NEW APPROACHES TO NEUROBLASTOMA THERAPY (NANT) CONSORTIUM

NANT 2011-01: RANDOMIZED PHASE II STUDY OF ^{131}I-MIBG VS. ^{131}I-MIBG WITH VINCRISTINE AND IRINOTECAN VS. ^{131}I-MIBG WITH VORINOSTAT FOR RESISTANT/RELAPSED NEUROBLASTOMA.

IND# 120414

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ABSTRACT

^{131}I-Metaiodobenzylguanidine (^{131}I-MIBG) is one of the most active agents for patients with relapsed or refractory neuroblastoma. A large multicenter phase 2 study yielded an estimated overall response rate in this population of 36% using single-agent ^{131}I-MIBG. Recent NANT clinical trials have demonstrated the feasibility of combining ^{131}I-MIBG with systemic radiation sensitizers, with the aim of enhancing the antitumor activity of ^{131}I-MIBG.

NANT 04-06 was a phase 1 dose escalation study of ^{131}I-MIBG together with vincristine and protracted irinotecan (20 mg/m^2/day IV x 5 days x 2 weeks) as a radiation sensitizer. This combination was tolerable using ^{131}I-MIBG doses up to 18 mCi/kg. However, more patients than anticipated from either drug alone developed protocol-associated diarrhea, a known toxicity of protracted irinotecan exposures. Furthermore, the new national standard shown to be effective in other tumor types has been a 5-day higher dose irinotecan schedule. Therefore, a pilot study at UCSF evaluated ^{131}I-MIBG together with vincristine and irinotecan given at higher doses for a shorter exposure (50 mg/m^2/day IV x 5 days x 1 week). This study confirmed that vincristine and irinotecan could be safely combined with ^{131}I-MIBG up to doses of 18 mCi/kg, with lower rates of diarrhea compared to NANT 04-06. Promising antitumor activity was seen in both studies of this combination.

NANT07-03 was a phase 1 dose escalation study of ^{131}I-MIBG together with vorinostat, an oral histone deacetylase (HDAC) inhibitor. The rationale for this study included three main points. First, vorinostat has modest activity against neuroblastoma in preclinical models. Second, vorinostat has been shown to sensitize tumor cells, including neuroblastoma, to the effects of radiation therapy. Third, vorinostat upregulates the expression of the norepinephrine transporter on neuroblastoma cell lines. This phase 1 study demonstrated that 14 days of vorinostat could safely be combined with ^{131}I-MIBG up to doses of 18 mCi/kg.

While these combination trials have demonstrated promising antitumor activity, these small phase 1 studies have not clarified whether combination therapy results in increased response rates compared with single-agent ^{131}I-MIBG. The current randomized phase 2 pick the winner study is therefore designed to select the ^{131}I-MIBG treatment regimen associated with the highest objective response rate. Patients with relapsed or refractory neuroblastoma and MIBG-avid disease will be randomized at study entry to one of three treatment arms: single-agent ^{131}I-MIBG; ^{131}I-MIBG with vincristine and irinotecan; and ^{131}I-MIBG with vorinostat. All patients will receive 18 mCi/kg ^{131}I-MIBG with prophylactic autologous stem cell support two weeks following ^{131}I-MIBG infusion. Patients enrolling prior to Amendment 7A may receive up to two courses of therapy. Patients enrolling with Amendment 7A and after may receive only one course of therapy. The primary endpoint is objective response following the first course of therapy, using standard NANT response criteria. Secondary and other endpoints will include toxicity, site specific response, best response for patients treated with two courses of therapy (if applicable), overall survival, and correlative biology endpoints related to radiation exposure. Up to a total of 105 eligible and evaluable patients will be randomized to one of the three arms.

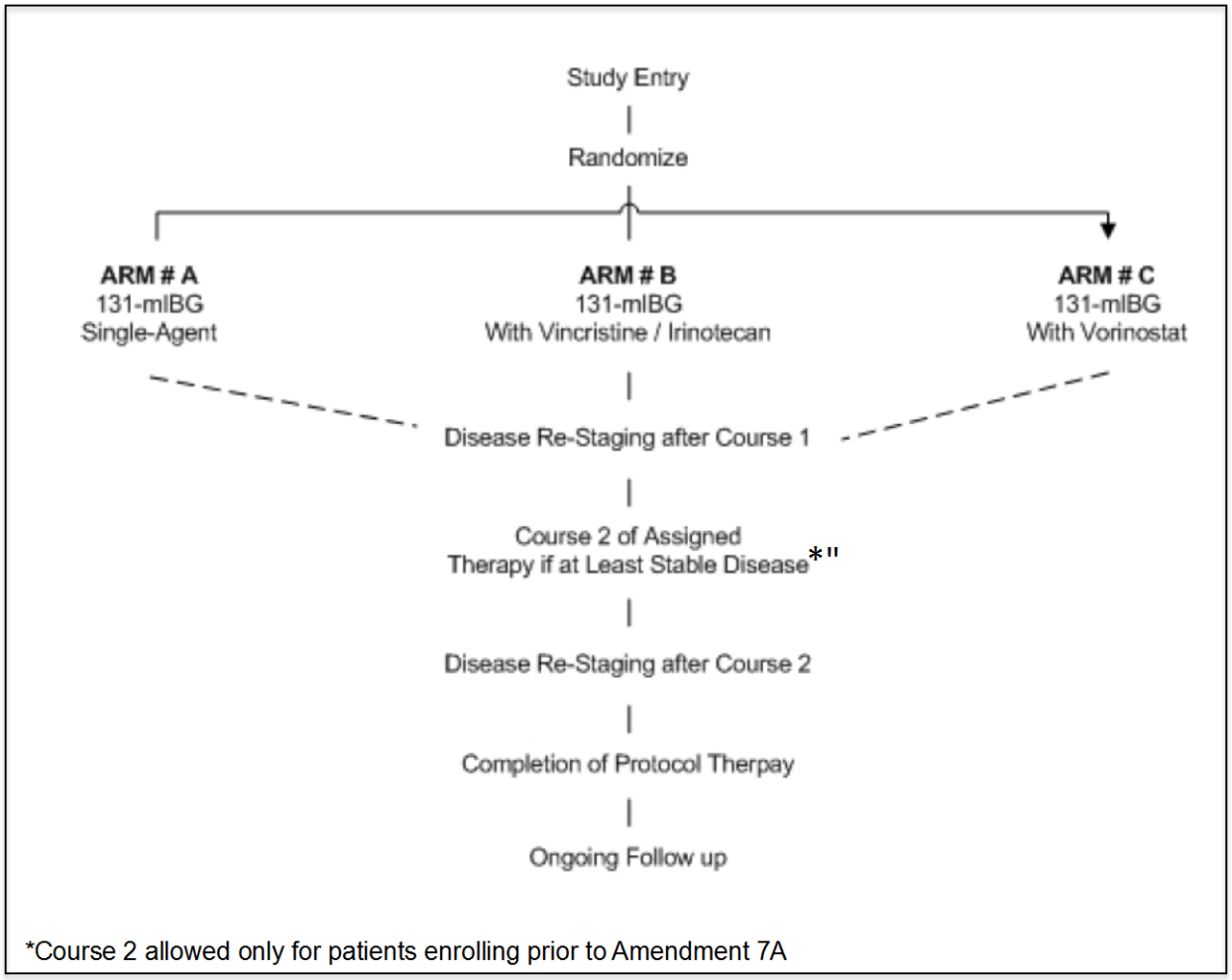
Hypothesis:

We hypothesize that:

- ^{131}I-MIBG, combined with either vorinostat or irinotecan, will be associated with a higher objective response rate compared with single-agent ^{131}I-MIBG;
- ^{131}I-MIBG, combined with either vorinostat or irinotecan, will be tolerable but associated with an increase in protocol-associated toxicity compared with single-agent ^{131}I-MIBG;
- ^{131}I-MIBG, combined with either vorinostat or irinotecan, will be associated with increased measures of radiation exposure as assessed by peripheral blood markers.

TREATMENT SCHEMA

Patients will be randomized at study entry to one of three treatment arms.



Patients assigned to single-agent ^{131}I-MIBG (Arm A) will receive 18 mCi/kg ^{131}I-MIBG on Day 1 and autologous stem cell infusion on Day 15.

Patients randomized to receive vincristine / irinotecan / ^{131}I-MIBG (Arm B) will receive vincristine 2 mg/m^2 (maximum dose 2 mg; see 4.2.2.1 for dosing if < 3 years old) intravenously on Day 0. They will receive irinotecan 50 mg/m^2 (maximum dose 100 mg) intravenously on Days 0 to 4. Patients will also receive diarrhea prophylaxis with cefixime 8 mg/kg/day orally on Days -1 to +6. Patients will receive 18 mCi/kg ^{131}I-MIBG on Day 1 and autologous stem cell infusion on Day 15.

Patients randomized to receive vorinostat / ^{131}I-MIBG (Arm C) will receive vorinostat orally once daily on Days -1 to +12 (14 total doses) at a dose of 180 mg/m^2 (maximum dose 400 mg). Patients will receive 18 mCi/kg ^{131}I-MIBG on Day 1 and autologous stem cell infusion on Day 15.

All patients will undergo disease restaging between Day 43 and 50, 6-7 weeks after ^{131}I-MIBG infusion. Patients enrolling prior to Amendment 7A with at least stable disease after the first cycle can receive a second cycle of therapy, provided they meet specified protocol criteria.

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

Primary Aim:

1.1
To identify the ^{131}I-MIBG treatment regimen associated with the highest overall response rate after one course of treatment on the following three arms: single-agent ^{131}I-MIBG; vincristine / irinotecan / ^{131}I-MIBG; or vorinostat / ^{131}I-MIBG

Secondary Aim:

1.2
To compare the toxicity profiles associated with each of the following three ^{131}I-MIBG treatment regimens: single-agent ^{131}I-MIBG; vincristine / irinotecan / ^{131}I-MIBG; or vorinostat / ^{131}I-MIBG

Exploratory Aims:

1.3
To describe best overall response for patients treated with two courses of therapy, site-specific response of bone marrow, soft tissue, and MIBG-avid lesions as well as overall survival and progression-free survival following each of three ^{131}I-MIBG treatment regimens

1.4
To assess peripheral blood and bone marrow minimal residual disease at study entry and following each of three ^{131}I-MIBG treatment regimens quantified using a neuroblastoma-specific NB 5-gene detection assay (NB5 assay) by TaqMan® low density array (TLDA)

1.5
To compare whole body radiation exposure in patients with neuroblastoma treated with each of three ^{131}I-MIBG treatment regimens

1.6
To evaluate peripheral blood lymphocyte γ-H2AX foci levels, expression of a panel of DNA damage response genes in peripheral blood mononuclear cells, plasma Flt-3 ligand, serum amylase, and circulating tumor DNA as markers of radiation exposure, response, and toxicity in patients with neuroblastoma treated with each of three ^{131}I-MIBG treatment regimens

1.7
To evaluate the performance of a computerized MIBG scan scoring system in patients with relapsed or refractory neuroblastoma treated with ^{131}I-MIBG therapy

1.8
To evaluate the association between tumor norepinephrine transporter (NET) protein expression and response to protocol therapy.

1.9
To describe the concordance rate between institutional Curie score and centrally reviewed Curie score in patients with relapsed or refractory neuroblastoma.

1.10
To describe the incidence and severity of sinusoidal obstruction syndrome (SOS) in patients with neuroblastoma who receive ^{131}I-MIBG therapy on this protocol and subsequently receive high-dose chemotherapy with stem cell rescue as part of their clinical care.

