unilateral tumor with contralateral regional lymph node involvement; or,
midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement. The midline is defined as the vertebral column. Tumors originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.

Stage 4: Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin and/or other organs (except as defined for Stage 4S).

Stage 4S: Localized primary tumor (as defined for Stage 1 or 2A or 2B), with dissemination limited to liver, skin, and/or bone marrow (limited to infants < 1 year of age). Marrow involvement should be < 10% of total nucleated cells identified as malignant on bone marrow biopsy or on marrow aspirate. MIBG scan (if performed) should be negative in the marrow.

7.4 International Response Criteria
Measurable tumor is defined as the product of the longest x widest perpendicular diameter. The third dimension is added when possible. Elevated catecholamine metabolite levels and tumor cell invasion of bone marrow also is considered measurable tumor.

7.4.1 Complete Response (CR)**
No evidence of primary tumor, no evidence of metastases (chest, abdomen, liver, bone, bone marrow, nodes, etc.), and HVA/VMA normal.

7.4.2 Very Good Partial Response (VGPR)
Greater than 90% reduction of primary tumor; no metastatic tumor (as above except bone); no new bone lesions, all pre-existing lesions improved on bone scan; HVA/VMA normal.

7.4.3 Partial Response (PR)
Fifty-90% reduction of primary tumor; 50% or greater reduction in measurable sites of metastases; 0-1 bone marrow samples with tumor; number of positive bone sites decreased by > 50%.

7.4.4 Mixed Response (MR)
Greater than 50% reduction of any measurable lesion (primary or metastases) with, < 50% reduction in any other site; no new lesions; <25% increase in any existing lesion (exclude bone marrow evaluation).

7.4.5 No Response (NR)
No new lesions; <50% reduction but <25% increase in any existing lesion (exclude bone marrow evaluation).

7.4.6 Progressive disease (PD)**
Any new lesion or increase of a measurable lesion by > 25%; previous negative marrow positive for tumor.

** MIBG scan must be negative for patient to be classified as having a complete response. New site of disease documented by MIBG scan qualifies patient as having progressive disease.

8.0 CRITERIA FOR DISCONTINUATION OF PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Discontinuation of Protocol Therapy
a. Completion of the planned therapy.
   b. Toxicity as defined in Section 6.4.3.3 (in either GM-CSF or aldesleukin (IL-2) courses).
   c. Refusal of further protocol therapy by patient, parent, or guardian.
   d. Physician determines it is in patient’s best interest.
e. Recurrent or progressive disease at any time.
f. Use of other anti-cancer agents.
g. Grade 4 QTc or Grade 4 ventricular arrhythmia.

8.2 Off Study Criteria
a. Death
b. Lost to follow-up
c. Patient enrollment onto another COG study with tumor therapeutic intent (e.g., at recurrence).
d. Withdrawal of consent for any further data submission.
e. Tenth anniversary of study entry

9.0 REGISTRATION AND STUDY MONITORING

9.1 IRB Approval
Local IRB/REB approval of this study must be obtained by a site prior to enrolling patients. Sites must submit IRB/REB approvals to the NCI’s Cancer Trials Support Unit (CTSU) Regulatory Office and allow 3 business days for processing. The submission must include a fax coversheet (or optional CTSU IRB Transmittal Sheet) and the IRB approval document(s). The CTSU IRB Certification Form may be submitted in lieu of the signed IRB approval letter. All CTSU forms can be located on the CTSU web page (https://www.ctsu.org). Any other regulatory documents needed for access to the study enrollment screens will be listed for the study on the CTSU Member’s Website under the RSS Tab.

IRB/REB approval documents may be faxed (1-215-569-0206), emailed (CTSURegulatory@ctsu.coccg.org) or mailed to the CTSU Regulatory office.

When a site has a pending patient enrollment within the next 24 hours, this is considered a “Time of Need” registration. For Time of Need registrations, in addition to marking your submissions as ‘URGENT’ and faxing the regulatory documents, call the CTSU Regulatory Helpdesk at: 1-866-651-CTSU. For general (non-regulatory) questions, call the CTSU General Helpdesk at: 1-888-823-5923.

9.2 Training
In addition to obtaining IRB approval, all physicians, nurses, and pharmacists involved in the conduct of ANBL0032 must complete web based training. The training module with appropriate dosing, administration guidelines and toxicity management for the agents used in this trial, along with instructions for the use of the application, can be found in power-point (PPT) in the study page of the COG website. Upon completion of the training by an institution’s staff, attested to by the Principal Investigator, patient enrollment on ANBL0032 via RDE will be permitted. Sites participating in the ECG cohort must complete training regarding the performance of 12-Lead ECGs prior to the conduct of study related ECGs.

9.3 Patient Registration
Prior to study enrollment, all patients must have been enrolled via the RDE system into the COG Cancer Registry (Diagnosis/Registry). The patient registration application is available 24 hours a Day, 7 days a week. If you have problems with registration, please refer to the online help in the eRDE area of the COG website.

A Biopathology Center (BPC) number will be assigned as part of the registration process. Each patient will be assigned only one BPC number per COG Patient ID. Please use this number as part of the labeling information on all banking and biology specimens sent to the Biopathology Center or a COG Reference
Laboratory. If you have a question about a patient’s BPC Number, please call the Biopathology Center at (800) 347-2486.

9.4 Study Enrollment
Patients may be enrolled on the study once all eligibility requirements for the study have been met. Study enrollment is accomplished by going to the Enrollment application in the RDE system.

Prior to enrollment on this study, patients must be assigned a COG patient ID number. This number is obtained via the eRDE system once authorization for the release of protected health information (PHI) has been obtained. The COG patient ID number is used to identify the patient in all future interactions with COG. If you have problems with the registration, please refer to the online help.

In order for an institution to maintain COG membership requirements, every newly diagnosed patient needs to be offered participation in ACCRN07, Protocol for the Enrollment on the Official COG Registry, The Childhood Cancer Research Network (CCRN).

9.5 Timing
Patients must be enrolled before treatment begins. The date protocol therapy is projected to start must be no later than ten (10) calendar days after the date of study enrollment.

9.6 Data
This study will use the COG Remote Data Entry System (RDE) for data entry. If you are unable to access the RDE, call 352-273-0562 or 273-0550.

10.0 ADVERSE EVENT REPORTING REQUIREMENTS
The descriptions and grading scales found in the revised CTCAE version 4.0 will be utilized for reporting beginning October 1st, 2011.

10.1 Purpose
Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Certain adverse events must be reported in an expedited manner to allow for timelier monitoring of patient safety and care. The following sections provide information about expedited reporting.

10.2 Determination of Reporting Requirements
Reporting requirements may include the following considerations: 1) whether the patient has received an investigational or commercial agent; 2) the characteristics of the adverse event including the grade (severity), the relationship to the study therapy (attribution), and the prior experience (expectedness) of the adverse event; 3) the Phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

An investigational agent is a protocol drug administered under an Investigational New Drug Application (IND). In some instances, the investigational agent may be available commercially, but is actually being tested for indications not included in the approved package label.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. The NCI, rather than a commercial distributor, may on some occasions distribute commercial agents for a trial.

When a study includes both investigational and commercial agents, the following rules apply.

- Concurrent administration: When an investigational agent is used in combination with a
commercial agent, the combination is considered to be investigational and expedited reporting of adverse events would follow the guidelines for investigational agents.

- **Sequential administration:** When a study includes an investigational agent and a commercial agent on the same study arm, but the commercial agent is given for a period of time prior to starting the investigational agent, expedited reporting of adverse events which occur prior to starting the investigational agent would follow the guidelines for commercial agents. Once therapy with the investigational agent is initiated, all expedited reporting of adverse events follow the investigational agent reporting guidelines.

10.3 **Steps to Determine if an Adverse Event is to be Reported in an Expedited Manner**

**Step 1:** Identify the type of event using the NCI Common Terminology Criteria v. 3.0 (CTCAE) (v 4.0 will be used starting from October 1st, 2011). The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be downloaded from the CTEP home page ([http://ctep.cancer.gov](http://ctep.cancer.gov)). All appropriate treatment locations should have access to a copy of the CTCAE.

**Step 2:** Grade the event using the NCI CTCAE.

**Step 3:** Determine the attribution of adverse event in relation to the protocol therapy. Attribution categories are: Unrelated, Unlikely, Possible, Probable, and Definite.

**Step 4:** Determine the prior experience of the adverse event.

*Expected events for a CTEP IND agent are defined as those listed in the ASAEL* (Agent Specific Adverse Event List), a subset of the CAEPR (Comprehensive Adverse Event and Potential Risks). For investigational agents that are not commercially available and are being studied under a company’s IND, expected AEs are usually based on the Investigator’s Brochure.

*Unexpected events for a CTEP IND agent are defined as those NOT listed in the ASAEL.*

Guidance on expectedness of the agent is provided in the Drug Information Section of this protocol.

**Step 5:** Review Tables A and/or B in this section to determine if:

- there are any protocol-specific requirements for expedited reporting of specific adverse events that require special monitoring; and/or
- there are any protocol-specific exceptions to the reporting requirements.

**Step 6:** Determine if the protocol treatment given prior to the adverse event included an investigational agent, a commercial agent, or a combination of investigational and commercial agents.

**Note:** If the patient received at least one dose of investigational agent, follow the guidelines in Table A. If no investigational agent was administered, follow the guidelines in Table B.

10.4 **Reporting Methods**

- Use the NCI’s Adverse Event Expedited Reporting System (AdEERS). The NCI’s guidelines for AdEERS can be found at [http://ctep.cancer.gov](http://ctep.cancer.gov).

  An AdEERS report must be submitted by one of the following methods:

  - Electronically submit the report via the AdEERS Web-based application located at [http://ctep.cancer.gov/reporting/adeers.html](http://ctep.cancer.gov/reporting/adeers.html), or
  - Fax supporting documentation for AEs related to investigational agents to:
• The NCI for agents supplied under a CTEP IND only (fax # 301-230-0159).
• and to COG for all IND studies (fax # 626-241-1795; attention: COG AE Coordinator).

- DO NOT send the supporting documentation for AEs related to commercial agents to the NCI. Fax this material to COG (fax # 626-241-1795; attention: COG AE Coordinator).
- ALWAYS include the ticket number on all faxed documents.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

10.5 When to report an event in an expedited manner
- Some adverse events require notification within 24 hours (refer to Table A) to NCI via the web based application and/or notify the Study Chair.

In the rare situation where Internet connectivity is disrupted, the 24-hour notification is to be made to the NCI for agents supplied under a CTEP IND by telephone call to 301-897-7497. In addition, once Internet connectivity is restored, a 24-hour notification that was phoned in must be entered into the electronic AdEERS system by the original submitter of the report at the site.

- Submit the report within 5 calendar days of learning of the event.

10.6 Other recipients of adverse event reports
COG will forward reports and supporting documentation to the Study Chair, to the FDA (when COG holds the IND) and to the pharmaceutical company (for industry sponsored trials).

Adverse events determined to be reportable must also be reported according to the local policy and procedures to the Institutional Review Board responsible for oversight of the patient.
10.7 Reporting of Adverse Events for investigational agents – AdEERS 24-hour notifications, and complete report requirements

Reporting requirements are provided in Table A. The investigational agent used in this study is Chimeric human/murine ch14.18 monoclonal antibody (MAB Ch14.18) (NSC #623408), IND# 4308.

**Table A** Phase 2 and 3 Trials and COG Group-wide Pilot Studies utilizing an Agent under a CTEP IND or a Non-CTEP IND: AdEERS Expedited Reporting Requirements for Adverse Events That Occur Within 30 Days\(^1\) of the Last Dose of the Investigational Agent

<table>
<thead>
<tr>
<th></th>
<th>Grade 1 Unexpected</th>
<th>Grade 1 Expected</th>
<th>Grade 2 Unexpected</th>
<th>Grade 2 Expected</th>
<th>Grade 3 Unexpected</th>
<th>Grade 3 Expected</th>
<th>Grades 4 &amp; 5 (^2) Unexpected</th>
<th>Grades 4 &amp; 5 (^2) Expected</th>
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<td>Unrelated Unlikely</td>
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<td>Not Required</td>
<td>5 Calendar Days</td>
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<td>5 Calendar Days</td>
<td>Not Required</td>
<td>5 Calendar Days</td>
<td>5 Calendar Days</td>
</tr>
<tr>
<td>Possible Probable</td>
<td>Not Required</td>
<td>5 Calendar Days</td>
<td>Not Required</td>
<td>5 Calendar Days</td>
<td>5 Calendar Days</td>
<td>5 Calendar Days</td>
<td>24-Hour; 5 Calendar Days</td>
<td>5 Calendar Days</td>
</tr>
<tr>
<td>Definite</td>
<td>Not Required</td>
<td>Not Required</td>
<td>5 Calendar Days</td>
<td>5 Calendar Days</td>
<td>Not Required</td>
<td>5 Calendar Days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND or non-CTEP IND require reporting as follows:

AdEERS 24-hour notification (via AdEERS for CTEP IND agents; via e-mail to COG AE Coordinator for agents in Non-CTEP IND studies) followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events
- AdEERS 5 calendar day report:
  - Grade 3 unexpected events with hospitalization or prolongation of hospitalization (see exceptions below)
  - Grade 5 expected events

\(^2\) Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

\(^3\) Please see instructions for special monitoring and exceptions below under section entitled “Additional Instructions or Exceptions.”

Note: All deaths on study require timely reporting to COG via RDE regardless of causality. Attribution to treatment or other cause must be provided.

- **Expedited AE reporting timelines defined:**
  - “24 hours; 5 calendar days” – The investigator must initially report the AE (via AdEERS for CTEP IND agents; via e-mail to COG AE Coordinator for agents in non-CTEP IND studies) within 24 hours of learning of the event followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.
  - “5 calendar days” - A complete AdEERS report on the AE must be submitted within 5 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE Grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS if the event occurs following treatment with an agent under a CTEP IND.

March 2005
• Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

• Protocol specific reporting of AEs, in addition to the AdEERS requirements, are to be entered in the COG remote data entry system.

Additional Instructions or Exceptions to AdEERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing an Agent under a CTEP IND or Non-CTEP IND:

Additional AdEERS reporting segment:

• Any death that occurs more than 30 days after the last dose of treatment with an investigational agent which can be attributed (possibly, probably, or definitely) to the agent and is not due to cancer recurrence/progression must be reported via AdEERS for an agent under a CTEP IND [and via AdEERS for non-CTEP IND agent] per the timelines outlined in the table above.

• All the following ≥ Grade 3 AEs regardless of their attribution to study drug, whether they are expected or unexpected and regardless of whether they required hospitalization or prolonged hospitalization should be reported as AdEERS. These include Allergic reactions/hypersensitivity, Hypotension, Urticaria, Adult respiratory distress syndrome (ARDS), Dyspnea, Cytokine release syndrome/Acute infusion reaction, and Acute vascular leak syndrome.

Note: Allergic reaction/hypersensitivity will be referred as ‘Allergic reaction and anaphylaxis’ and ‘Acute vascular leak syndrome will be referred as ‘Capillary leak syndrome’ per the CTCAEv4.0 followed from October 1st, 2011.

Exceptions to AdEERS:

• ≤ Grade 4 lymphocyte.

• ≤ Grade 4 hematologic toxicity, which improves to at least Grade 2 or baseline pretherapy values within one week of completing Interleukin-2 infusion.

• ≤ Grade 4 hematologic toxicity, which improves to at least Grade 2 or baseline pretherapy values within one week of completing Ch.14.18 on Cycles 1, 3, and 5.

• Grade 2 hematologic toxicity

10.8 Reporting of Adverse Events for commercial agents – AdEERS abbreviated pathway

The following are expedited reporting requirements for adverse events experienced by patients on study who have not received any doses of an investigational agent on this study. Commercial reporting requirements are provided in Table B.

COG requires the AdEERS report to be submitted within 5 calendar days of learning of the event.
Table B
Reporting requirements for adverse events experienced by patients on study who have NOT received any doses of an investigational agent on this study.

AdEERS Reporting Requirements for Adverse Events That Occur During Therapy With a Commercial Agent or Within 30 Days¹

<table>
<thead>
<tr>
<th>Attribution</th>
<th>Grade 4</th>
<th>Grade 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexpected</td>
<td>AdEERS</td>
<td>AdEERS</td>
</tr>
<tr>
<td>Expected</td>
<td>Unexpected</td>
<td>AdEERS</td>
</tr>
<tr>
<td>Unrelated or Unlikely</td>
<td>Unexpected</td>
<td>AdEERS</td>
</tr>
<tr>
<td>Possible, Probable, Definite</td>
<td>AdEERS</td>
<td>AdEERS</td>
</tr>
</tbody>
</table>

¹This includes all deaths within 30 days of the last dose of treatment with a commercial agent, regardless of attribution. Any death that occurs more than 30 days after the last dose of treatment with a commercial agent which can be attributed (possibly, probably, or definitely) to the agent and is not due to cancer recurrence must be reported via AdEERS.

10.9 Routine Adverse Event Reporting

Note: The guidelines below are for routine reporting of study specific adverse events on the COG case report forms.

The NCI defines both routine and expedited AE reporting. Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database. For this study, non-targeted routine reporting will include all AdEERS reportable events and Grade 3 and higher AEs. Targeted routine reporting will include all grades of AEs to be reported including Allergic reaction/hypersensitivity, Hypotension, Urticaria, Adult respiratory distress syndrome (ARDS), Dyspnea, Cytokine release syndrome/Acute infusion reaction, and Acute vascular leak syndrome. (Allergic reaction/hypersensitivity will be referred as ‘Allergic reaction and anaphylaxis’ and ‘Acute vascular leak syndrome will be referred as ‘Capillary leak syndrome’ per the CTCAEv4.0 followed from October 1st, 2011).

In addition, we will be collecting the lot numbers of ch14.18 antibody, Altesleukin (IL-2) and sargramostim, whether or not there are any adverse events.

10.10 Reporting secondary AML/MDS

As of August 25, 2010, all secondary malignancies should be reported via AdEERS. In addition, secondary malignancies should be reported to the COG SDC in the eRDE using the Secondary Malignancy Report form.
11.0 STATISTICAL CONSIDERATIONS:
The primary objective of this study is to determine if monoclonal antibody Chl4.18 + cytokines + 13-cis-retinoic acid (GD2 + RA) improves event-free survival (EFS) after myeloablative therapy and stem cell rescue as compared to RA alone (RA). This will be evaluated in all randomized patients who achieve a CR, VGPR, or PR prior to ASCT and in a subset of Stage 4 patients older than one year at diagnosis who achieve a CR, VGPR, or PR prior to ASCT. The primary objective of the study was achieved when the Lan-DeMets interim monitoring boundary (Table 11.6) for EFS was met (per data frozen January 12, 2009), and randomization was halted.

The “randomized portion of the trial” is the portion prior to January 12, 2009 when strata 01-06 and 08-25 patients were randomly assigned to Treatment 01 vs 02. The “non-randomized portion of the trial” is the portion as of amendment #9, when all newly enrolled patients were non-randomly assigned to Treatment 02. Note that amendment #9 also makes allowance for patients who were previously enrolled and randomized to Treatment 01 (RA-only) to crossover to receive the ch14.18 antibody. These patients will be designated as the “crossover patients”. Crossover patients will be monitored for safety, but there will be no inferential efficacy analyses of the crossover subset.

Secondary objectives address a comparison of the two treatment regimens in terms of overall survival (OS), the decrease of the MRD burden, as well as the determination of factors that are significantly prognostic of EFS and S.

Total patient enrollment expected
ANBL0032 was originally designed to enroll 386 eligible randomized patients to address whether the addition of ch14.18 antibody improves outcome for patients with high risk neuroblastoma who attain at least a partial response before transplant. Enrollment of patients to the randomized portion of the study was permanently suspended in January 2009, when the COG Phase III Data and Safety Monitoring Committee released the interim study results demonstrating that ch14.18 antibody statistically significantly improved event-free survival for these patients. Prior to the suspension of enrollment, annual accrual was about 40 patients per year. Enrollment of patients to ANBL0032 is now (February 2011) about 120 per year. In addition, enrollment of similar patients to ANBL0931 (A Comprehensive Safety Trial of Chimeric Antibody 14.18 (ch14.18) with GM-CSF, IL-2 and Isotretinoin in High-Risk Neuroblastoma Patients Following Myeloablative Therapy, expected to complete enrollment in June 2011) is 80 per year. Thus, total COG enrollment of patients eligible for ch14.18 antibody treatment is presently about 200 per year.

ANBL0032 and ANBL0931 are the present mechanisms for patients to receive ch14.18 antibody treatment, until such time as a commercial product becomes available. The availability of the commercial product depends on several factors, including FDA approval of the agent (based, in part, on the adverse event data being collected through ANBL0931) and demonstration that the commercial product is clinically equivalent to that presently being provided by the National Cancer Institute. Until such time the commercial product is approved (and once ANBL0931 completes enrollment), ANBL0032 will be the primary mechanism available for patients to receive ch14.18 antibody treatment.

It is estimated that it might take until the end of 2013 for commercial ch14.18 antibody product to be available. Enrollment to ANBL0032 will continue until commercial product is available to collect adverse event and outcome data to better refine toxicity and outcome estimates for ch14.18 immunotherapy in a larger population of children with high-risk neuroblastoma. Given the present enrollment of ANBL0032-eligible patients to ANBL0032 and ANBL0931, ANBL0032 enrollment could reach up to 1150 patients.

Executive Summary
The primary analysis will be in an intent-to-treat comparison of EFS rates between treatment arms. Three-hundred and eighty six eligible patients will be randomized in equal proportions in a stratified
fashion to either GD2+RA or RA alone. A sample size of 386 gives 80% power for a two-sided log-rank test comparison at a level of 0.05 (or a one-sided test at a level of 0.025) to detect a 15% EFS difference (50% vs. 65%) between treatment arms. For the purposes of licensing the antibody, the definitive analysis will be performed after the occurrence of 137 events or after the last patient enrolled has been followed for 3 years, or when the efficacy monitoring boundary has been reached, whichever comes first. Sequential monitoring will be performed for early stoppage of the trial if the GD2+RA arm is either significantly worse or significantly better than RA alone based on Lan-Demets boundaries (Table 11.6) or if the conditional power falls below 20%. The trial will either be stopped or the therapy modified if the stopping rule for unacceptable toxicities is met.

Important secondary analyses include comparison of OS by treatment arm (to be performed only if the EFS treatment arm comparison is found to be significant) and comparison of the change from baseline of MRD by treatment arm.

**Endpoints**

The primary study endpoint to address Objective 1.1 will be Event-free survival (EFS). An event is defined as a relapse, progressive disease, secondary malignancy, or death. The time to event is calculated from the time of study enrollment until the first occurrence of an event or until last contact with the patient if no event occurs.

The secondary endpoint to address Objective 1.21 will be Overall Survival (OS). The event is death. The time to event is calculated from the time of study enrollment until death, or until last contact with the patient if the patient does not die. Due to crossover of patients randomized to Treatment 01 to receive ch14.18 antibody treatment, it is anticipated that the OS treatment difference will be diluted. The power to detect a statistically significant difference in the final analysis of OS is reduced by the crossovers.

EFS is accepted as a more accurate measure of treatment arm difference than OS because patients often receive multiple active salvage agents after their first relapse/PD when they go off protocol therapy. The heterogeneity of these off protocol therapies can bias, dilute, or completely obscure the OS comparison by treatment arm.

Despite the influence of off protocol therapy on OS, EFS remains an excellent surrogate for OS. That is because, unfortunately, virtually all high risk NBL patients who relapse will eventually succumb to their disease and die, albeit several years later. The 5-year OS rate of 119 patients on COG NBL relapse study P9462 is 10.4% (+/- standard error of 4.9%) (Unpublished COG Fall 2003 Progress Report for P9462). Therefore, it is the primary objective of the ANBL0032 trial to prevent relapse/PD; thus, the choice of EFS as the primary endpoint.

A practical reason for using EFS instead of OS is because of the additional follow-up time that would be required to observe enough deaths to have sufficient power. Based on P9462 data, that additional observation time would add an extra 1.5 years to the total study duration, which is not feasible.

### 11.1 Estimated annual accrual

**Randomized portion of the study**

185 high risk patients per year could potentially be accrued. However, because the patients to be enrolled are not newly diagnosed and must complete induction therapy and transplant prior to enrollment, there will be some attrition (death, relapse, drop-outs, or response less than PR).

It is estimated that 124 patients per year will be newly diagnosed, high risk who are enrolled on a COG front-line high risk study. Of these, about 45% are expected to subsequently enroll on ANBL0032. In
addition, it is estimated that at least 28 patients per year who complete induction and transplant without enrolling on a COG front-line high risk study will be eligible for and enroll on ANBL0032.

Net: 84 CR, VGPR, and PR patients per year who will be randomized post-transplant; 72 of these will be INSS stage 4; 61 of these will be either a CR or a VGPR, and 52 of the 84 are anticipated to be INSS Stage 4 with a response of either a CR or a VGPR.

For the randomized portion of the trial, randomization will be stratified by pre-ASCT response status, CR versus VGPR vs. PR and by pre-ANBL0032 treatment, as follows:

a) patient was randomized to receive purged stem cells in study A3973;
b) patient was randomized to receive unpurged stem cells in study A3973;
c) patient was not enrolled on but was treated per A3973 with purged stem cells;
d) patients was not enrolled on but treated per A3973 with unpurged stem cells;
e) patient was treated per the POG 9341/9342 or CCG-3891 protocols;
f) patient was enrolled on or treated per ANBL02P1;
g) patient was enrolled on or treated per ANBL0532;
h) patient was treated per 9640;
i) patient was treated per ANBL00P1;
j) patient was treated per CHP594/DFCI34-DAT;
k) patient was treated per NANT 2001-02;
l) patient was enrolled on or treated per ANBL07P1 or,
m) other treatment.

In addition, patients with persistent disease documented by biopsy post-ASCT/XRT (Stratum 07) will be enrolled on the study and assigned to receive RA+anti-GD2 (Treatment 02). Based on results from CCG-3891, 11.6% of patients will fall into Stratum 07, i.e., about 37 patients. These patients will be excluded from the assessment of Objective 1.1 for the analysis of the comparison of the two treatment arms.

**Strata (pre-ASCT response; pre-ANBL0032 treatment group)**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>CR; A3973 purged arm</td>
</tr>
<tr>
<td>02</td>
<td>CR; A3973 not purged arm</td>
</tr>
<tr>
<td>03</td>
<td>VGPR; A3973 purged arm</td>
</tr>
<tr>
<td>04</td>
<td>VGPR; A3973 not purged arm</td>
</tr>
<tr>
<td>05</td>
<td>PR; A3973 purged arm</td>
</tr>
<tr>
<td>06</td>
<td>PR; A3973 not purged arm</td>
</tr>
<tr>
<td>07</td>
<td>post-ASCT persistent disease documented by biopsy (assigned to Treatment 02)</td>
</tr>
<tr>
<td>08</td>
<td>CR; not enrolled on but treated per A3973, purged stem cells</td>
</tr>
<tr>
<td>09</td>
<td>CR; not enrolled on but treated per A3973, unpurged stem cells</td>
</tr>
<tr>
<td>10</td>
<td>VGPR; not enrolled on but treated per A3973, purged stem cells</td>
</tr>
<tr>
<td>11</td>
<td>VGPR; not enrolled on but treated per A3973, unpurged stem cells</td>
</tr>
<tr>
<td>12</td>
<td>PR; not enrolled on but treated per A3973, purged stem cells</td>
</tr>
<tr>
<td>13</td>
<td>PR; not enrolled on but treated per A3973, unpurged stem cells</td>
</tr>
<tr>
<td>14</td>
<td>CR; treated on or per POG 9341/9342 or CCG-3891</td>
</tr>
<tr>
<td>15</td>
<td>VGPR; treated on or per POG 9341/9342 or CCG-3891</td>
</tr>
<tr>
<td>16</td>
<td>PR; treated on or per POG 9341/9342 or CCG-3891</td>
</tr>
<tr>
<td>17</td>
<td>CR; treated with SINGLE TRANSPLANT on or per ANBL02P1, NANT2001-02, ANBL0532 or ANBL07P1</td>
</tr>
<tr>
<td>18</td>
<td>VGPR; treated with SINGLE TRANSPLANT on or per ANBL02P1, NANT2001-02, ANBL0532 or ANBL07P1</td>
</tr>
<tr>
<td>19</td>
<td>PR; treated with SINGLE TRANSPLANT on or per ANBL02P1, NANT2001-02, ANBL0532 or ANBL07P1</td>
</tr>
<tr>
<td>20</td>
<td>CR; other treatment</td>
</tr>
</tbody>
</table>
21 = VGPR; other treatment
22 = PR; other treatment
23 = CR; treated with TANDEM TRANSPLANT on or per ANBL0532, 9640, ANBL00P1, CHP594, or DFCI34-DAT
24 = VGPR; treated with TANDEM TRANSPLANT on or per ANBL0532, 9640, ANBL00P1, CHP594, or DFCI34-DAT
25 = PR; treated with TANDEM TRANSPLANT on or per ANBL0532, 9640, ANBL00P1, CHP594, or DFCI34-DAT

Prior to stopping the randomization, only the Stratum 07 patients were non-randomly assigned to Treatment 02. After stopping the randomization, all patients enrolled are non-randomly assigned to Treatment 02, and will be clearly identified by their response to a question about the timing of their enrollment, something like, “Is patient being enrolled after the randomized portion of the trial was halted?”

Treatment:
01 = RA only (permanently closed to accrual as of amendment # 9)
02 = RA + ch14.18

11.2 Sample Size
For the non-randomized portion of the study, there are no power calculations. The sample size is based on the availability of the ch14.18 antibody, as described in Section 11.1.

For the randomized portion of the study:
In consideration of the potential for study drug licensure, this study will be powered at a level of 0.025 (i.e., a level of 0.05 in a two-sided test) to address Objective 1.1, involving the intent-to-treat cohort of all eligible randomized patients. This requires n=386, as shown in Table 11.2. However, a result of p<0.05 in a one-sided test will be considered successful by COG members. At a level of 0.05 in a one-sided test, a sample size of 328 INSS Stage 4 patients will provide sufficient power for inferential testing within that cohort. Within each of the cohorts shown in Table 11.2, the following criteria were used to determine the required sample size:

Primary endpoint for Objective 1.1: Event-free survival (EFS)
Control 3-year EFS: 50% (Post Transplant)
Experimental 3-year EFS: 65% (Post Transplant)
Monitoring Method: Lan-Demets each 6 months, starting after 20% of the planned events have occurred (See Section 11.6 for details.)
Assumptions:
  a) Proportional Hazards with average post versus pre 3-year hazard ratio of 25%;
  b) That approximately 10% of patients will be lost to follow-up; and,
  c) That about 85% of study patients will be INSS Stage 4.
Follow-up: To event, or study end, whichever is first.
Note that the ‘clock starts’ for duration of survival time at the time of post-transplant randomization, i.e., study enrollment.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Sample Size</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025 level test for All Eligible Patients:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR, VGPR, &amp; PR</td>
<td>386</td>
<td>80%</td>
</tr>
<tr>
<td>CR &amp; VGPR</td>
<td>281</td>
<td>62%</td>
</tr>
<tr>
<td>0.05 level test for INSS Stage 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR, VGPR, &amp; PR</td>
<td>328</td>
<td>85%</td>
</tr>
<tr>
<td>CR &amp; VGPR</td>
<td>238</td>
<td>74%</td>
</tr>
</tbody>
</table>
In summary, to achieve 80% power at a level of 0.025 in the one-sided (i.e., 0.05 level two-sided) EFS comparison of the two treatment arms, the sample size of randomized eligible patients required is 386. In addition, we expect to accrue 37 non-randomized Stratum 07 patients. This gives a total of 423 patients on study and can be accrued in approximately 5 years.