2.0 BACKGROUND

2.1 Neuroblastoma

Neuroblastoma is the most common extracranial solid cancer of childhood.[1] Most patients are initially diagnosed prior to 10 years of age. Approximately 50% of patients present with initially metastatic disease. Many of these patients are either refractory to initial therapy or develop recurrent disease after receiving multimodal therapy. The outcome for patients with recurrent or refractory disease remains poor. Novel approaches to treating these patients are required to improve their outcome.

2.2 ^{131}I-MIBG: Clinical Data in Neuroblastoma

^{131}I-metaiodobenzylguanidine (MIBG) is a guanethidine analog with specific uptake in sympathetic nervous system tissues. The norepinephrine transporter mediates uptake of ^{131}I-MIBG into the cells of these tissues.[2] As a tumor derived from the sympathetic nervous system, neuroblastoma cells avidly accumulate ^{131}I-MIBG.[3-5] Low doses of both ^{131}I-MIBG and ^{123}I-MIBG are widely used as nuclear medicine imaging agents in the diagnostic evaluation of patients with neuroblastoma.

Given that neuroblastoma is radiosensitive, high-dose ^{131}I-MIBG has been evaluated as a targeted radiopharmaceutical in the treatment of patients with neuroblastoma for the past 25 years. Unlike standard external beam radiotherapy, ^{131}I-MIBG is administered intravenously and then distributes throughout the body.[6] The biological or effective half-life of the administered radiation in the blood of patients with neuroblastoma ranges from 9-130 hours, reflecting clearance mainly in the urine.[6-8] Radiation that is not cleared in the urine or stool remains in the body and decays with a half-life of approximately 8 days, the physical half-life of ^{131}I.[9] Since ^{131}I-MIBG taken up by neuroblastoma cells is not cleared in the urine or stool, these cells are exposed to radiation with a half-life that more closely follows the physical half-life of ^{131}I. Therefore, three weeks following therapeutic ^{131}I-MIBG administration, tumor cells may still be exposed to small amounts of radiation.

The whole body radiation dose received is approximately linear with ^{131}I-MIBG dose administered. After a dose of 12 mCi/kg, patients receive a calculated whole-body radiation dose of approximately 2 Gy.[10] As a targeted radiopharmaceutical, the dose delivered to the tumor is much higher.[10]

Aside from transient nausea and xerostomia, the main early toxicity of ^{131}I-MIBG therapy is myelosuppression. With higher doses (> 12 mCi/kg ^{131}I-MIBG), hematologic toxicity becomes dose-limiting and hematopoietic stem cell infusion has been used to promote hematologic recovery.[11] Thrombocytopenia occurs more often and is more prolonged than neutropenia, particularly for those patients with bone marrow disease prior to ^{131}I-MIBG treatment.[12] In one study using 18 mCi/kg ^{131}I-MIBG, the median time to platelet nadir was 24 days and patients who did not receive hematopoietic stem cell support required platelet transfusions for a median of 5 weeks.[12] Nonhematologic toxicity has been minimal, even at higher doses. In a phase I dose escalation study of ^{131}I-MIBG, no grade 3 or 4 nonhematologic toxicities were observed following a single course of therapy.[11] In a phase II study in which patients received 18 mCi/kg ^{131}I-MIBG, 22% of patients had a grade 3 non-hematologic toxicity and 9% of patients had a grade 4 non-hematologic toxicity.[13] The majority of these toxicities consisted of febrile neutropenia and other infectious complications. Patients on these studies have been able to receive multiple courses of therapy without unanticipated toxicity.[13-15] In one series, 28 patients received at least two courses of therapy with a median ^{131}I-MIBG dose of 18 mCi/kg given at a median interval of 98 days between treatments.[15] Of the 24 patients who received two courses of ^{131}I-MIBG, only 8 patients received stem cell reinfusion to support hematologic recovery. Non-hematologic toxicity was as expected based upon patients treated with only one course of ^{131}I-MIBG. Potential late toxicities include hypothyroidism, secondary malignancy, and reduced fertility.[16-18]

Early studies evaluated ^{131}I-MIBG as monotherapy for patients with recurrent or refractory disease. The objective response rate in these studies ranged from 30% to 37%.[11, 13, 14, 19] The response rate with monotherapy may be even higher in patients with newly diagnosed disease.[20, 21] A recent large phase II trial demonstrated that ^{131}I-MIBG is one of the most active agents in patients with progressive, relapsed, or refractory neuroblastoma.[13] Of 164 patients, of whom 148 were treated with 18 mCi/kg, 36% had an objective response. Patients > 12 years of age and those with disease limited to either bone/bone marrow or soft tissue, but not both, were more likely to achieve response following ^{131}I-MIBG as single-agent therapy. A more recent study has confirmed that older patients appear to have a higher response rate to ^{131}I-MIBG.[22]

The use of multiple ^{131}I-MIBG treatments to higher cumulative doses has also been assessed. Multiple treatments of ^{131}I-MIBG at 56 day intervals have been shown to be tolerable.[23] NANT protocol 2000-01 evaluated double infusion of ^{131}I-MIBG given two weeks apart to cumulative doses of up to 42 mCi/kg, supported by PBSC infusion.[24] Doses of 18 mCi/kg x 2 doses (36 mCi/kg) were well-tolerated. Cumulative lifetime doses of 54 mCi/kg have also been used, mainly on compassionate access protocols. There have not been any late liver, renal, or cardiac toxicity in patients with multiple infusions. Recent studies from the Children's Hospital of Philadelphia have shown that ^{131}I-MIBG can be given at 42 day intervals without increased toxicity.[25]

Based on these encouraging results, the NANT consortium has conducted phase 1 trials evaluating ^{131}I-MIBG in combination with radiation sensitizers (see below). In addition, the Children's Oncology Group has opened a pilot study incorporating ^{131}I-MIBG therapy into the upfront treatment of children with high-risk neuroblastoma.