**Twelve Lead ECG in Cardiac Safety Sub-Study Sample Size Considerations**

Sixty evaluable subjects will be enrolled within the ECG cohort. This sample size is selected based upon the size of similar studies in the patient population. Due to the morbidity and the concomitant medication use in these patients, the sample size selected will allow for a gross characterization of ECG parameters in regards to heart rate, PR, QRS, QT, QTc and morphologic changes.

Based on an inter-subject variability estimate of 20 msec using 3 replicates, 60 subjects will provide a 90% confidence interval half-width of 4.3 msec in terms of the change from baseline in corrected QT. Variability is assumed to be larger in this patient population due to medications given concurrently with the study drugs and the fluctuations in heart rate upon treatment administration.

The list of COG limited institutions allowed to participate in the ECG sub-study cohort is given below.

<table>
<thead>
<tr>
<th>No.</th>
<th>ECG STUDY PARTICIPATING INSTITUTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Children's Hospital of Philadelphia</td>
</tr>
<tr>
<td>2</td>
<td>St. Jude Children's Research Hospital Memphis</td>
</tr>
<tr>
<td>3</td>
<td>Seattle Children's</td>
</tr>
<tr>
<td>4</td>
<td>Children's Healthcare of Atlanta - Eagleton</td>
</tr>
<tr>
<td>5</td>
<td>Children's Hospital of Los Angeles</td>
</tr>
<tr>
<td>6</td>
<td>Dana-Farber Cancer Institute</td>
</tr>
<tr>
<td>7</td>
<td>Cook Children's Medical Center</td>
</tr>
<tr>
<td>8</td>
<td>C S Mott Children's Hospital</td>
</tr>
<tr>
<td>9</td>
<td>Cincinnati Children's Hospital Medical Center</td>
</tr>
<tr>
<td>10</td>
<td>Duke University Medical Center</td>
</tr>
<tr>
<td>11</td>
<td>Washington University School of Medicine</td>
</tr>
<tr>
<td>12</td>
<td>Vanderbilt University</td>
</tr>
<tr>
<td>13</td>
<td>University of California San Francisco Medical Center-Parnassus</td>
</tr>
</tbody>
</table>

**Projected accrual duration**

Randomized portion of the study:
Maximal anticipated Accrual Duration of the randomized portion of the study: 5 years
Maximal anticipated Study duration of the randomized portion of the study: 8 years (5 for accrual plus 3.0 follow-up.)

For the non-randomized portion of the study:
It is estimated that it might take until the end of 2013 for commercial ch14.18 antibody product to be available. Enrollment to ANBL0032 will continue until commercial product is available to collect adverse event and outcome data to better refine toxicity and outcome estimates for ch14.18 immunotherapy in a larger population of children with high-risk neuroblastoma. Given the present enrollment of ANBL0032-eligible patients to ANBL0032 and ANBL0931, ANBL0032 enrollment could reach up to 1150 patients.

**Accrual documented by prior trials (similar tumor types/PS/prior Rx patients)**
75 patients per year for POG 9341. (9341 eligibility is slightly more restrictive). 125-135 patients per year for CCG-3881, and 99-113 patients per year for CCG 3891 in the last 4 years.
11.5 **Timing of Registration**
It is strongly recommended that consent for ANBL0032 be obtained prior to transplant. If persistent
disease is suspected, biopsy of suspected disease is recommended but not required. As soon as the results
of the radiographic studies and the biopsy results (if done) are known, enrollment on ANBL0032 should
be performed. This should occur preferably within a window from Day 56 to Day 85 post-ASCT, but no
later than Day 100. All patients who have persistent disease documented by biopsy will be enrolled to
Stratum 07.

11.6 **Early Stopping Rules**
1) For the randomized portion of the study, there will be cause to stop the trial early if the ch14.18 + RA
arm appears to be insufficiently efficacious, significantly more efficacious, or if there are unacceptable
toxicities. The need for curtailment for non-significance of the ch14.18 + RA arm will be assessed using
Lan-DeMets boundaries for the hazard ratio (RA only: ch14.18+RA) at each interim analysis. The
interim analyses of EFS will take place every six months starting after 20% (29) of the planned events
have occurred. The lower bound is calculated based on repeated testing of the alternative hypothesis that
the relative risk is equal to 1.6 at a p-value of 0.005. This relative risk is calculated as
critcontrol:experimental using the planning parameters for 3-year EFS. The upper bound uses an alpha*t²
spending function for a cumulative alpha level of 0.025.

<table>
<thead>
<tr>
<th>Monitoring timepoint</th>
<th>Observed cumulative number of events</th>
<th>Observed proportion of total expected information</th>
<th>Observed upper boundary z-value</th>
<th>Upper boundary z-value, cum alpha=.025</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring 2006</td>
<td>29</td>
<td>0.212</td>
<td>1.963</td>
<td>3.00</td>
</tr>
<tr>
<td>Fall 2006</td>
<td>39</td>
<td>0.285</td>
<td>1.905</td>
<td>3.00</td>
</tr>
<tr>
<td>Spring 2007</td>
<td>49</td>
<td>0.360</td>
<td>2.257</td>
<td>2.91</td>
</tr>
<tr>
<td>Fall 2007</td>
<td>57</td>
<td>0.416</td>
<td>2.450</td>
<td>2.82</td>
</tr>
<tr>
<td>Spring 2008</td>
<td>62</td>
<td>0.453</td>
<td>2.120</td>
<td>2.81</td>
</tr>
<tr>
<td>Fall 2008</td>
<td>70</td>
<td>0.511</td>
<td>2.550</td>
<td>2.70</td>
</tr>
<tr>
<td>Spring 2009</td>
<td>83</td>
<td>0.606</td>
<td>2.528</td>
<td>2.55</td>
</tr>
</tbody>
</table>

These values will be used to project the conditional power of the trial when run to completion. If the
conditional power falls below 20%, the Data Monitoring Committee (DMC) will consider termination of
the trial. This curtailment has virtually no impact on the operating characteristics of the trial.

2) In the randomized portion of the trial, the need for curtailment due to toxicity was assessed using
a frequency tabulation of the unacceptable toxicities. This rule will be repeated during the non-
randomized portion of the trial for the cohort of newly enrolled patients.
- allergic reaction, grade >=4
- acute vascular leak syndrome, grade >=4
- neuropathy-motor, grade >=3, duration >= 2 weeks
  At grade >=4, requiring narcotics/lidocaine and persists >= 4 days after the end of ch14.18
  infusion:
- Pain (where xxx represents all possible values)
- Pain – Other, Specify

This tabulation will be performed twice: 1) after the first 48 patients have completed the anti-GD2 + RA
arm; and, 2) after the first 90 patients have completed the anti-GD2 + RA arm. The expected (null)
unacceptable true toxicity rate is 20% (10 out of 48 or 18 out of 90). Within the first 48 patients, if 12
(25%) or more patients have at least one such toxicity on the anti-GD2 + RA arm, then this will be cause
for a review by the DMC for consideration of modification of the dosing regimen.

Within the first 90 patients, if 23 (26%) or more patients have at least one such toxicity on the anti-GD2 +
RA arm, then this will be cause for review by the DMC. These stopping limits were determined using an
exact calculation from a two-stage design at a level of 0.09 and power of 91.8% to detect a true toxicity
rate of 34%.

3) There will also be interim monitoring of the Stratum 07 patients who have persistent disease
documented by biopsy and who are assigned to receive RA+ch14.18. It is estimated that a total of 37
such patients will be accrued. The 1-year (from the time of RA treatment start) EFS rate of the
documented disease RA patients on CCG-3891 is 35%, i.e., the “early” (post-ASCT and RA start) event
rate in this cohort is about 65%. It is hoped that the addition of ch14.18 will improve survival; otherwise,
alternative treatment options should be considered for these patients. Therefore, an acceptable early event
rate of 50% has been chosen for patients treated with RA+ch14.18. An unacceptable event rate is 75%.
A two-stage rule will be used. For the first stage, if there are 10 or more event within the first 14 Stratum
07 patients (≥ 71.4%) (followed for at least six months or until death), then this will be cause for a review
by the DMC for consideration of other treatment alternatives for this cohort of patients. For the second
stage, if there are a total of 16 or more event out of a total of 26 Stratum 07 patients (≥ 61.5%) (followed
for at least six months), this will be cause for a DMC review. These stopping limits were determined
using an exact calculation from a two-stage design with a significance level of 0.192 and power of 96.4%.
An event is defined to be a relapse, progressive disease, second malignancy, or death.

Additionally, in adherence with standard procedure, the DMC will review all ADRs reported on both the
ch14.18 +RA arm and the RA only arm.

4) For the non-randomized portion of the study, and separately for the crossover patients, the number of
toxic deaths will be monitored. A single toxic death due to IL2 overdose occurred on the randomized
portion of the trial. If at any time a toxic death occurs a) within the patients enrolled after activation of
amendment #9 on the non-randomized portion of the trial; or, b) within the crossover patients, the study
will be referred to the DSMC and the Neuroblastoma Committee for consideration of therapy
modifications.

5) For the crossover patients, unacceptable toxicities will be defined the same way as in Rule 2) above.
We will monitor the occurrence of unacceptable toxicities in the crossover patients using the following
rule:

**Stage 1:** Accrue 11 crossover patients. If at any time, 5 or more crossover patients have at least one
unacceptable toxicity, then stop for review of dosing modifications. If 4 or fewer patients, continue to
stage 2.

**Stage 2:** Accrue 13 more crossover patients, for a total of 24 crossover patients. If at any time, 6 or more
crossover patients have at least one unacceptable toxicity, then stop for review of dosing modifications. If
5 or fewer, then conclude that the toxicity is acceptable in the crossover patients.

This rule tests the null hypothesis that the unacceptable toxicity rate is <=13% versus the alternative that
it is >=38% with 92% power and alpha=0.06.

6) For the crossover patients, the occurrence of allergic reaction/hypersensitivity (CTV code 1010.000)
events of grade >=4 will be monitored using the following rule.
Stage 1: Of the first 11 crossover patients, if at any time 2 or more patients report at least one "allergic reaction/hypersensitivity" event, then stop for dosing modifications. If 1 or 0 patients, continue to Stage 2.

Stage 2: Accrue 13 more crossover patients, for a total of 24 crossover patients. If at any time 3 or more patients report at least one "allergic reaction/hypersensitivity" event, then stop for dosing modifications. If 2 or fewer, then conclude that the "allergic reaction/hypersensitivity" toxicity is acceptable in the crossover patients.

This rule tests the null hypothesis that the allergic reaction/hypersensitivity events rate is <=3% versus the alternative that it is >=20% with 90% power and alpha=0.10.

Allergic reaction/hypersensitivity will be referred as ‘Allergic reaction and anaphylaxis’, Acute vascular leak syndrome as ‘Capillary leak syndrome’ and Neuropathy-motor will be ‘Peripheral motor neuropathy’ per CTCAE v 4.0 followed from October 1st, 2011.

11.7 Methods
For the randomized portion of the study, descriptive analyses below will be performed separately in the cohort of patients who were non-randomly assigned to the immunotherapy + RA group. For the randomized portion of the trial, inferential analyses will be performed within the cohorts of patients listed in Table 11.2. No inferential analyses will be performed within strata, although descriptive analyses may be performed within strata. All analyses will be performed in each cohort as intent-to-treat. For the non-randomized portion of the trial, no inferential analyses of EFS or OS will be performed.

11.7.1 Objective 1.1, 1.21 and 1.22
The definitive assessment of the EFS primary objective 1.1 in the randomized portion of the study was completed when the interim stopping boundary for efficacy was met. The final analyses of EFS (in the stage 4 subset (b)) and of OS (overall (a) and in the stage 4 subset (b)) will be performed based on a data snapshot taken two years after the last randomized patient was enrolled, i.e., in early 2011.

The assessment of EFS primary objective 1.1 and OS Secondary objective 1.21 will be performed in two different randomized eligible patient cohorts based on the patient’s INSS stage, although the primary analysis is based on cohort a) below. The two cohorts of patients are:

a) Intent-to-treat cohort of all those randomized eligible patients with pre-ASCT response of CR,VGPR, or PR (estimated accrual of 84 patients per year); (for Objectives 1.1 and 1.21) and,

b) Intent-to-treat cohort of INSS Stage 4 eligible patients randomized with pre-ASCT response of CR, VGPR, or PR (estimated accrual of 72 patients per year) (for objectives 1.22).

In each cohort, a one-sided log-rank test with a significance level of 0.05 will be used to test for a difference of at least 15% between the EFS rates of the anti-GD2 + RA group (65%) versus the RA only group (50%). In consideration of the potential for study drug licensure, a two-sided log-rank test at a level of 0.05 (i.e., 0.025 level one-sided) will also be used within the cohort of all randomized patients. The power to detect these differences within each cohort is shown in Table 11.2. With 386 eligible randomized patients the study will have 88% power using a 0.05 level one-sided test to detect a 15% difference (60% vs. 75%) in 3-year OS. However, the power to detect a statistically significant difference in the final analysis of OS will be reduced by the crossovers. Due to crossover of patients randomized to Treatment 01 to receive ch14.18 antibody treatment, it is anticipated that the OS treatment difference will be diluted.

In secondary analyses of EFS and OS, three approaches will be taken to account for crossovers in intent-to-treat comparisons of RA-only versus RA+anti-GD2 for EFS and OS:
i. Generate Kaplan-Meier curves and perform log rank tests, censoring the RA-only patients who crossed over at the time they crossed over;

ii. Generate Kaplan-Meier curves and perform log rank tests, censoring the RA-only randomized patients who were within one year of ASCT at the time that Amendment #9 was activated; and

iii. Using inverse probability-weighted estimators, a multivariable Cox model will be constructed to balance the treatment groups with respect to confounding baseline characteristics of the crossovers. [Curtis et al, Medical Care 45:(supp 2) Oct 2007 S103-S107 and Chlebowski et al, NEJM 360 (6): 573-587.]

The final analysis will be performed after the occurrence of 137 events, after the last patient enrolled has been followed for 3 years, or when the efficacy monitoring boundary has been reached, whichever comes first. The EFS and OS analyses will be performed in sequence. If the two-sided log-rank test comparison of intent-to-treat randomized treatment arms for EFS yields $p<0.05$, then analysis of OS will proceed.

By randomizing at the point of divergence, the resulting trial is very powerful even though it is looking for a relatively large difference of 15%. Consider the fact that we expect a large fraction of patients to be lost prior to randomization. If this number is about 50%, it means that this study is sensitive to an overall difference in EFS of only 7.5% (25% for the RA only arm versus 32.5% for the ch14.18 + RA arm). For example, a study randomized at diagnosis with 530 patients would have 80% power to detect a difference of 8.9% in 3-year EFS (25% vs. 33.9%).

11.7.2 Objective 1.23

The analysis to address secondary objective 1.23 will be performed within the cohort of eligible randomized patients with pre-ASCT response of CR, VGPR, or PR, i.e., excluding Stratum 07 patients, who have at least one specimen time-point after baseline at which they have not progressed/died. For immunocytology and RT-PCR, the MRD measurements collected are the number of neuroblastoma cells per $10^5$ blood or marrow cells. A descriptive analysis of the change from baseline of MRD will be performed for ch14.18 + RA versus RA only. Also, a Wilcoxon rank-sum test will be performed to compare the median change from baseline of MRD between the two treatment arms. MRD will be measured at two time points: 1) up to 4 weeks before starting therapy on ANBL0032 and, 2) after completion of RA. To assess the change in MRD due to immunotherapy, the change from time point 1) to time point 2) will be used. This statistical testing will be performed using MRD measurements made with immunocytology and repeated using MRD measurements made with RT-PCR.

For immunocytology, the level of detectable MRD post-transplant is 1 neuroblastoma cell per $10^5$ marrow or blood cells. For RT-PCR, the minimum detection limit is 1 neuroblastoma cell per million marrow or blood cells. (Seeger et al., Journal of Clinical Oncology, (in press)).

It is estimated that 70% of patients (n=270) will have sufficient immunocytology and RT-PCR data for these analyses (10% loss due to occurrence of events, and 20% loss due to non-compliance with sample submission). The power provided by these 270 will be extremely dependent upon the standard deviation of values of MRD change from baseline. This standard deviation is unknown at this time because this is the first study to attempt MRD measurements within this cohort of patients. Post-hoc power calculations will be performed once the standard deviation is determined. There are no plans to adjust the accrual goal for power to address this MRD objective; the study is powered for the primary EFS analysis.

For MIBG, the MRD assessment is an ordinal variable with four categories: No evidence of disease, improved, no change, progression/new lesions. The comparison between the two treatment arms will be performed using an ordinal logistic regression model. To assess the change in MRD (based on MIBG) due to immunotherapy, the assessment at time point 2) will be used in the model as the dependent
variable, the assessment at time point 1) ("baseline") will be included on the model, and the term for
treatment will be tested as an additional term in the model.

The MRD treatment arm comparisons will be performed independently for immunocytology, RT-PCR,
and MIBG. No adjustment for multiple comparisons will be made.

A compliance rate of at least 80% will be targeted for submission of immunocytology data. If samples
are not submitted or if samples are non-evaluable, they will be counted as not compliant. The compliance
rate will be reported in each agenda report, available for review by the DMC. The following two-stage
test will be used to flag the study for review should the compliance rate fall significantly below 80%. The
first stage will be performed using the first 84 patients. A one sample proportion test, monitoring for
compliance less than 80% (alternative hypothesis of 70%), will be performed. There are three possible
outcomes of this test:

- \( Z < -1.95 \) \( \rightarrow \) compliance is significantly less than 80% - study is flagged for review by
  the DMC for potentially stopping the immunocytological testing;
- \( Z > -0.38 \) \( \rightarrow \) compliance is good - no need to continue monitoring;
- \( -1.95 < Z < -0.38 \) \( \rightarrow \) compliance is borderline – proceed to second stage

If proceeding to the second stage is justified, data for the next 90 patients will be monitored for a total of
174 patients. The following outcomes are possible from the second stage of the one sample proportion
test:

- \( Z < -1.78 \) \( \rightarrow \) compliance is significantly less than 80% - study is flagged for review by
  the DMC for potentially stopping the immunocytological testing;
- \( Z > -1.78 \) \( \rightarrow \) compliance is good - no need to continue monitoring.

This two-stage test is one-sided, uses a significance level of .05, and has overall power of 80%. A test of
one proportion using a fixed sample size would require \( n=154 \) in order to have 80% power (one-sided
with .05 significance). The maximum expected sample size required by this two-stage test is 134. No
two-stage test has a smaller maximum expected sample size.

11.7.3 Objectives 1.24 and 1.25
All patients from the randomized portion of the trial (including stratum 07 patients from the randomized
portion of the trial) will be included in the analyses to address secondary objectives 1.24 and 1.25. A
multivariate Cox proportional hazards regression model will test to see if the change in MRD burden, the
Ch14.18 serum level, HACA titer, effector cell function, or serum marker for effector cell activation are
associated with EFS. Significant biologic prognostic factors will also be included in the model.

11.7.4 Objective 1.26
To address secondary objective 1.26, descriptive analyses of toxicity will be performed over all patients
from the randomized portion of the trial (including stratum 07 patients from the randomized portion of the
trial), comparing the ch14.18 + RA regimen to the RA only arm. Without treatment arm comparison,
descriptive analyses of toxicity will also be performed a) within the patients from the non-randomized
portion of the trial who received the ch14.18+cytokines under the new administration guidelines; and, b)
within the crossover patients regarding their ch14.18 treatment.

11.7.5 Objectives 1.27 and 1.28
To address Objective 1.27, EFS curves for various levels of ADCC will be descriptively compared. To
address Objective 1.28, the average level of HACA at each collection time point during immunotherapy
will be calculated.
11.7.6 **Objective 1.29**

To address Objective 1.29, a historical comparison will be made. Due to the small sample sizes, this comparison will be a descriptive one only. (However, inferential testing in the direction of concern will have already been performed using the stopping rule for DMC review of the Stratum 07 patients.) The historical control is the n=37 patients on CCG-3891 with documented residual disease who were treated with RA and who had a 3-year EFS rate of 12% ± 6%. This rate will be compared to the 3-year EFS rate for the ANBL0032 patients with persistent disease documented by biopsy who are treated with RA + ch14.18. For the CCG-3891 cohort, the “clock started” for survival time when the RA treatment started, and for the ANBL0032 patients, the clock will start at the time of ANBL0032 registration. Randomized treatment on ANBL0032 should begin within a few days after registration, so the few days difference between the two studies should be negligible.

11.7.7 **Objective 1.30**

The cohort of patients for this analysis of the 13-cis-RA PK data are patients enrolled on either A3973, ANBL0032, or other high risk studies who receive 13-cis-RA. The analyses will be conducted in the overall cohort as well as within the subset of patients who receive only one transplant and cis-RA without the addition of antibody. Patients will be pooled across these studies until at least 195 patients are available for analysis. We would not stop consenting patients to this aim or collecting blood specimens in the middle of a study simply because the minimum accrual goal of n=195 had been reached; accrual of patients towards this aim would continue until that study’s overall accrual was completed. A larger cohort would permit this aim to be addressed with greater accuracy.

For each patient, the peak serum concentration level will be determined. Pilot data\(^2\) indicate the mean peak serum concentration level will be approximately 7 µM. Given a mean peak serum concentration level of 7 µM, a sample size of n=195 will permit placement of a 90% confidence interval on the mean peak serum concentration level with a precision of +/- 0.24 µM. This confidence interval will provide a descriptive assessment of the inter-patient variability of the cis-RA plasma levels.

To determine if there is a relationship of the peak serum concentration level with event-free survival, the term for this level will be tested in a Cox proportional hazards model. If the level is statistically significant, then a) the hazard ratio will provide a measure of increased risk for an event for a unit change in the level; and, b) a cut-off in serum concentration level (low vs. high) will be identified that optimizes the difference in EFS between the low and high serum concentration groups.

To determine if there is a relationship of the peak serum concentration level with toxicity rates, Kendall’s Tau statistic will be calculated.

11.7.8 **Objective 1.31**

For the patients from the non-randomized portion of the trial, Kaplan-Meier curves of EFS and OS will be generated, including 95% confidence intervals on the curves. This will be done separately for a) patients who meet the original eligibility criteria (from the randomized portion of the trial); and, b) patients who meet the revised expanded eligibility criteria (criteria which were incorporated after the randomization was halted). To place the EFS and OS of the non-randomized cohort into context, characteristics at the time of ANBL0032 enrollment (such as MYCN, age, INSS stage, pre-ASCT response, and other factors thought to be potentially prognostic) will be compared for patients randomized to Treatment 02 (from the randomized portion of the study) vs patients non-randomly assigned to Treatment 02 (from the non-randomized portion of the study, and excluding stratum 07). This analysis of EFS and OS will be performed 2 years after the last patient was enrolled after activation of amendment #9 to the non-randomized portion of the study.
11.7.9 **Objective 1.32**  
**Safety and Toxicity:**

a. A Wilcoxon test will be used to compare the number of courses of therapy delivered for A) patients randomized to Treatment 02 (from the randomized portion of the study) vs B) patients non-randomly assigned to Treatment 02 (from the non-randomized portion of the study, and excluding stratum 07).

b. The number of patients who required a dose reduction of IL-2 or stopping of IL-2 will be tabulated.

c. Within the patients on the non-randomized portion of the trial: A) the number of toxic deaths will be monitored via rule 4 in Section 11.6; and B) the number of unacceptable toxicities will be monitored via rule 2 in Section 11.6. For the crossover patients, A) the number of toxic deaths will be monitored via rule 4 in Section 11.6 and c) the number of allergic reactions/hypersensitivity events (‘allergic reaction and anaphylaxis’ per CTCAE v 4.0 followed from October 1st, 2011).

d. Will be monitored via rule 6 in Section 11.6.

11.7.10 **Objective 1.33**  
**Twelve-Lead ECG and Corresponding Pharmacokinetic Data Analysis in Cardiac Safety Sub-study**

a. **Endpoints**

   a. ECG Endpoints

      i. QTcF (Fridericia’s) calculated as QT/RR^{1/3}
      ii. QTcB (Bazett’s) calculated as QT/RR^{1/2}
      iii. PR Interval [msec]
      iv. RR Interval [msec]
      v. QRS Duration [msec]
      vi. Heart Rate [beats/min]
      vii. QT Uncorrected.

   b. Other Endpoints

      i. ch14.18 serum concentrations taken at Cmax and troughs.
      ii. ECGs will also be evaluated for morphology and abnormalities with respect to the following:

         • Atrial fibrillation or flutter
         • Second degree heart block,
         • Third degree heart block,
         • Complete right bundle branch block,
         • Complete left bundle branch block,
         • ST segment depression,
         • T wave abnormalities (negative T waves only),
         • Myocardial infarction pattern, and
         • U waves.

   c. Analysis Considerations

      i. In general, descriptive summaries will include n, mean, standard deviation, median, minimum, maximum and 90% confidence intervals for continuous variables, and n and percent for categorical variables. Summaries will present data by assessment time when appropriate.

d. Analysis Population

   i. ECGs will be evaluated using the ECG Safety Population which consists of all ECG cohort patients dosed with ch14.18 and with at least one pre-dose ECG evaluation. Pharmacokinetics of the ECG cohort will also be evaluated using the ECG safety population. If patients have an assessment time with a
pharmacokinetic assessment but not a corresponding ECG, this assessment time will not be included in the analysis.

1. Patients meeting the following criteria will be excluded from the ECG analysis population:
   a. Patients with evidence of clinically significant cardiac disease (NYHA - class > 2) including unstable angina, acute myocardial infarction within six months of Day 1, congestive heart failure, and arrhythmia requiring therapy, with the exception of extrasystoles or minor conduction abnormalities.
   b. Patients with a baseline ECG QTcF >480 ms.

ii. Pharmacokinetics of the ECG cohort will also be evaluated using the ECG safety population. If patients have an assessment time with a pharmacokinetic assessment but not a corresponding ECG, this assessment time will not be included in the analysis.

e. ECG Summaries
   i. Continuous Assessments
      1. A descriptive summary of ECG safety data (QTcF, QTcB, PR, RR, QRS, HR, QT) including change from baseline at each planned time point and overall post-dose time points will be presented along with 90% confidence intervals. Additional analyses may be performed as broken down by gender and age. Change from baseline will be calculated for individual subjects and then summarized.
      2. QTcB and QTcF will be evaluated for correction integrity by plotting QTcF and QTcB against RR to determine any bias at the extreme heart rates.

   ii. Categorical Assessments
      1. Additional outlier analyses will be performed to determine the number and percentage of subjects with an increase from baseline in QTc > 30 msec and > 60 msec at each time point and overall (maximum change), and the number and percentage of subjects at each time point and overall (maximum value) with QTc>450 msec, >480 msec, >500 msec where QTc is in reference to both QTcF and QTcB.

   outfitter Criteria for QTc (QTcF, QTcB)

<table>
<thead>
<tr>
<th>Type of Value</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed QTc (ms)</td>
<td>≥450 and &lt;480</td>
</tr>
<tr>
<td></td>
<td>≥480 and &lt;500</td>
</tr>
<tr>
<td></td>
<td>≥500</td>
</tr>
<tr>
<td>Change in QTc (ms)</td>
<td>≥30 and &lt;60</td>
</tr>
<tr>
<td></td>
<td>≥60</td>
</tr>
</tbody>
</table>

Listings will also be provided.

   iii. Pharmacokinetic Analyses
      1. Serum concentration-time data for ch14.18 will be summarized and listed for each collection time point.
      2. Pharmacokinetic/Pharmacodynamic Analyses
         a. The relationship between actual values and time-matched changes in QTcF and QTcB, against serum concentrations of ch14.18 will be explored graphically using concentration data
from individual subjects with a fitted regression line superimposed.

b. The pk/pd analysis technique will be a repeated measures analysis of the change from baseline in QTcF and QTcB using the SAS Mixed Procedure. Concentration maybe natural log-transformed prior to analysis. Initial covariates to be included in the model will be serum concentration levels, baseline QTc, age, weight, nominal time point, and cycle. A forward selection process will be used and covariates with p-values greater than 0.15 will be considered to be removed from the model (except for concentration). AIC scores will also be taken into consideration in terms of model value and covariate predictability. Covariance structure will be set as autoregressive.

c. Additional criteria for comparing the goodness-of-fit of potential models include:
   i. improved fit of the diagnostic scatter plots (observed vs. predicted QT, residual/weighted residual vs. predicted QT or time). This type of evaluation will lend itself to possible outlier analyses and transformations.
   ii. convergence of the minimization process
   iii. completion of the covariance step without warning messages

d. In the event that a relationship between ch14.18 concentration and QT interval is observed, simulations and additional statistical analysis will be performed to assess anticipated changes in QT interval at clinically relevant doses or exposure.

11.7.11 To address Objective 1.34:
A Fisher’s exact test will be performed to determine if the presence of naturally occurring anti-glycan antibodies correlates with allergic reactions. A Wilcoxon test will be performed to determine if the presence of naturally occurring anti-glycan antibodies correlates with blood levels of ch14.18.

11.7.12 To address Objective 1.35:
Kaplan-Meier plots of EFS will be generated for the three genotype subgroups of FcR as well as for the three genotype subgroups of Kir/Kir-Ligand. In addition, a log rank test comparison will be made in a pairwise fashion of each of the genotypes within FcR and within Kir/Kir-Ligand.

12.0 GENDER/MINORITY ANALYSIS:
POG’s recent five-year retrospective analysis of female and minority accrual reveals that accrual rates for women and African Americans mirror that of Caucasian males. However, we have identified other ethnic groups (Hispanic, Native American, Asian/Pacific Islander) that are under represented due to cultural and/or socioeconomic factors. POG has implemented various geographic strategies to increase enrollment of these under represented patient subgroups, as well as methods to monitor protocol compliance with racial/ethnic guidelines.

COG will assess potential access barriers through the use of a specialized data collection form to capture extensive demographic and socioeconomic characteristics. Geocoding for the patient’s residence will be used as a proxy to characterize socioeconomic status. Also, COG investigators are encouraged to participate in the ongoing non-therapeutic cancer control study (COG 9284/9285) that evaluates barriers to patient enrollment on frontline therapeutic studies. Data from new minority subjects enrolled as a result of these efforts will be reviewed shortly after subjects begin treatment, and throughout the course of
therapy, to monitor protocol compliance with racial/ethnic guidelines. If obstacles resulting in decreased compliance are identified, appropriate measures will be instituted to boost racial/ethnic enrollment.