2.3 Rationale for Combination of ^{131}I-MIBG with Irinotecan

2.3.1 Clinical Use of Irinotecan and Irinotecan / Vincristine in Neuroblastoma

Irinotecan has been shown to be active against pediatric solid tumors in several clinical trials. A COG phase II trial treated 171 children with solid tumors with irinotecan at a dose of 50 mg/m^2/dose IV x 5 days.[26] This regimen was found to be tolerable and 1/18 patients with neuroblastoma had an objective partial response. Subsequent trials evaluated irinotecan in combination with other chemotherapy agents. A COG rhabdomyosarcoma trial established that the combination of irinotecan (20 mg/m^2/dose IV for 5 days/week x 2 weeks) with vincristine was associated with greater activity compared to irinotecan alone.[27]

Several studies have evaluated the combination of irinotecan and temozolomide specifically in patients with neuroblastoma. A Children's Oncology Group phase II study was conducted in 59 patients with recurrent or refractory disease using temozolomide 100 mg/m^2/day orally x 5 days and irinotecan 10 mg/m^2/day IV once daily for 5 days each week for two weeks.[28] The objective response rate was 16%. Stable disease was documented as best response to therapy in an additional 47% of patients. A NANT phase I study in patients with neuroblastoma evaluated oral irinotecan together with temozolomide.[29] This combination resulted in 1 patient out of 14 total patients with a complete response as well as stabilization of disease in an additional 6 patients. A report of a single institution study describes 49 patients with neuroblastoma treated with temozolomide 150 mg/m^2/day orally x 5 days and irinotecan 50 mg/m^2/day IV once daily for 5 days for one week.[30] One third of patients (12/36) treated in this series showed tumor regression, although only 3 of the 36 evaluable patients had a complete or partial response.

The incidence of thrombocytopenia or neutropenia $\geq$ grade 3 associated with this combination appears to be relatively low. In the COG phase II study in neuroblastoma, 9% and 25% of patients experienced $\geq$ grade 3 thrombocytopenia or neutropenia, respectively.[28] In one case series in patients with Ewing sarcoma, 11% and 12% of cycles were associated with $\geq$ grade 3 thrombocytopenia or neutropenia, respectively.[31] These results suggest that an additional myelosuppressive agent could be added to irinotecan-based therapy. Moreover, the incidence of grade 3 or 4 diarrhea was < 5% with this regimen, suggesting that non-hematologic toxicity is also manageable with irinotecan at this dose and schedule.[28, 31]

This protocol utilizes irinotecan administered once daily for 5 days for one week. This schedule has been selected for several reasons. First, irinotecan administered with temozolomide on this schedule has activity in patients with neuroblastoma.[30] Second, experience in patients with rhabdomyosarcoma on COG protocol ARST0121 has shown that the antitumor activity of this combination is not compromised by the use of a one week schedule. Third, the one week schedule is advantageous in terms of cost and patient convenience. Fourth, the risk of diarrhea is anticipated to be lower with this schedule.

2.3.2 Camptothecins as Radiation Sensitizers

The combination of a topoisomerase I inhibitor with radiotherapy has an attractive rationale. The presumed mechanism is potentiation of radiation-induced double stranded DNA breaks (in G_2M-phase) through inhibition of DNA repair by removal of topoisomerase-I activity (in the subsequent S-phase).[32]

Studies have shown that radiation-induced cytotoxicity in non-actively dividing cells requires an intact stereo-specific interaction between camptothecin and topoisomerase-I.[33] The process is initiated by topoisomerase-I complex with DNA. Sub-lethal DNA damage occurs when this complex prevents re-sealing of the topoisomerase-I mediated single strand breaks. Collision of the replication fork and drug-stabilized single strand DNA or the addition of radiation greatly enhances tumoricidal activity.

Pre-clinical studies have evaluated the timing of drug incubation with irinotecan-radiosensitized breast cancer and melanoma cells. Drug given before or during radiation demonstrated an enhanced effect over radiation alone.[33] Radiosensitization experiments using 9-aminocamptothecin (9-AC) used a low dose with radiation.[34] With a constant total dose, dosing on multiple days weekly was more effective than a single dose given weekly.

Given the preclinical data for radiosensitization, a large number of studies have evaluated irinotecan in combination with external beam radiotherapy in adults with a range of carcinoma diagnoses. These studies have specifically evaluated patients with: esophageal[35]; head/neck squamous cell[36]; non-small cell lung[37-39]; pancreatic[40]; rectal[41-47]; and small cell lung[48-50] cancers. Irinotecan has typically been given on a weekly schedule in these studies, in several cases with additional chemotherapy agents. The overall conclusion from most of these studies is that there are no toxicities linked specifically to the combination of irinotecan with external beam radiation.

One study evaluated the combination of irinotecan and cisplatin together with external beam radiotherapy in patients with small cell lung cancer.[51] Irinotecan was given once every three weeks and this regimen resulted in excessive non-hematologic toxicity (diarrhea, dehydration, and esophagitis). In contrast, daily irinotecan was tolerable up to 18 mg/m^2 for 5 weeks concurrent with radiotherapy in patients with pancreatic cancer.[40]

One study evaluated the combination of irinotecan and temozolomide together with external beam radiotherapy in patients with glioblastoma and anaplastic astrocytoma.[52] This combination resulted in excessive hematologic and infectious toxicity and has not been developed further.

In pediatrics, the combination of vincristine and irinotecan has been used concurrently with external beam radiotherapy in patients with high-risk rhabdomyosarcoma. This combination has been shown to be tolerable in this setting.[53] Importantly, this experience has been with identical vincristine and irinotecan dosing as is used in the current protocol.