**Target Accrual**

The gender and ethnic distribution of the study population is expected to be:

<table>
<thead>
<tr>
<th>Ethnic Category</th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hispanic or Latino</td>
<td>33</td>
<td>54</td>
<td>87</td>
</tr>
<tr>
<td>Not Hispanic or Latino</td>
<td>427</td>
<td>636</td>
<td>1063</td>
</tr>
<tr>
<td>Ethnic Category: Total of all subjects</td>
<td>460</td>
<td>690</td>
<td>*1150</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Racial Category</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>American Indian or Alaskan Native</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Asian</td>
<td>23</td>
<td>34</td>
<td>57</td>
</tr>
<tr>
<td>Black or African American</td>
<td>46</td>
<td>71</td>
<td>117</td>
</tr>
<tr>
<td>Native Hawaiian or other Pacific Islander</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>White</td>
<td>388</td>
<td>579</td>
<td>967</td>
</tr>
<tr>
<td>Racial Category: Total of all subjects</td>
<td>460</td>
<td>690</td>
<td>*1150</td>
</tr>
</tbody>
</table>

* These totals must agree

This distribution was derived from A3973.

In this study we will define and compare patient outcome within and between the following groups: (1) males; (2) females; (3) whites; (4) Hispanics; and (5) African-Americans. The result in any one of the subgroups will be considered at odds with the overall results only if the subgroup is found to be a significant predictor in a relative risk analysis (utilizing the regression method of Cox), even after taking account of possible differences by subgroup in terms of known prognostic variables.
REFERENCES


85. Samokyszyn VM, Gall WE ZG, al e: 4-hydroxyretinoic acid, a novel substrate for human liver microsomal UDP-glucuronosyltransferase(s) and recombinant UGT2B7. J Biol Chem 275:6908-6914, 2000
APPENDIX I: CRITERIA FOR EVALUATING PERFORMANCE STATUS

The Play-Performance Scale for children is designed to provide a standardized measure of the performance status of the child with cancer. Appropriate for use with children aged 1 to 16.

Have parent select description which best describes child's play during the past week, averaging out good days and bad days.

### PERFORMANCE STATUS CRITERIA

Karnofsky and Lansky performance scores are intended to be multiples of 10

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
<th>ECOG (Zubrod) Score</th>
<th>Karnofsky Score</th>
<th>Lansky* Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fully active, able to carry on all pre-disease performance without restriction.</td>
<td>100</td>
<td>100</td>
<td>Fully active, normal.</td>
</tr>
<tr>
<td></td>
<td>90 Able to carry on normal activity, minor signs or symptoms of disease.</td>
<td>90</td>
<td>90</td>
<td>Minor restrictions in physically strenuous activity.</td>
</tr>
<tr>
<td>1</td>
<td>Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.</td>
<td>80</td>
<td>80</td>
<td>Normal activity with effort; some signs or symptoms of disease.</td>
</tr>
<tr>
<td></td>
<td>70 Cares for self, unable to carry on normal activity or do active work.</td>
<td>70</td>
<td>70</td>
<td>Both greater restriction of and less time spent in play activity.</td>
</tr>
<tr>
<td>2</td>
<td>Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours</td>
<td>60</td>
<td>60</td>
<td>Up and around, but minimal active play; keeps busy with quieter activities.</td>
</tr>
<tr>
<td></td>
<td>50 Requires considerable assistance and frequent medical care.</td>
<td>50</td>
<td>50</td>
<td>Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.</td>
</tr>
<tr>
<td>3</td>
<td>Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.</td>
<td>40</td>
<td>40</td>
<td>Mostly in bed; participates in quiet activities.</td>
</tr>
<tr>
<td></td>
<td>30 Severely disabled, hospitalization indicated. Death not imminent.</td>
<td>30</td>
<td>30</td>
<td>In bed; needs assistance even for quiet play.</td>
</tr>
<tr>
<td>4</td>
<td>Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.</td>
<td>20</td>
<td>20</td>
<td>Often sleeping; play entirely limited to very passive activities.</td>
</tr>
<tr>
<td></td>
<td>10 Moribund, fatal processes progressing rapidly.</td>
<td>10</td>
<td>10</td>
<td>No play; does not get out of bed.</td>
</tr>
</tbody>
</table>

*The conversion of the Lansky to ECOG scales is intended for NCI reporting purposes only.
APPENDIX II: RECOMMENDED PROCEDURE FOR PERFORMING MIBG SCANS

MIBG (meta-iodobenzylguanidine) was developed by Dr. Donald Wieland of the University of Michigan in the late 1970’s for scintigraphic imaging of neuroendocrine tumors, specifically pheochromocytomas and neuroblastomas. MIBG is an aralkylguanidine which bears structural similarity to the neurotransmitter and catecholamine hormone norepinephrine and the ganglionic blocking drug guanethidine. When MIBG is labeled with radioactive iodine, gamma camera imaging of patients injected with this compound produces images of the sites of the tumors and of the related structures of the sympathetic nervous system. The I-123 labeled form of this agent has been approved for use by the US Food and Drug Administration for the diagnostic imaging of neuroblastoma and pheochromocytoma, and this agent is commercially available. MIBG has considerably assisted the diagnostic evaluation of patients with known or suspected neuroblastoma, since it provides information about the tumor behavior all along the course of the disease from diagnosis to completion of therapy. Scores of publications from around the world have documented its utility in the diagnosis and monitoring of neuroblastoma. It alone is the single best imaging test for monitoring disease activity. MIBG imaging is performed routinely at many institutions. The following protocol is recommended, to assure high quality images are obtained at all institutions which participate in neuroblastoma evaluations.

Patient preparation: Iodides, usually SSKI (saturated solution of potassium iodide), are administered to reduce thyroidal accumulation of free radioiodine, preferably beginning the day prior to injection. For infants and children, one drop t.i.d. is sufficient, for adolescents 2 drops t.i.d., and for adults 3 drops t.i.d. Patients and/or parents are always asked about exposure to potential interfering agents. If none is noted, an indwelling intravenous line is established. The dose of MIBG is administered by slow intravenous injection over 90 seconds.

Images from the head to the distal lower extremities should be obtained.

A. I-123MIBG scintigraphy is performed to obtain both planar and tomographic images.
   1. Planar – Anterior and posterior views from the top of the head to the proximal lower extremities are obtained for 10 minutes at 24 hours and occasionally at 48 hours following injection of 10 mCi/1.7 square meters of body surface area (~150µCi/kg, maximum 10 mCi). Anterior views of the distal lower extremities are adequate. A large field of view dual head gamma camera with low energy collimators is preferred.
   2. SPECT - Most patients receiving I-123 MIBG also undergo SPECT at 24 hours, using a single or multi-headed camera with a low energy collimator. The camera is rotated through 360 degrees, 120 projections at 25 seconds per stop. Data are reconstructed using filtered back projections with a Butterworth filter and a cut off frequency of 0.2-.0.5.
APPENDIX III: GUIDELINES FOR ALDESLEUKIN (IL-2) ADMINISTRATION AND MANAGEMENT OF TOXICITIES ASSOCIATED WITH CH14.18 + CYTOKINES

The GD-2 antigen is highly expressed on neuroblastoma tumors. Ch14.18 is a human-mouse chimeric ch14.18 antibody. It can cause tumor cell lysis through complement activation or ADCC (antibody dependent cellular cytotoxicity) reactions mediated by neutrophils, monocytes and natural killer cells. Aldesleukin (IL-2) augments ADCC of lymphocytes and the generation of LAK cells from natural killer cells, and GM-CSF augments ADCC of neutrophils.

Before starting immunotherapy, please obtain a copy of management recommendations and algorithms for anaphylaxis and hypotension provided in this appendix and the training module on COG website. It is recommended that these be reviewed, printed and available on the inpatient unit to facilitate treatment decisions should these symptoms occur.

Aldesleukin (IL-2) and morphine are Y-site compatible (visually and with retention of Aldesleukin (IL-2) activity), according to 10th edition of Handbook on Injectable Drugs.

Two lines should be sufficient. Patients on 0935A have received Morphine by continuous infusion with aldesleukin (IL-2) without problem (Y connection). If you need to give bolus meds, you can interrupt the aldesleukin (IL-2), flush with 5% dextrose, give the med, flush with 5% dextrose and then restart the infusion.

The most common toxicities associated with ch14.18 + GM-CSF/ Aldesleukin (IL-2) are listed below with suggested management strategies.

FEVER:
Persistent fevers to 40°C can occur. The fevers can occur during ch14.18 and Interleukin-2 infusions. Fevers have also been seen when neither Interleukin-2 nor ch14.18 is running. Patients generally do not have chills or other symptoms and signs of infections with these fevers.

MANAGEMENT: Draw blood cultures from both lumens of the central catheter for FEVER: routine bacteria ONLY with first T spike > 38.5°C then observe only, unless further workup is indicated by specific patient history or symptoms. FURTHER WORKUP MUST BE DISCUSSED WITH THE ATTENDING MD BEFORE ORDERING.

NEUROPATHIC PAIN:
Antibody binding to peripheral nerve fibers results in mild to severe pain. The severity is dependent on the dose and rate of antibody. It is usually reversible, and resolve a few hours after the infusion is completed.

MANAGEMENT: Continuous infusion narcotic drip with Morphine or lidocaine (morphine may be substituted by fentanyl or hydromorphone) is started each day with the 4 daily antibody treatment course and is weaned off over 2 hours s/p the end of antibody infusion. Consult with attending MD before switching a child from morphine sulfate to fentanyl or hydromorphone.

A. BREAKTHROUGH PAIN WITH ANTIBODY INFUSION: Give bolus dose of narcotic which is written in orders. If the patient requires consistent bolus dosing over a 4-6hr period, increase the drip rate. In addition, may consider decreasing the antibody infusion rate. CONSULT WITH ATTENDING MD PRIOR TO DECREASING INFUSION RATE.

B. PAIN WHEN ANTIBODY INFUSION BEGINS: Despite morphine bolus before antibody infusion, some patients have an initial rush of pain within 1st hour of starting antibody infusion. Can try cutting antibody infusion rate by 50% for the first hour, then increase gradually to the initial rate.
C. NARCOTIC WEAN WHEN ANTIBODY COURSE IS COMPLETED: Children should not be tolerant to this narcotic dose so they should be able to wean fairly quickly. Wean narcotics over 2–3 hrs after completion of last antibody infusion, as tolerated. OPTIMAL GOAL is to discharge the following day off narcotics.

IF PATIENT NOT TOLERATING IV NARCOTIC WEAN: Calculate total current dose of narcotic to that point and change to oral morphine sulfate given q4hr and discharge on this dose. The oral morphine sulfate dose is 3x the IV dose. This will be weaned as an outpatient after d/c.

CAPILLARY LEAK SYNDROME:
Signs and symptoms include weight gain (+1-2 kg over 24-48hrs), I&O's unbalanced (+500 mL or more) with increased specific gravity (1.030), HYPOALBUMINEMIA (loss of 1.5 gms over 4 days), puffiness, changes in VS (BP, HR, RR, decreased pulse ox reading (not usually < 90%). Mechanism for the cause of the capillary leak is not entirely understood. This syndrome may be heightened in the course containing aldesleukin (IL-2) and ch14.18 since aldesleukin (IL-2) is known to cause a capillary leak syndrome.

MANAGEMENT: Monitor 24hr I&O closely-particularly middle 2 days (Day 1 & 2) and weight, minimize additional IV fluid administration (ie: hydration line). Do not necessarily need to treat low albumin unless experiencing SIGNIFICANT VS CHANGES (including significant drop in urine output. Most children have tolerated this albumin drop and rebound once the antibody is stopped. IF YOU ARE CONCERNED ABOUT VITAL SIGN DETERIORATION, discuss the case with the attending MD. It may be necessary to decrease the antibody infusion rate, give NS boluses or albumin.

ELECTROLYTE IMBALANCE:
HYPONATREMIA with Na wasting in the urine (To date we have not seen a serum Na drop less than 130). HYPOKALEMIA, mechanism of action is unknown.

MANAGEMENT: Provide appropriate supplementation keeping in mind fluid concerns as discussed above. Fine tuning results with TID to QID monitoring of laboratory values and urine lytes is usually not necessary. In General, increasing the NaCl and KCL in the IV fluid has been sufficient (ie: 1/2NS to 3/4NS to NS). Serum sodium and potassium are daily standard orders during antibody treatment. If ordering more frequent checks, order Na and a K instead of complete electrolyte panel.

ALLERGIC-TYPE REACTIONS:
Respiratory signs/symptoms seem to initiate with a dry persistent cough with "tightness" in lower airways. Skin reactions can manifest as erythmatous discreet or confluent hives that can be pruritic. Such rash can occur with antibody or narcotics infusion.

MANAGEMENT: Guaifenesin is ineffective for the cough in this situation. Give diphenhydramine or hydroxyzine for both respiratory and skin reactions and observe for response. In case of persistent symptoms, continue diphenhydramine or hydroxyzine q4hr throughout entire antibody treatment course. Also consider decreasing antibody infusion rate. Attempt to avoid steroids (hydrocortisone). PLEASE TALK WITH ATTENDING MD WHEN CONSIDERING WHETHER TO STOP, HOLD OR PROLONG ANTIBODY INFUSION.

VISUAL CHANGES:
Fixed & dilated pupils, c/o photophobia, c/o difficulty reading small print (visual accommodation paralysis) may occur, esp. with repeated courses.

MANAGEMENT: ALERT ATTENDING MD FOR ANY OF THE ABOVE CHANGES. These changes usually resolve over time; do not seem to be permanent. Perform baseline visual exam on admission Day 0.
and evaluate for changes over 4 day admission. Record a specific comment regarding pupillary responses on day of discharge.

**WEIGHT LOSS/ANOREXIA/JOINT PAINS:**

**MANAGEMENT:** Some patients have experienced significant anorexia and weight loss (2 kg x 1mo.). This should be noted in admitting history and referred to primary managing MD and/or case manager to handle as an outpatient. TPN is not required since this syndrome seems to be self limiting.
HYPOTENSION ALGORITHM:

SIGNIFICANT HYPOTENSION
[defined as symptomatic and/or those with systolic blood pressure < 80 mm/hg for age > 12 years old), < 70 (age 1-12 years old), <65 for infant OR a decrease that is more than 15% below baseline]

Hold ch14.18 infusion
Hold cytokine (IL-2, GM-CSF) if infusing
Decrease the dose of narcotic if possible
Give normal saline 10 ml/kg over 30 min. Keep patient in Trendelburg position, if possible

IF SIGNIFICANT HYPOTENSION RESOLVES
Resume ch14.18 infusion at 1.25 mg/m2/hr

IF BP STABLE for 2hrs, resume cytokine

IF SIGNIFICANT HYPOTENSION RECURS
IF BP STABLE for 2hrs, increase ch14.18 infusion to 2.5 mg/m2/hr

IF SIGNIFICANT HYPOTENSION PERSISTS
Give another fluid bolus as any of the following:
1) Normal saline 10mL/kg over 30 minutes
2) Normal saline 3-5 mL/kg with albumin (25%) 1 gm/kg over 30-60 min (recommended as an alternative to NS alone if albumin <= 3.0)
3) PRBC 10 ml/kg over 2-3 hrs (can be in place of or in addition to the above and is recommended if Hgb < 10)

If hypotension is severe at any time (eg. poor perfusion, acidemia, impairment of organ function), may use pressors in addition to fluid resuscitation. Adrenergic pressors, rather than dopamine, are recommended.
ANAPHYLAXIS ALGORITHM:

- ANAPHYLAXIS (>Grade 3 hypersensitivity)
  Acute onset (minutes to hours) with skin/mucous membranes involvement (hives, generalized itch/flushing, swollen lips/tongue/uvula) and at least one of the following:
  a) Respiratory compromise: dyspnea, wheezing, broncospasm, stridor, hypoxia
  b) Reduced blood pressure and/or associated symptoms of end-organ dysfunction: hypotonia, syncope, incontinence

DISCONTINUE ch14.18 and IL-2/GM-CSF
Assessment of airway, breathing, circulation
Anticipate need for CPR

1. EPINEPHRINE (1:1000 0.01 mg/kg (IM preferred over SQ) q15-20 min as needed (treatment of choice); may also give (1:10,000) 0.01mg/kg or 0.1cc/kg: IVP (max 5cc) q3-5 minutes
2. Antihistamines such as diphenhydramine 1 mg/kg IV
3. Oxygen even if O₂ saturation normal
4. Hydrocortisone 1-2 mg/kg IV
5. Inhaled Albuterol for bronchospasm
6. Atropine 0.01 – 0.02 mg/kg IV if bradycardia
7. Give fluid bolus as (note: >50% of blood volume may extravasate in the first 10 minutes):
   1) Normal saline 10mL/kg over 30 minutes
   2) Normal saline 3-5 mL/kg with albumin (25%) 1 gm/kg over 30-60 min (recommended as an alternative to NS alone if albumin <= 3.0)
   3) Vasopressors (norepinephrine) for hypotension resistant to volume replacement and epinephrine

Position with lower extremities elevated, if possible (Trendelenburg position)

If hypotension is severe at any time (eg, poor perfusion, acidemia, impairment of organ function), may use pressors in addition to fluid resuscitation. Adrenergic pressors, rather than dopamine, are recommended.
This model informed consent form has been reviewed by the DCT/NCI and is the official consent document for this study. Institutions should use the sections of this document which are in bold type in their entirety. Editorial changes to these sections may be made as long as they do not change information or intent. If the local IRB insists on making deletions or more substantive modifications to any of the sections in bold type, they must be justified in writing by the investigator at the time of the institutional audit.

SAMPLE RESEARCH INFORMED CONSENT/PARENTAL PERMISSION FORM

ANBL0032, Phase III Randomized Study of Chimeric Antibody 14.18 (Ch14.18) in High Risk Neuroblastoma Following Myeloablative Therapy and Autologous Stem Cell Rescue

CONSENT 1: FOR NEWLY ENROLLED PATIENTS

If you are a parent or legal guardian of a child who may take part in this study, permission from you is required. The assent (agreement) of your child may also be required. When we say “you” in this consent form, we mean you or your child; “we” means the doctors and other staff.

WHY ARE YOU BEING INVITED TO TAKE PART IN THIS STUDY?

You are being asked to take part in this research study because you have been undergoing treatment for high risk neuroblastoma. This study is for a continuing treatment for high risk neuroblastoma.

This study is called a clinical trial. A clinical trial is a research study involving treatment of a disease in human patients. This study is organized by Children's Oncology Group (COG). COG is an international research group that conducts clinical trials for children with cancer. More than 200 hospitals in North America, Australia, New Zealand, and Europe are members of COG.

It is common to enroll children and adolescents with cancer in a clinical trial that seeks to improve cancer treatment over time. Clinical trials include only people who choose to take part.

Please take your time to make your decision. You may want to discuss it with your friends and family. We encourage parents to include their child in the discussion and decision to the extent that the child is able to understand and take part.

WHAT IS THE CURRENT STANDARD OF TREATMENT FOR THIS DISEASE?

The standard treatment for neuroblastoma consists of anti-cancer drugs (chemotherapy), surgery, and radiation therapy. At the end of this intensive treatment, it is standard for children with high risk neuroblastoma to take the drug, isotretinoin, by mouth for 6 months.

Isotretinoin (Accutane®, or 13 cis retinoic acid) works as a continuing treatment to maintain the response to previous treatments. Isotretinoin is a drug closely related to vitamin A and has been shown to help stop the multiplication of remaining neuroblastoma cells.
WHY IS THIS STUDY BEING DONE?

Children with high-risk neuroblastoma often respond to standard treatment at first, but there is a high risk that the cancer will come back. This study is being done to try and improve the likelihood that the cancer will not come back in children with high-risk neuroblastoma.

Prior to participation in this study you received chemotherapy, surgery, radiation therapy, and stem cell transplant treatment. You responded to treatments. The purpose of this companion study is to evaluate an experimental treatment aimed at maintaining or improving your response to previous treatments.

This study involves the use of an investigational biologic therapy, ch14.18, a monoclonal antibody. Monoclonal antibodies are proteins made in the lab, designed to attach to specific cancer cells. Ch14.18 was designed to attach to neuroblastoma cells and other cancer cells that have GD-2 on their cells. When ch14.18 attaches to the neuroblastoma cells, the body’s immune system is stimulated to attack and kill the neuroblastoma cells. Ch14.18 represents a new kind of cancer therapy that, unlike chemotherapy and radiation, targets the destruction of cancer cells without destroying nearby healthy cells.

GM-CSF is a substance that is similar to a substance made by the body in all individuals. Under normal circumstances, the body makes small amounts of GM-CSF that helps it to produce normal infection fighting white blood cells. It is now possible to make GM-CSF outside of the body and give humans much higher doses than their own body makes. There is some evidence that, in the lab and in animals, GM-CSF increases the anti-cancer effect of monoclonal antibodies like ch14.18.

Aldesleukin (IL-2) is a substance that is similar to a substance made by the body in all individuals. Under normal circumstances, the body makes small amounts of aldesleukin (IL-2) that helps white blood cells fight infection. It is now possible to make aldesleukin (IL-2) outside of the body and give humans much higher doses than their own body makes. There is some evidence that, in the lab and in animals, aldesleukin (IL-2) increases the anti-cancer effect of monoclonal antibodies like ch14.18. We wish to study whether aldesleukin (IL-2) can help improve the efficacy of ch14.18 in humans.

Once you are registered on this study, you will receive the experimental treatment ch14.18 + Interleukin-2 + GM-CSF + Isotretinoin (Accutane®). There is no “randomization” and all patients entered onto the study will get the same treatment.

It is important to keep in mind that ch14.18 is an experimental drug and it is impossible to predict whether or not you will benefit from its use. However, early results from this study show that the experimental treatment works better than standard treatment to help get rid of any remaining neuroblastoma cells for as long as possible.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

It is expected that a maximum of 893 patients will be treated on this non-random portion of the study.
WHAT IS INVOLVED IN THIS STUDY?

Methods for Giving Drugs
Various methods will be used to give drugs to you. Some drugs will be given by capsule through the mouth. Other drugs will be given using a needle inserted into a vein. Still others may be given by a shot underneath the skin.

Central Line
For drugs to be given by vein, you doctor will likely recommend that you have a central venous line placed. A central line is a special type of tubing inserted into a large vein in the chest by a surgeon during a short operation. You would be anesthetized for this procedure and receive pain medication afterwards to keep you comfortable. The central line is used to administer chemotherapy drugs and to withdraw small amounts of blood for testing during treatment. If you are to have a central line inserted, the risks associated with central lines will be explained to you, all of your questions will be answered and you will be given a separate informed consent document to read and sign.

The treatment given is described below.

Ch14.18, Aldesleukin (IL-2), GM-CSF, Isotretinoin (cis-RA)

Approximately 2 months following stem cell transplant, subjects will begin a six-month treatment. The treatment consists of six 28-day courses. The drugs to be given in each course are shown below.

Treatment Schema for Courses with GM-CSF (Courses 1, 3, and 5)

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14-23</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Begin</td>
<td></td>
</tr>
<tr>
<td>ch14.18</td>
<td>⬆️</td>
<td>⬆️</td>
<td>⬆️</td>
<td>⬆️</td>
<td>⬆️</td>
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<td>⬆️</td>
<td>⬆️</td>
<td>⬆️</td>
<td>⬆️</td>
<td>Course 2 &amp; 4</td>
<td></td>
</tr>
</tbody>
</table>

cis-RA

GM-CSF: GM-CSF will be given as an injection under the skin (preferred) or directly into a vein for 2 hours daily from Day 0 through Day 13.

Ch14.18 Monoclonal Antibody: Ch14.18 will be given directly into a vein for 10 hours daily for 4 days (Days 3-6).

Isotretinoin (cis-RA): Isotretinoin (cis-RA) will be given by mouth twice a day for 14 days (Days 10-23).
Treatment Schema For Courses With Aldesleukin (IL-2)  (Courses 2 & 4)

Day  0  1  2  3  4-6  7  8  9  10  11-13  14  15  16  17  18-27  
IL-2  X  X  X  X  X  X  X  X  
Ch14.18  ☞  ☞  ☞  ☞  

cis-RA

Aldesleukin (IL-2): Aldesleukin (IL-2) will be given by vein continuously for 4 days during the first week of each course (Days 0-3) and will then be given by vein continuously for 4 days during the second week of each course (Days 7-10).

Ch14.18 Monoclonal Antibody: Ch14.18 will be given directly into a vein for 10 hours daily for 4 days (Days 7-10). Patients will need to be hospitalized for administration of the antibody and may need to go to the Intensive Care Unit in the event of serious side effects from the antibody.

Isotretinoin (cis-RA): Isotretinoin (cis-RA) will be given by mouth twice a day for 14 days (Days 14-27).

Treatment For Course With Isotretinoin Only  (Course 6)

Isotretinoin: Isotretinoin will be given by mouth twice a day for 14 days (Days 0-13).

Additional Medications
To minimize the side effects of ch14.18 + aldesleukin (IL-2) or GM-CSF, the following medications may be administered: acetaminophen, morphine (or fentanyl or hydromorphone), hydroxyzine, epinephrine (Susphrine), meperidine, furosemide, diphenhydramine, lidocaine and albumin.

Standard Medical Tests
Before treatment on this study begins, during and after treatment, you will receive a series of standard medical tests:

- Physical exam
- Blood tests
- Bone marrow tests
- Various scans
- Tests of kidney function
- Tests of lung and heart function
- Urine tests
- In addition, when blood and bone marrow are taken for routine purposes, an extra 1 teaspoonful of bone marrow and an extra 1 tablespoonful of blood will be collected and sent to research labs for special studies to assess the effects of treatment.

Research Study Test
If you have a serious allergic reaction to the ch14.18 antibody during a course of treatment, you will have no more than 3 teaspoonfuls of blood taken at that time. Study doctors will test the blood to learn more about how a person will respond to the antibody. The test results will not be given back to you, and no treatment decisions will be based on the results.
HOW LONG WILL I BE ON THIS STUDY?

The treatment portion of the study will last about 6 months. We would like to keep track of your medical condition for ten years after you enter the study. Keeping in touch with you and checking on how your health is every year for a while after you complete treatment helps us understand the long-term effects of the study.

The researchers may decide to remove you from the study if your cancer gets worse, or you experience effects from the treatment that are considered too severe.

You can remove yourself from the study at any time. However, if you consider removing yourself from the study, we encourage you to talk to your regular physician and to the research physician before making a final decision.

WHAT ARE THE RISKS OF THE STUDY?

The experimental study treatment may cause significant side effects. One of these side-effects is a possible serious allergic reaction to ch14.18 antibody or IL-2. These allergic reactions may range from fever, aches and pains in the joints, and skin rash to low blood pressure, shortness of breath and difficulty breathing.

While on the study, you are at risk for the side effects listed below. There may also be other side effects that cannot be predicted. Some of the increased side effects may occur during therapy (such as low blood counts and increased risk of infection). Other side effects that could occur later might also be life threatening, or even fatal. You should discuss these potential risks with the researcher and/or your regular doctor. Other drugs will be given to make side effects less serious and less uncomfortable. Patients are watched carefully and treatment is stopped if serious side effects develop.

Reproductive risks: The use of isotretinoin can cause birth defects to unborn children if taken during pregnancy. It is unknown what effect(s) the other treatments may have on an unborn child. For this reason, if you are of child-bearing age, you will be asked to practice an effective method of birth control while participating on this study.

Side effects of study drugs:

Risks and side effects related to isotretinoin include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare But Serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Dryness of your skin and mucous membranes</td>
<td>• Rash and itching</td>
<td>• Severe allergic reaction which can be life-threatening with shortness of breath, low blood pressure, rapid heart rate, chills and fever</td>
</tr>
<tr>
<td>• Dry, cracked and bleeding lips</td>
<td>• Headache</td>
<td>• Irritation of the small airways in your lungs that can make you cough and wheeze</td>
</tr>
<tr>
<td>• An increased tendency to sunburn</td>
<td>• Increase in cholesterol and a decrease in the good fat in the blood</td>
<td>• An allergic reaction in the blood vessels of the skin which turn the skin red, inflamed and bumpy and</td>
</tr>
<tr>
<td>• Bloody nose from dry membranes of the nose</td>
<td>• Red eyes</td>
<td></td>
</tr>
<tr>
<td>• Aches and pains in the joints</td>
<td>• Elevation in the blood of certain enzymes found in the liver which may mean liver irritation or damage</td>
<td></td>
</tr>
<tr>
<td>• Back pain</td>
<td>• Fewer red blood cells and white blood cells and platelets in the blood</td>
<td></td>
</tr>
</tbody>
</table>
- Elevation of the fats in your blood
- Increase in calcium in your blood which may require decreasing the dose
- An increase in a laboratory test on your blood that may measure some non-specified inflammation which may or may not be of any importance
- A low number of red blood cells can make you feel tired and weak
- A low number of white blood cells can make it easier to get infections
- A low number of platelets causes you to bruise and bleed more easily
- Too many platelets in the blood
- Loss or thinning of hair
- Appetite disturbances causing you not to feel hungry or to feel unusually hungry
- Weight loss
- Increase in blood sugar levels
- A darkening or lightening of your skin
- Finger and toe nail changes including breaking or splitting more easily
- The sudden appearance of little yellow raised bumps on the skin usually because the cholesterol in the blood is too high (xanthomas)
- Dizziness
- Difficulty falling asleep or staying asleep and strange dreams
- A feeling of tiredness or not feeling well
- Nervousness
- Numbness and tingling in the fingers and toes
- Difficulty hearing clearly or a ringing in the ears
- Changes in vision including more difficulty seeing at night, blurred vision, changes in color vision, pain or squinting
- Which may lead to skin breakdown
- A severe lowering of the white blood count which can make you very susceptible to infections which could be life-threatening
- Convulsions
- Brain swelling that can give you symptoms of severe headache, nausea and vomiting, and changes to your vision including blurriness and pressure behind the eyes
- Life-threatening or fatal changes in moods have occurred including severe depression or feelings of suicide and feelings of aggressiveness and violent behavior
- Thinning of the bone (osteoporosis) which could lead to weakness of the bone, bone fractures or delay in healing of fractures
- Inflammation of the pancreas which can lead to severe abdominal pain and in some very rare cases can be fatal
- Damage to the muscle which can release a protein that can cause severe damage to the kidneys
- Inflammation of the intestinal tract which can result in diarrhea and bleeding
- Serious skin reactions that can range from red bumpy rash to severe rash with blistering of the skin. The rash may spread and can result in loss of skin and damage to mucous membranes and may be life-threatening.
- This drug can cause severe birth defects in a developing fetus, if you are capable of
in bright light, and cataract formation
- Fluid retention
- Chest pain
- Inflammation of the gums
- A dry throat which could lead to a change in your voice and more throat infections
- Slowed growth
- Irregular periods
- Mild kidney damage which could lead to blood or protein in the urine or renal stones
- Extra bone growth along the spine and a tendency for calcium deposits in the tendons and ligaments where they attach to the bone which can lead to pain or stiffness and arthritis of the back and tendonitis

becoming pregnant or of child bearing age, you must practice 2 forms of reliable birth control, sign the Patient Information/Informed Consent form(s), have regular pregnancy tests, be able to keep appointments and agree to follow the iPLEDGE program steps (a special program required by the manufacturers and approved by the Food and Drug Administration (FDA) which your doctor will explain to you).