Irinotecan has also been safely combined with yttrium-90 microspheres for the treatment of patients with liver metastases from colorectal cancer, indicating that it is feasible to combine irinotecan with other forms of radiotherapy aside from external beam radiotherapy.[54]

2.3.3 Camptothecins with ^{131}I-MIBG

Two cell lines expressing the norepinephrine transporter were used to evaluate three treatment schedules with topotecan and ^{131}I-MIBG with the goal of determining the efficacy of the combination.[55] Topotecan administration given before, during, or after ^{131}I-MIBG therapy was evaluated in *in vitro* and *in vivo* model systems. Supra-additive cytotoxicity was achieved when the topotecan was administered before, during, but not after the ^{131}I-MIBG therapy. The mean times required for a 10-fold increase in tumor volume were 18.6 days (untreated), 31.9 days (^{131}I-MIBG alone), 25.3 days (topotecan alone), 37.1 days (topotecan before ^{131}I-MIBG), and 49.7 days (topotecan after ^{131}I-MIBG). 100% of tumors were cured with no myelotoxicity when topotecan was given simultaneously with ^{131}I-MIBG.

A pilot study performed in children with advanced neuroblastoma used 12 mCi/kg of ^{131}I-MIBG given on days 1 and 15 along with 5 days of topotecan starting on days 1 and 15, followed by stem cell rescue.[56] Eight patients were treated without significant toxicity and all had marrow reconstitution. Efficacy was not reported.

Based on these data, the NANT consortium initiated protocol 04-06 evaluating vincristine, irinotecan, and ^{131}I-MIBG. In this phase I trial, escalating doses of ^{131}I-MIBG were given on Day 1 together with fixed doses of vincristine (1.5 mg/m^2 on days 0 and 7) and irinotecan (20 mg/m^2 on days 0-4 and 7-11). All patients treated with ^{131}I-MIBG doses > 12 mCi/kg received autologous hematopoietic stem cell support

on day 13. The dose escalation portion of this study (NANT04-06) demonstrated that this combination was tolerable at a maximum ^{131}I-MIBG dose of 18 mCi/kg. Dose-limiting toxicities included grade 3 diarrhea (dose level 15 mCi/kg), grade 3 ALT elevation (dose level 18 mCi/kg), and grade 3 hallucination (dose level 18 mCi/kg). There were no unexpected hematologic toxicities. The objective response rate among patients treated at dose levels 8-18 mCi/kg was 25%.[57]

Closer evaluation of NANT 04-06 has suggested that the incidence of diarrhea was higher than would have been anticipated based on prior experience using irinotecan at that dose and schedule, particularly since all patients received cefixime or cefpodoxime for diarrhea prophylaxis.[57, 58] The overall incidence of grade 3 diarrhea was 6 / 18 patients, or 33%. In contrast, the incidence of grade 3 or 4 diarrhea was < 5% when irinotecan was given at a similar dose and schedule together with temozolomide in 79 patients with either neuroblastoma or Ewing sarcoma.[28, 31]

Since ^{131}I-MIBG uptake is often seen in the bowel, it is possible that the combination of irinotecan with ^{131}I-MIBG directly increased the risk of diarrhea. The schedule-dependence of irinotecan toxicity is well-documented, with more myelosuppression observed with shorter higher-dose therapy and more diarrhea observed with protracted lower-dose therapy.[59] Therefore, investigators at UCSF conducted a pilot study of the combination of ^{131}I-MIBG together with vincristine and irinotecan on a 5 day x 1 week schedule (irinotecan given as 50 mg/m^2/day). Two dose levels of ^{131}I-MIBG were evaluated, 15 and 18 mCi/kg. This irinotecan schedule was tolerable with ^{131}I-MIBG doses of 18 mCi/kg. Preliminary assessment of the first 12 patients treated revealed that only 6% of patients had grade 3 or 4 diarrhea, which compares favorably with the results obtained on NANT 04-06.[60] The objective response rate in the first 12 patients was 25%, suggesting that the anti-neuroblastoma activity of this combination is not compromised by this irinotecan dose and schedule.

2.4 Rationale for Combination of Vorinostat with ^{131}I-MIBG

2.4.1 Vorinostat

Vorinostat is a potent inhibitor of HDAC activity, binding directly to the catalytic pocket of HDAC enzymes and ultimately blocking enzymatic deacetylation. Active at low nanomolar concentrations, vorinostat has been found to inhibit the enzymatic activity of several HDACs including HDAC1, HDAC2, HDAC3 (Class I variants) and HDAC6 (Class II variant).[61, 62] All 4 core nucleosomal histones (H2A, H2B, H3 and H4) become hyperacetylated following culture of normal and malignant cells with vorinostat.[62] Vorinostat inhibition of HDAC activity and subsequent accumulation of acetylated histones, leads to enhanced transcription of genes that promote differentiation and apoptosis as well as inhibition of tumor growth.[61, 62] Vorinostat induces apoptosis in human myelomonocytic cells and acute T cell leukemia. It also inhibits the proliferation of cultured transformed human cells derived from leukemias, non-small cell lung carcinoma, colon carcinoma, central nervous system tumors, melanomas, ovarian carcinomas, renal cell carcinomas, prostate and breast cancer.[63] Vorinostat has also been shown to have significant antitumor activity in xenograft models of human prostate carcinoma, breast carcinoma and colon carcinoma. Activity has also been demonstrated in a transgenic murine model of APL. In all models, activity occurred in the absence of significant toxicity.[63]

2.4.2 Pre-clinical Studies of Histone Deacetylase (HDAC) Inhibitors in Neuroblastoma

Several groups have evaluated HDAC inhibitors other than vorinostat in neuroblastoma cell lines. A variety of HDAC inhibitors (including trichostatin A, CBHA, butyrate, valproic acid, MS-27-275, and BL1521) promote apoptosis and differentiation in a range of neuroblastoma cell lines.[64-70] Treatment of neuroblastoma cells in culture with HDACI sensitizes these cells to TRAIL-mediated apoptosis.[71] HDAC inhibition increases the activity of pro-apoptotic Bax and increases cell-cycle regulator p21 in neuroblastoma cells.[64, 67, 68, 70] Use of the HDAC inhibitor depsipeptide was shown to sensitize chemotherapy-resistant neuroblastoma cells to chemotherapy.[72]

Other groups have also evaluated HDAC inhibitors in neuroblastoma xenografts. Treatment of neuroblastoma mouse xenografts with the HDAC inhibitor CBHA suppressed tumor growth compared with vehicle-treated tumors. Some tumors regressed with this treatment and this group observed a synergistic effect with addition of all-trans retinoic acid.[73] Mouse neuroblastoma xenografts treated with HDAC inhibitor MS-27-275 showed decreases in tumor growth, decreases in tumor vascularity, and decreases in tumor p21 and MYCN oncoprotein expression.[66]

Additional preclinical studies have focused specifically on vorinostat and have demonstrated modest single-agent activity against neuroblastoma cell lines. One group treated SH-SY5Y neuroblastoma cells in culture with vorinostat (0.5-2 micromolar) and documented consistent increases in histone H3 acetylation.[74] This treatment increased expression of the cyclin dependent kinase inhibitor p21. After 48 hours of incubation with 1-2 micromolar vorinostat, these cells showed decreased cell viability. The addition of all-trans retinoic acid appeared to potentiate this effect.[74] Studies performed at UCSF by Dr. Haas-Kogan have established that the IC_{50} of vorinostat in the MYCN amplified neuroblastoma cell line NB1691 is approximately 2 micromolar.[75]

The NIH-sponsored Pediatric Preclinical Testing Program has also evaluated vorinostat in mouse xenografts established from six different neuroblastoma cell lines. Xenografts derived from four of these lines showed statistically significant prolongation in event-free survival compared to control-treated mice.[76]

Additional studies conducted at UCSF have demonstrated that neuroblastoma cell lines exposed to vorinostat at clinically attainable concentrations results in an increase in norepinephrine transporter mRNA and protein expression.[77] Vorinostat treated neuroblastoma cells also showed an increase in norepinephrine and ^{123}I-MIBG uptake compared with control treated cells. This effect was seen in three neuroblastoma cell lines exposed to clinically relevant concentrations of vorinostat. These results suggest that vorinostat will increase the uptake of ^{131}I-MIBG by neuroblastoma cells.

2.4.3 Preclinical Studies of HDAC Inhibitors as Radiation Sensitizers

A large body of preclinical data indicates that HDAC inhibitors other than vorinostat potentiate the effects of radiation on tumor cells across a range of human cancers.[78] The mechanism by which HDAC inhibitors enhance the effect of radiation appears to involve three distinct processes. Firstly, HDAC inhibitors lead to an open chromatin conformation allowing more significant DNA damage through double and single strand breaks. Secondly, HDAC inhibitors alter the production of cellular reactive oxygen species generating increased free radicals in addition to modulating the cell redox potential. Thirdly, HDAC inhibitors down-regulate the expression of DNA repair proteins. These studies have indicated that treatment with the HDAC inhibitor prior to radiation therapy provides the optimal level of synergy, though treatment following radiation exposure may also be important.

Vorinostat has been evaluated as a radiosensitizing agent in preclinical models of melanoma, glioma, prostate cancer, and colon cancer.[79-81] Pretreatment with vorinostat increases apoptosis in response to radiation in these tumor types. As with other HDAC inhibitors, the ability of vorinostat to enhance the radiation effect appears to result from impairment of DNA repair mechanisms. Specifically, vorinostat has been shown to decrease DNA repair proteins (including Ku70, Ku86, and Rad50) and prolong expression of γ-H2AX.

Dr. Haas-Kogan's laboratory at UCSF has demonstrated enhanced efficacy of vorinostat when combined with low-dose radiotherapy in neuroblastoma.[75] Her group treated NB1691 neuroblastoma cell lines with vorinostat across a range of concentrations. After 24 hours of exposure to vorinostat, cells were treated with 1 Gy radiation and remained exposed to vorinostat. The effect of this treatment on cell viability was then determined at hour 72. Control cells treated with radiation alone had 100% relative viability. Pretreatment with vorinostat at 1 micromolar reduced cell viability to approximately 20%, such that the IC_{50} for this combination was < 1 micromolar. This result compares favorably with IC_{50} of 2 micromolar for vorinostat alone. When this experiment was repeated using 2 Gy radiation, cells treated with 1 micromolar had less than 20% viability and no viable cells were observed at vorinostat concentrations of 2.5 micromolar and higher.

Dr. Haas-Kogan's laboratory also evaluated the combination of vorinostat and external beam radiation in a mouse neuroblastoma xenograft model. In this metastatic model of neuroblastoma, NB1691 cells are injected into nude mice and metastatic deposits reliably develop. Mice were then treated with vorinostat 150 mg/kg by intraperitoneal injection, whole body radiation 1 Gy, or vorinostat 150 mg/kg by intraperitoneal injection plus whole body radiation 1 Gy. Treatment was started 17 days after tumor cell injection and treatments were administered every other day for total of 3 treatments. Tumor volumes were measured and the average tumor volume per treatment group (5 mice in control group; 10 mice in treatment groups) was determined. Mice treated with the combination of vorinostat and radiation had significantly smaller tumors compared to the other treatment groups ($p < 0.05$).[75]

2.4.4 Clinical Experience with Vorinostat

Vorinostat has been used extensively in adults and has been granted US FDA approval for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma who have progressive, persistent, or recurrent disease on or following two systemic therapies (Vorinostat Package Insert).[82] The approved adult dose is 400 mg orally once daily. This dose was approved based on a series of phase I and II studies.

The first human experience with vorinostat was in an adult phase I study of an IV formulation.[83] A total of 37 patients were treated. Patients with solid tumors were evaluable for hematologic toxicity. In these patients, appreciable thrombocytopenia only occurred with multiple cycles of therapy. Platelets typically recovered within 7 days off drug. Other toxicities observed included mild gastrointestinal symptoms, fatigue, tumor pain during drug infusion, and mild renal insufficiency that resolved off drug.