Risks and side effects related to Chimeric MoAb 14.18 include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare But Serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Fever (high temperature)</td>
<td>• A bleeding disorder in which small blood clots develop throughout the bloodstream blocking small blood vessels and decreasing platelets and proteins needed to control bleeding. This condition can lead to bleeding from many areas of the body and can be life threatening.</td>
<td>• The heart stops pumping blood</td>
</tr>
<tr>
<td>• Pain</td>
<td>• Fast heartbeat; regular rhythm</td>
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</tr>
<tr>
<td>• Abnormal levels of a protein (C-reactive protein) that is associated with inflammation (swelling and redness)</td>
<td>• Pain in the abdomen (belly)</td>
<td></td>
</tr>
<tr>
<td>• Cough</td>
<td>• Diarrhea</td>
<td></td>
</tr>
<tr>
<td>• Skin rash with the presence of macules (flat discolored area) and papules</td>
<td>• Nausea</td>
<td></td>
</tr>
<tr>
<td>• Serum sickness: an allergic reaction that causes fever, aches and pains in the joints,</td>
<td>• Vomiting</td>
<td></td>
</tr>
<tr>
<td>• Swelling of the arms and/or legs</td>
<td>• Allergic reaction to the drug that can occur immediately or may be delayed and may be life threatening. The reaction may include fever, chills, hives, skin rash, low blood pressure, fast heart rate, wheezing, swelling of the throat, and difficulty breathing.</td>
<td></td>
</tr>
<tr>
<td>(raised bump) skin rash and swollen lymph glands.</td>
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<td></td>
</tr>
<tr>
<td>Infections, including those caused by a bacteria, virus, or fungus.</td>
<td></td>
<td></td>
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<tr>
<td>Increase in the blood level of certain enzymes (ALT or AST) which could indicate liver irritation or damage.</td>
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</tr>
<tr>
<td>Increased levels of a chemical (creatinine) in the blood which could mean kidney damage.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fewer white blood cells (lymphocytes) and platelets in the blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o a low number of white blood cells can make it easier to get infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o a low number of platelets causes you to bruise and bleed more easily</td>
<td></td>
<td></td>
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<tr>
<td>Loss of appetite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormally low level of the protein albumin in the blood. Low albumin may result in leaking of fluid from the blood into the tissue.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low level of potassium in the blood which may cause weakness, tiredness, muscle cramps, constipation, and abnormal heart beat and may require that you take extra potassium by mouth or through the vein.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nerve pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nerve damage that may cause pain, burning, numbness, and tingling in the hands and feet and may affect the ability to perform tasks that require fine movements.</td>
<td></td>
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</tr>
<tr>
<td>Presence of excessive protein in the urine which may indicate kidney damage.</td>
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<td></td>
</tr>
<tr>
<td>Blockage of the bronchus (an air tube from the windpipe to the lungs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortness of breath</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low levels of oxygen in the blood which may make you feel short of breath, confused dizzy or drowsy.</td>
<td></td>
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</tr>
<tr>
<td>Abnormal, high-pitched, musical breathing sound caused by a blockage in the throat or voice box</td>
<td></td>
<td></td>
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<tr>
<td>Itching</td>
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<td></td>
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<tr>
<td>Hives, red and sometimes itchy bumps on the skin.</td>
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<td></td>
</tr>
<tr>
<td>A condition in which fluid and proteins leak out of tiny blood vessels and flow into surrounding tissues, resulting in dangerously low blood pressure. Vascular or capillary leak syndrome may lead to multiple organ failure</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
such as kidney, heart or liver failure and shock
- High blood pressure
- Low blood pressure

**Risks and side effects related to GM-CSF (Sargramostim) include those which are:**

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare but serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>Stomach or abdominal pain or cramps</td>
<td>Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, and a rapid heart beat</td>
</tr>
<tr>
<td>Bone pain</td>
<td>Weakness</td>
<td>A severe reaction which can cause shortness of breath, a low blood pressure, a rapid heart rate, fever, a feeling of warmth and back pain which may occur only with the first dose and not with further doses</td>
</tr>
<tr>
<td>Muscle and joint pains</td>
<td>Loss of appetite</td>
<td>An abnormally rapid heat beat</td>
</tr>
<tr>
<td>Fever and Chills</td>
<td>Nausea and/or vomiting</td>
<td>Leakage of fluid into the lungs which may result in shortness of breath and difficulty breathing and/or leakage of fluid into body tissues with puffiness of legs, arms or abdomen, weight gain and a drop in blood pressure</td>
</tr>
<tr>
<td>Rash and itchiness</td>
<td>Diarrhea</td>
<td>Inflammation of the lungs which may lead to pain and shortness of breath</td>
</tr>
<tr>
<td>A feeling of discomfort or not feeling well and/or tiredness</td>
<td>Excessive sweating</td>
<td>A build-up of fluid around the heart which may be painful</td>
</tr>
<tr>
<td></td>
<td>Inflammation of a vein through which the drug was given</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Redness and pain at the injection site</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weight gain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fewer platelets in the blood.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>o A low number of platelets causes you to bruise and bleed more easily</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase in the blood of certain enzymes or bilirubin (a substance that comes from the liver breaking down waste products) which could indicate liver irritation or damage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elevation in the blood of creatinine which normally is removed from the blood by the kidney and could indicate kidney damage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluid build-up in the tissues usually of the lower legs</td>
<td></td>
</tr>
</tbody>
</table>
Risks and side effects related to Aldesleukin (IL-2) include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare But Serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Fever and chills including shaking chills</td>
<td>• Nausea and vomiting</td>
<td>• A severe allergic reaction that can be life-threatening and may lead to difficulty in breathing, a drop in blood pressure, and an irregular heart-beat.</td>
</tr>
<tr>
<td>• Flu like symptoms with headache, tiredness, aches and pains</td>
<td>• Low levels of certain salts in the body like sodium, calcium, magnesium and phosphate which may require treatment</td>
<td>• Heart attack or severe pain in the chest (angina) that can be life-threatening or fatal.</td>
</tr>
<tr>
<td>• Diarrhea</td>
<td>• High levels of uric acid in the blood which could damage the kidneys</td>
<td>• Abnormal electrical conduction within the heart which can cause irregular heartbeat and may be life threatening</td>
</tr>
<tr>
<td>• Loss of Appetite</td>
<td>• Upset of the normal acid levels in your blood</td>
<td>• Inflammation of the heart muscle which could lead to heart failure</td>
</tr>
<tr>
<td>• A feeling of weakness and/or tiredness not relieved by sleep or rest</td>
<td>• Low levels of sugar in the body which may require that you take replacement</td>
<td>• A severe drop in blood pressure that will require treatment</td>
</tr>
<tr>
<td>• A mild drop in blood pressure</td>
<td>• A condition (vascular or capillary leak syndrome) in which fluid and proteins leak out of tiny blood vessels and flow into surrounding tissues, resulting in dangerously low blood pressure which may lead to multiple organ failure such as kidney, heart or liver failure and shock.</td>
<td>• A decrease in the factors in the blood that help your blood to clot normally</td>
</tr>
<tr>
<td>• Skin rash including rash with the presence of macules (flat discolored areas) and papules (raised bumps)</td>
<td>• Heart problems including an irregular or rapid heartbeat leading to unpleasant sensation in the chest</td>
<td>• Bleeding which can occur in the head, stools, the nose, urine and other parts of the body</td>
</tr>
<tr>
<td>• Itching</td>
<td>• Decrease in heart's ability to pump blood during the &quot;active&quot; phase of the heartbeat (systole)</td>
<td>• Convulsion or Seizures</td>
</tr>
<tr>
<td>• Fluid retention and build-up in the tissues usually of the lower legs leading to an increase in weight</td>
<td>• Hearing loss</td>
<td>• Prolonged loss of consciousness (coma)</td>
</tr>
<tr>
<td>• Increased levels of a chemical (creatine) in the blood which could mean kidney damage</td>
<td>• Dizziness</td>
<td>• Inflammation of your colon (large bowel) which could lead to bloody diarrhea and may be life threatening or a hole may develop in the intestines which would cause leakage into the abdomen (belly) with pain and infection</td>
</tr>
<tr>
<td>• A temporary decrease in the amount of urine which could mean the kidneys are not working as well</td>
<td>• Cough</td>
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<tr>
<td></td>
<td>• Stuffy or runny nose, sneezing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Dry mouth</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Headache or head pain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Enlarged abdomen (belly)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Weight gain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Pain in the abdomen (belly) or other parts of the body</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• High blood sugar which may require treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Mood changes including depression, inability to sleep or excessive sleepiness, irritability and agitation, anxiety, confusion, and mood swings such as feelings</td>
<td></td>
</tr>
</tbody>
</table>
- Elevation in the blood of certain enzymes or bilirubin which could indicate liver irritation or damage
- An increase in the blood of a type of white blood cell called an eosinophil. These are sometimes associated with allergic reactions
- An increase in the number of white blood cells in the blood
- Fewer red blood cells and platelets in the blood
  - a low number of red blood cells can make you feel tired and weak
  - a low number of platelets causes you to bruise and bleed more easily
- Of suicide, feelings of aggressiveness
- Noticeable changes in a person's personality, behavior, and thinking
- Trouble with memory
- Aches and pains in the muscles and joints, sleep difficulties, a feeling of extreme tiredness or not feeling well
- Muscular discomfort or pain from infection or an unknown cause
- Nerve damage that may cause pain, burning, numbness, and tingling
- Inflammation and/or sores in the mouth that may make swallowing difficult and are painful (painful mouth sores)
- Severe rashes which can result in loss of skin
- Hair loss
- Infections including those caused by bacteria, virus, and fungus which may require treatment
- Blurred vision
- A flushing of your skin with redness and a feeling of warmth
- Widening of blood vessel
- Fewer white cells in the blood
  - a low number of white blood cells may make it easier to get infections
- Decrease or increase in your thyroid hormones
- Shortness of breath
- Poor blood supply to the arms and legs
- Inflammation of your pancreas which could cause pain in your abdomen and may be life-threatening
- Severe kidney damage (which may be permanent)
- If you have ever been told that you have a disease such as lupus, rheumatoid arthritis or other disease that is caused by a disturbance in your immune system “autoimmune disease”, aldesleukin (IL-2) may cause these to be worse.
- Damage to your lungs which could lead to fluid in the lungs and affect your ability to breathe and the levels of oxygen in the blood
- Stopping of breathing
- Clotting of blood vessels which can lead to pain and swelling in the area of the clot. Such clots may break loose and cause damage or be life-threatening depending on where they go
- Sudden death
WILL I BENEFIT FROM THIS STUDY?

While the hope is that the experimental treatment on this study will help get rid of the neuroblastoma for as long as possible, there may not be any direct medical benefit to you if you participate in the study.

It is hoped that the information learned from this study may help future patients with high-risk neuroblastoma.

ARE THERE OTHER TREATMENT OPTIONS?

Yes, there are other options. Instead of participating in this study, you have these options:

- Treatment with isotretinoin alone.
- Taking part in another study.
- Treatment that is considered in your best interest according to your treating physician.

Please talk to your doctor about these and other options.

WILL MY MEDICAL INFORMATION BE KEPT PRIVATE?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

It is very unlikely that the research testing might uncover important information about you or your current or future health. If this unlikely event occurs, the researchers may contact your doctor through Children’s Oncology Group about what the test results might mean. Only your doctor will be notified and the information will remain confidential. Your doctor may discuss this unexpected finding with you, and may recommend consultation with a genetic counselor and/or repeat testing in a clinical (not research) laboratory if necessary. It is possible that your doctor may recommend that no additional action is necessary.

The Children’s Oncology Group has received a Certificate of Confidentiality from the federal government, which will help us protect the privacy of our research subjects. Information about the certificate is attached at end of this consent.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as:

- Children's Oncology Group
- Representatives of the National Cancer Institute (NCI), Food and Drug Administration (FDA), and other U.S. and international governmental regulatory agencies involved in overseeing research
- The Institutional Review Board of your Hospital
- The drug companies that are involved in the production of the study drug, including United Therapeutics, and their designated reviewers
WILL I HAVE TO PAY FOR THIS TREATMENT?

Taking part in this study may lead to added costs to you or your insurance company. There are no plans for the study to pay for medical treatment. Please ask about any expected added costs or insurance problems. Staff will be able to assist you with this.

The study agent ch14.18 will be provided free of charge by the Cancer Therapy Evaluation Program, NCI while you are taking part in this study. The NCI does not cover the cost of getting Ch14.18 ready and giving it to you, so you or your insurance company may have to pay for this. Even though it is unlikely, there is a possibility that at some point the supply of ch14.18 may run out. If this happens, your study doctor will talk with you. You may have to be taken off study. Sargramostim (GM-CSF), aldesleukin (IL-2), and isotretinoin are commercially available, and you or your insurance company will need to pay for the costs of these drugs.

In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge. No funds have been set aside to compensate you in the event of injury. However, by signing this form, you are not giving up any legal rights to seek to obtain compensation for injury.

You or your insurance company will be charged for continuing medical care and/or hospitalization.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute’s Web site at http://cancer.gov/clinicaltrials/understanding/insurance-coverage. You can print a copy of the “Clinical Trials and Insurance Coverage” information from this Web site.

FUNDING SUPPORT

If you choose to enroll on this study, this institution will receive some money from the Children’s Oncology Group and may receive some funds from United Therapeutics to help cover the research costs. There are no plans to pay you for taking part in this study.

This study includes providing specimens to the researcher, there are no plans for you to profit from any new product developed from research done on your specimens.

WHAT ARE MY RIGHTS AS A STUDY PARTICIPANT?

Taking part in this study is voluntary. You may choose not to take part in this study. If you participate, you may withdraw from the study at any time. If you withdraw from the study, physicians and hospital personnel will still take care of you. You will not be penalized and you will not lose any benefits to which you are entitled.

You also have the right to know about new information that may affect your health, welfare, or your willingness to participate in the study. You will be provided with this information as soon as it becomes available.

Whether you participate or not, you will continue to get the best medical care this hospital can provide.
WHAT IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related problem, or if you think you have been injured, you may contact Dr. XXXX or your doctor at XXXXX.

If you have any questions about your rights as a research participant or any problems that you feel you cannot discuss with the investigators, you may call XXXX IRB Administrator at (XXXX).

If you have any questions or concerns that you feel you would like to discuss with someone who is not on the research team, you may also call the Patient Advocate at XXXX.

WHERE CAN I GET MORE INFORMATION?
The COG Family Handbook for Children with Cancer has information about specific cancers, tests, treatment side effects and their management, adjusting to cancer, and resources. Your doctor can get you this Handbook, or you can get it at: http://www.childrensoncologygroup.org/familyhandbook.


If you are in the United States, you may call the NCI's Cancer Information Service at: 1-800-4-CANCER (1-800-422-6237).

Information about long term follow-up after cancer treatment can be found at: http://www.survivorshipguidelines.org/.

A description of this clinical trial will be available on the internet at: http://www.ClinicalTrials.gov, as required by U.S. Law. This web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time.

Optional Testing of Blood and Bone Marrow Samples for Experimental Purposes:

Optional test of isotretinoin (13 Cis Retinoic Acid) pharmacokinetics and pharmacogenomics

A single blood sample (one teaspoon) will be taken from either a central venous catheter (if in place) or a vein (a small needle is injected into the vein and blood is drawn into a syringe) to find out how much isotretinoin (13 cis retinoic acid) is found in your blood 4 hours after taking the dose on the 14th day of the first 2 week course that the isotretinoin (13 cis retinoic acid) is taken (this kind of test is called pharmacokinetics).

In addition, tests will be done on the same blood sample to look at differences in the level of enzymes that break down isotretinoin (13 cis retinoic acid) in the body and the differences in genes that control these enzymes (this kind of test is called pharmacogenomics). Investigators will look to see if enzyme levels or variations in genes affect the severity of side effects or the effectiveness of the drug in patients.

Although these studies are very important to improving our understanding of isotretinoin (13 cis retinoic acid) use in children with neuroblastoma, the results of these studies will not directly influence your treatment. These samples will not be identified with your name. Neither you nor your doctor will be told about the results of these tests.
You may choose not to participate in the pharmacokinetic and pharmacogenomic studies and will still be eligible to receive the experimental treatment.

Please indicate below whether you choose to participate in the pharmacokinetic and pharmacogenomic studies.

________ Yes, I agree to provide this additional blood sample.

________ No, I do not agree to provide this additional blood sample.

**Optional Test of Minimal Residual Disease**

These tests will be done only if you agree to give the extra blood and bone marrow samples. You can choose to take part in this clinical trial without taking part in the following tests.

Very small amounts of neuroblastoma can be present that are too small to be detected by a microscope. These small amounts of neuroblastoma are called Minimal Residual Disease, or MRD, for short. Special tests are available that can detect MRD.

If you agree, before you begin treatment and again at the end of treatment, we would like to collect 2 teaspoonfuls of bone marrow and 2 teaspoonfuls of blood. In addition, if bone marrow is collected during a follow-up test after the study treatment has ended, we would like to collect 1 teaspoonful of bone marrow. If the cancer goes away and then comes back (a relapse) we would also like to collect 1 teaspoonful of bone marrow.

These samples will be sent to research labs for special studies to measure MRD and assess the effects of treatment. The test results will not be given back to you, and no treatment decisions will be based on the results.

Please indicate below whether you choose to participate in the Minimal Residual Disease tests:

________ Yes, I agree to provide this additional blood and bone-marrow sample.

________ No, I do not agree to provide this additional blood and bone-marrow sample.

**Optional test of FcR and KIR genotyping**

If you agree, a single blood sample (one and a half teaspoons) will be taken to find out about differences in the genes of certain cells in your body. We think the differences may impact the ability to destroy neuroblastoma cells with ch14.18.

Investigators will look to see if variations in genes affect the effectiveness of the drug in patients. The test results will not be given back to you, and no treatment decisions will be based on the results. But the results may help to predict if patients with certain gene types may benefit from this treatment in the future.

Please indicate below whether you choose to participate in the FcR and KIR genotyping tests:

________ Yes, I agree to provide this additional blood sample.

________ No, I do not agree to provide this additional blood sample.
Use of Test Samples and Results for Related Studies:
In the case of tissue (specimen) banking, patients are being asked to grant approval at this time for unspecified, future research studies.

The results of tissue (specimen) bank research may help find new ways to learn about, prevent, or treat cancer and other diseases. Please read each sentence below and think about your choice. After reading each sentence, circle and initial the answer that is right for you. If you have any questions, please talk to your doctor or nurse, or call the National Cancer Institute's Cancer Information Service at 1-800-422-6327 (1-800-4-CANCER).

My tissue (specimen) may be kept for use in research to learn about, prevent, or treat cancer.
Yes    No    Initials________

My tissue (specimen) may be kept for use in research to learn about, prevent, or treat other health problems (for example: diabetes, Alzheimer's disease, or heart disease).
Yes    No    Initials________

STATEMENT OF CONSENT
I have been given a copy of all _____ [insert total number of pages] pages of this form. The form includes one (1) attachment.

I have already read the above information. I have asked all my questions and I have gotten answers. I agree to enroll in this study.

A copy of this consent form will be provided to you. You will be given a copy of the protocol (full study plan) upon request.

PATIENT NAME ________________________________________

_____________________________________      ______________
SIGNATURE OF PARENT OR GUARDIAN               DATE

____________________________________        ______________
SIGNATURE OF PHYSICIAN OR RESPONSIBLE INVESTIGATOR  DATE
The Children's Oncology Group has received a Certificate of Confidentiality from the federal government, which will help us protect the privacy of our research subjects. The Certificate protects against the involuntary release of information about subjects collected during the course of our covered studies. The researchers involved in the studies cannot be forced to disclose the identity or any information collected in the study in any legal proceedings at the federal, state, or local level, regardless of whether they are criminal, administrative, or legislative proceedings. However, the subject or the researcher may choose to voluntarily disclose the protected information under certain circumstances. For example, if the subject or his/her guardian requests the release of information in writing, the Certificate does not protect against that voluntary disclosure. Furthermore, federal agencies may review our records under limited circumstances, such as a DHHS request for information for an audit or program evaluation or an FDA request under the Food, Drug and Cosmetics Act. The Certificate of Confidentiality will not protect against the required reporting by hospital staff of information on suspected child abuse, reportable communicable diseases, and/or possible threat of harm to self or others.
This model informed consent form has been reviewed by the DCT/NCI and is the official consent document for this study. Local IRB changes to this document are allowed. (Institutions should attempt to use sections of this document which are in bold type in their entirety.) Editorial changes to these sections may be made as long as they do not change information or intent. If the local IRB insists on making deletions or more substantive modifications to the risks or alternatives sections, they must be justified in writing by the investigator and approved by the IRB.

SAMPLE RESEARCH INFORMED CONSENT/PARENTAL PERMISSION FORM

ANBL0032, Phase III Randomized Study of Chimeric Antibody 14.18 (Ch14.18) in High Risk Neuroblastoma Following Myeloablative Therapy and Autologous Stem Cell Rescue

CONSENT 2: FOR PATIENTS PARTICIPATING IN THE PLASMA ANALYSIS/ECG CARDIAC SAFETY SUB-STUDY

If you are a parent or legal guardian of a child who may take part in this study, permission from you is required. The assent (agreement) of your child may also be required. When the word "you" appears in this consent form, it refers to you or your child; “we” means the doctors and other staff.

WHY ARE YOU BEING INVITED TO TAKE PART IN THIS STUDY?

This study is called a clinical trial. A clinical trial is a research study involving treatment of a disease in human subjects. This study is organized by Children’s Oncology Group (COG). COG is an international research group that conducts clinical trials for children with cancer. More than 200 hospitals in North America, Australia, New Zealand, and Europe are members of COG.

You are being asked to take part in this study because you have been undergoing treatment for high risk neuroblastoma. This study is for a continuing treatment for high risk neuroblastoma.

WHAT IS THE CURRENT STANDARD OF TREATMENT FOR THIS DISEASE?

The standard treatment for neuroblastoma consists of anti-cancer drugs (chemotherapy), surgery, and radiation therapy. At the end of this intensive treatment, it is standard for children with high risk neuroblastoma to take the drug, isotretinoin, by mouth for 6 months.

Isotretinoin (Accutane®, or 13 cis retinoic acid) works as a continuing treatment to maintain the response to previous treatments. Isotretinoin is a drug closely related to vitamin A and has been shown to help stop the multiplication of remaining neuroblastoma cells.

Recently, the new experimental immunotherapy regimen in this study (that includes drugs ch14.18, IL2, and GM-CSF and isotretinoin) has been observed to work better than the standard treatment to help get rid of any remaining neuroblastoma cells.

WHY IS THIS STUDY BEING DONE?

The treatment for this study is described in the first consent form. You have already read the first consent form. In this separate part of the study, we would like to learn more about 2 things:

1. Any effect the chimeric antibody agent (ch14.18) might have your heart function.
2. Any effects the ch14.18 has on your body and metabolism.
ECG monitoring:
An electrocardiogram (ECG) is a test that measures the electrical activity of the heart. This test involves the placement of small patches, called electrodes, to several areas on your arms, legs and chest. These patches will be connected by wires to an ECG machine. The ECG machine will record the electrical activity of your heart in wavy lines which are reviewed by a doctor. ECG monitoring is being done to analyze the cardiac safety profile of the main investigational agent used in this study – ch14.18.

Ch14.18 plasma level analysis:
Blood tests will also be done to study the effects the chimeric antibody agent (ch 14.18) has on your body and metabolism. The amount of ch14.18 will be measured at certain time-points during therapy.

Each of the ch14.18 plasma level tests and ECG tests will be done at the same time-points in this study. This will allow us to look for a possible relationship between ECG findings and actual ch14.18 blood levels.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?
The total number of people enrolled on this sub-study is expected to be 60.

WHAT WILL HAPPEN ON THIS STUDY THAT IS RESEARCH?
ECG monitoring and ch14.18 plasma analysis will be done at select institutions during the course of this study treatment.

ECG machines capable of recording 10 seconds of digital data and sending the information to a central lab are installed in each of the selected institutions. Should the ECG show results that are of concern to the reviewer, the reviewer will provide that information to your study doctor. ECGs will be recorded 3 times at each of the following time-points:

- Cycle 1: Baseline, before getting GM-CSF (Day -1)
- Cycle 1: Day 6 at the end of the ch14.18 infusion
- Cycle 4: Day 80 prior to IL-2
- Cycle 4: Day 90 at the end of the ch14.18 infusion
- End of Study: within 2 weeks of Day 178

Blood tests will also be done at each of the 5 time-points listed above to examine how the study agent ch 14.18 is broken down. 5 mL (about 1 teaspoon) of blood will be collected to perform these tests. The blood samples will be sent to a central laboratory and the tests will be performed there.

HOW LONG WILL I BE ON THIS STUDY?
The ECG monitoring and blood tests will be done at various time-points during the entire course of the study (from cycle 1 to end of study). Therefore, it the length of this part of the study will be the same as the study treatment timeline – i.e. around 6 months.
WHAT ARE THE RISKS OF THE STUDY?

Risks of Blood Draws:
You may experience slight bruising or pain on your arm where blood samples will be taken. There is also the slight risk of infection, light-headedness, and/or fainting.

Risks of ECGs:
No electricity is sent through the body, so there is no risk of shock. In rare cases, some people may develop a rash or irritation where the electrodes were placed.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

We do not expect that there will be direct medical benefits to you from taking part in this study. We hope that the information learned from these research tests done in this study will benefit other subjects in the future.

WHAT OTHER OPTIONS ARE THERE?

You have the option not to participate in this part of the study. Refusal to participate or stopping participation will not affect the medical care you will receive.

WHAT ABOUT CONFIDENTIALITY?

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law.

The Children’s Oncology Group has received a Certificate of Confidentiality from the federal government, which will help us protect the privacy of our research subjects. Information about the certificate is included in Attachment #1.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as:

- Children’s Oncology Group
- Representatives of the National Cancer Institute (NCI), Food and Drug Administration (FDA), and other U.S. and international governmental regulatory agencies involved in overseeing research
- The Institutional Review Board of this hospital
- The drug companies that are involved in the production of the study drug, including United Therapeutics, and their designated reviewers

WHAT ARE THE COSTS?

Taking part in this study may lead to added costs to you or your insurance company. The study has no plans to pay for medical treatment. Please ask about any expected added costs or insurance problems. Staff will be able to assist you with this.

In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge. No funds have been set aside to compensate you in the event of injury. However by signing this form, you are not giving up any legal rights to seek to obtain compensation for injury.
You or your insurance company will be charged for continuing medical care and/or hospitalization.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute’s Web site at http://cancer.gov/clinicaltrials/understanding/insurance-coverage. You can print a copy of the “Clinical Trials and Insurance Coverage” information from this Web site.

**FUNDING SUPPORT**

If you choose to enroll on this study, this institution will receive some money from the Children's Oncology Group to do the research.

United Therapeutics is providing money to the Children's Oncology Group to do research, that will be distributed to the participating institutions.

This study includes providing specimens to the researcher, there are no plans for you to profit from any new product developed from research done on your specimens.

**WHAT ARE MY RIGHTS AS A PARTICIPANT?**

Taking part in this study is voluntary. You may choose not to be in this study. If you decide not to be in this study, you will not be penalized and you will not lose any benefits to which you are entitled. You will still receive medical care.

You can decide to stop being in the study at any time. Deciding to stop participating will not result in any penalty or loss of benefits to which you are entitled. Your doctor will still take care of you.

We will tell you about new information that may affect your health, welfare, or willingness to stay in this study. A committee outside of COG closely monitors study reports and notifies COG if changes must be made to the study. Members of COG meet twice a year to evaluate results of treatment and to plan new treatments.

During your follow-up visits after treatment, you may ask to be given a summary of the study results after they are written up. This may be several years from now since all of the people on the study need to complete treatment.

**WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?**

For questions about the study or a research-related problem, or if you think you have been injured, you may contact Dr. XXXX or your doctor at XXXXX.

If you have any questions about your rights as a research participant or any problems that you feel you cannot discuss with the investigators, you may call XXXX IRB Administrator at (XXXX).

If you have any questions or concerns that you feel you would like to discuss with someone who is not on the research team, you may also call the Patient Advocate at XXXX.

**WHERE CAN I GET MORE INFORMATION?**

The COG Family Handbook for Children with Cancer has information about specific cancers, tests, treatment side effects and their management, adjusting to cancer, and resources. Your
doctor can get you this Handbook, or you can get it at http://www.childrensoncologygroup.org/familyhandbook.

You may call the NCI's Cancer Information Service at:
1-800-4-CANCER (1-800-422-6237)

Visit the NCI's Web site at http://www.nci.nih.gov/cancerinfo/

Visit the COG Web site at http://www.curesearch.org

Information about long term follow-up after cancer treatment can be found at http://www.survivorshipguidelines.org/

You will get a copy of this form. You may also ask for a copy of the protocol (full study plan).

A description of this clinical trial will be available on the internet at: http://www.ClinicalTrials.gov, as required by U.S. Law. This web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time.

SIGNATURE

I have been given a copy of all _____ [insert total number of pages] pages of this form. The form includes one (1) attachment.

I have reviewed the information and have had my questions answered.
I agree to take part in this study on ECG monitoring and ch14.18 plasma level tests.

Participant ___________________________________________ Date ____________

Parent/Guardian________________________________________ Date ____________

Parent/Guardian________________________________________ Date ____________

Physician/PNP obtaining consent___________________________ Date ____________

IRB# IRB Approved:
Attachment #1: Certificate of Confidentiality Information

The Children’s Oncology Group has received a Certificate of Confidentiality from the federal government, which will help us protect the privacy of our research subjects. The Certificate protects against the involuntary release of information about subjects collected during the course of our covered studies. The researchers involved in the studies cannot be forced to disclose the identity or any information collected in the study in any legal proceedings at the federal, state, or local level, regardless of whether they are criminal, administrative, or legislative proceedings. However, the subject or the researcher may choose to voluntarily disclose the protected information under certain circumstances. For example, if the subject or his/her guardian requests the release of information in writing, the Certificate does not protect against that voluntary disclosure. Furthermore, federal agencies may review our records under limited circumstances, such as a DHHS request for information for an audit or program evaluation or an FDA request under the Food, Drug and Cosmetics Act. The Certificate of Confidentiality will not protect against the required reporting by hospital staff of information on suspected child abuse, reportable communicable diseases, and/or possible threat of harm to self or others.