This study was followed by an adult phase I study of oral vorinostat.[84] A total of 73 patients were treated. The maximum tolerated dose and schedule were 400 mg orally once daily or 200 mg orally twice daily or 300 mg orally twice daily for 3 days each week. x 3 days. Dose-limiting toxicities included anorexia, dehydration, diarrhea, elevated AST/ALT, and fatigue. The most common toxicities were fatigue, gastrointestinal symptoms, hyperglycemia, hypocalcemia, anemia, and thrombocytopenia. This study noted more thrombocytopenia with the twice daily continuous dosing schedule. Approximately 34% of patients reported dyspnea without consistent chest radiograph or electrocardiogram findings. A mild increase in creatinine was reported in 64% of patients. One patient developed a grade 3/4 increase in creatinine. The pharmacokinetic evaluations on this study indicated that vorinostat was 43% bioavailable. The exposure was linear across the range of 200-600 mg tested. The half-life was approximately 100 minutes. The mean concentration obtained after a 400 mg dose was approximately 1 micromolar (Investigator Brochure). An increase in histone H3 acetylation was noted at all dose levels within 2 hours of dose. The duration of this effect was dose dependent and extended up to 10 hours for the highest doses studied.

Another study sought to evaluate the effect of food on the pharmacokinetics of oral vorinostat.[85] Twenty-three adult patients were treated with vorinostat 400 mg orally once daily. A high-fat meal increased drug exposure by 38% and slowed the rate of absorption of the drug. After 22 days of therapy, the 24 hour exposure was similar to the initial exposure, suggesting that the drug does not accumulate and that the metabolism does not change over time. Most patients did not have drug detectable 24 hours after a dose. The most common toxicities on this study were gastrointestinal symptoms, anorexia, and fatigue. Approximately 13% of patients had grade 3 anemia and grade 3 thrombocytopenia.

An initial phase II study evaluated the activity of vorinostat in patients with refractory cutaneous T-cell lymphoma.[86] Thirty-three patients enrolled and were treated in one of three dosing arms: 400 mg orally once daily; 300 mg orally twice daily for 3 days each week for 4 weeks then 300 mg twice daily for 5 days each week; or 300 mg orally twice daily for 14 days then 200 mg orally twice daily. Eight patients had a partial response. The most common toxicities were fatigue, diarrhea, nausea, thrombocytopenia, taste changes, and dry mouth. Thrombocytopenia and dehydration were the most common grade 3 or 4 toxicities. Thrombocytopenia was most common in patients treated with 300 mg twice daily for 14 days. There appeared to be a lower response rate in patients who received discontinuous dosing.

A follow-up phase II study enrolled 74 patients with cutaneous T-cell lymphoma.[87] Patients received vorinostat 400 mg orally once daily. The study reported a 29.7% overall objective response rate and an additional 48% of patients were noted to have a clinical benefit from drug. The most common toxicities were diarrhea, fatigue, nausea, anorexia, taste disturbance, and thrombocytopenia. Grade 3 or 4 thrombocytopenia was noted in 5.4% of patients. There were three cases of QT_c prolongation.

One clinical trial utilizing vorinostat as a radiation sensitizer has been completed.[88] In that trial, 16 adult patients with pelvic gastrointestinal carcinoma were treated with the combination of vorinostat and external beam radiotherapy. The maximum tolerated dose of vorinostat was 300 mg once daily prior to daily radiation fractions for 2 weeks, with dose limiting fatigue and anorexia noted in one of six patients treated at that dose level. Other trials evaluating vorinostat in combination with external beam radiation are ongoing.

2.4.5 Clinical Experience with Vorinostat in Pediatric Patients

The clinical experience with vorinostat in children is limited. The Children's Oncology Group has completed an initial phase I study of vorinostat administered orally once daily.[89] The maximum tolerated dose was 230 mg/m^2/day. This dose corresponds closely to the adult dose of 400 mg/day, assuming an average adult body surface area of 1.8 m^2. The dose limiting toxicities included deep vein thrombosis, hypokalemia, neutropenia, and thrombocytopenia. Patients also experienced manageable hepatoxicity, including ALT, AST, and bilirubin elevations. This study was extended to also study the combination of vorinostat and cis-retinoic acid and to study a pediatric liquid formulation of vorinostat. Twelve patients were treated with the oral suspension at a dose of 230 mg/m^2/day. Four patients developed protocol-defined dose limiting toxicities in the first cycle of therapy (grade 4 thrombocytopenia in two patients; grade 3 ALT; grade 3 hyponatremia).

Following the first day's dose of vorinostat, patients reached a median peak concentration of 1.3 μM (range 0.37-2.2 μM), 0.66 μM (range 0.33-2.2 μM), and 1.2 μM (range 0.05-2.2 μM) after doses of 180 mg/m^2, 230 mg/m^2, and 300 mg/m^2, respectively. Most patients reached peak concentration within 2 hours of the oral dose. Peak drug concentrations were higher after 28 days of dosing, with mean values of 2.5 μM, 1.7 μM, and 1.7 μM at these same dose levels. These results indicate that a target peak plasma concentration of 1 μM is attainable in pediatric patients.

Follow-up pediatric studies have evaluated or are evaluating vorinostat combination regimens. These trials include NANT protocol N08-02 (vorinostat plus cis-retinoic acid), COG protocols ADVL0819 (vorinostat plus temozolomide), ADVL0916 (vorinostat plus bortezomib), and ACNS0822 (vorinostat plus external beam radiotherapy for pediatric high-grade glioma).

2.4.6 Clinical Experience with Vorinostat and ^{131}I-MIBG

The NANT consortium initiated protocol N07-03 based on three main pieces of evidence. First, vorinostat has modest activity against neuroblastoma in preclinical models. Second, vorinostat has been shown to sensitize tumor cells, including neuroblastoma, to the effects of radiation therapy. Third, vorinostat also appears to upregulate the expression of the norepinephrine transporter on neuroblastoma cell lines.

N07-03 was a phase 1 dose escalation study of ^{131}I-MIBG (Day 3) together with once daily vorinostat (Days 1-14). All patients received autologous stem cell rescue on Day 17. The recommended phase II doses were ^{131}I-MIBG 18 mCi/kg and vorinostat 180 mg/m^2/day. At this dose level, 0 of 5 patients had dose-limiting toxicity. The 6th patient at this dose level is without DLT mid-cycle 1 and will not impact final dose selection. Dose limiting toxicities included grade 4 hypokalemia in one patient treated with ^{131}I-MIBG 15 mCi/kg and vorinostat 230 mg/m^2/day, grade 3 bleeding in one patient treated with ^{131}I-MIBG 18 mCi/kg and vorinostat 230 mg/m^2/day, and grade 3 QTc prolongation and grade 5 CNS bleeding in one patient treated with ^{131}I-MIBG 18 mCi/kg and vorinostat 230 mg/m^2/day. Central review of response has not yet been completed.

2.5 Summary and Rationale for Current Trial

^{131}I-MIBG is a targeted radionuclide with one of the highest reported single-agent response rates in patients with relapsed or refractory neuroblastoma. More recent studies have attempted to combine ^{131}I-MIBG with systemic radiation sensitizers in an attempt to improve upon this single-agent activity. Since ^{131}I-MIBG will be incorporated into planned clinical trials for patients with newly diagnosed high-risk neuroblastoma, it is imperative to select the most active ^{131}I-MIBG treatment regimen for further evaluation.

Phase I studies of ^{131}I-MIBG with either irinotecan or vorinostat have demonstrated that these combinations are tolerable and active. Small sample sizes, patient heterogeneity, and escalating ^{131}I-MIBG doses do not allow the activity of these regimens to be fully assessed or compared with single-agent ^{131}I-MIBG. While we hypothesize that combination regimens will be superior to single-agent ^{131}I-MIBG, these regimens will likely be associated with increased toxicity. Therefore, the current study is a 3-arm randomized phase 2 trial designed to identify the optimal ^{131}I-MIBG treatment regimen for further study. The three treatment arms are single-agent ^{131}I-MIBG, ^{131}I-MIBG with vincristine/irinotecan, and ^{131}I-MIBG with vorinostat. Objective response rate following a single course of therapy will be the primary endpoint driving selection of the regimen to move forward into future studies.

2.6 Correlative and Exploratory Studies

2.6.1 Whole Body Radiation Exposure Following ^{131}I-MIBG Therapy

Previous studies of therapeutic ^{131}I-MIBG have derived estimates of whole body radiation exposure typically by using serial measurements of radiation exposure rate with an ionization chamber set at a fixed distance and geometry from the patient.[10] Patients treated with doses $\geq$ 10 mCi/kg typically have estimated whole body radiation doses of $\geq$ 200 cGy.[10, 90] In a group of 53 patients treated with 18 mCi/kg ^{131}I-MIBG, the median estimated whole body radiation dose was 292 cGy, with a broad range of 173 – 418 cGy.[12] Higher whole body radiation dose appears to correlate with increased hematologic toxicity[10, 12, 90], though the impact of whole body radiation dose on response to ^{131}I-MIBG therapy remains unclear.[10] Moreover, it is not clear whether the addition of irinotecan or vorinostat might impact whole body radiation dose. Two potential mechanisms by which these agents might impact whole body radiation dose include increasing tumor uptake and/or retention of ^{131}I-MIBG or by delaying physiologic clearance of ^{131}I-MIBG.

In the current study, all patients will have whole body radiation doses calculated based on serial measurements of radiation exposure rate with an ionization chamber set at a fixed distance and geometry. The primary analyses will compare whole body radiation dose between randomized treatment groups and also between responders vs. non-responders. Secondary analyses will compare whole body radiation dose between patients with and without grade 4 neutropenia, grade 4 thrombocytopenia, and grade $\geq$ 3 non-hematologic toxicity.

2.6.2 Five Gene (NB5) TLDA Assay for Neuroblastoma Tumor Cell Detection

Dr. Robert Seeger's laboratory developed a five gene (NB5) TLDA assay for sensitive detection of neuroblastoma tumor cells in peripheral blood and bone marrow. Five genes [chromogranin A (CHGA), doublecortin (DCX), dopadecarboxylase (DDC), paired-like homeobox 2B (PHOX2B), and tyrosine hydroxylase (TH)] are highly expressed by neuroblastoma cell lines and tumors. These same genes are rarely expressed by normal blood cells, peripheral blood stem cells (PBSC), and bone marrow. Four housekeeping genes are used for quality control and data analysis. Heterogeneity in expression of the detection genes among neuroblastoma cell lines (n=22) and primary untreated stage 4 neuroblastoma tumors (n=23) is minimal. However, the use of five genes assures that heterogeneity will not impact tumor cell detection.

Spiking experiments demonstrate the sensitivity of neuroblastoma cell detection using this 5-gene signature. Detection sensitivity with five genes (including TH) is superior to that of a single gene (TH). The five-gene detector has nearly 100% sensitivity to detect neuroblastoma RNA at a dose of 10^{-5}, whereas the TH-only detector has sensitivity of <60%. In terms of neuroblastoma cell detection, the 5-gene signature can detect a 10^{-6} neuroblastoma cell frequency in PBMC with 81% probability compared to <30% for a TH-alone detector.

TLDA Detection Gene Score (DG score) is highly correlated with Immunocytology Score (number of tumor cells/10^6 total cells) when Immunocytology is positive in bone marrow. Forty-four unselected fresh bone marrow specimens were tested by both assays. The rank correlation between the TLDA DG Score and Immunocytology Score was $r = -.93$ ($p < 0.001$), which demonstrates a clear relationship between the two assays. The NB5 assay also can detect tumor cells in bone marrow that are not detected by immunocytology. Thirty-six of the 44 bone marrows were negative by immunocytology, but 20 of these were positive by TLDA. These data further confirm that the NB5assay is more sensitive than immunocytology for detecting tumor cells. Moreover, detection of occult bone marrow tumor cells by TLDA appears to have clinical relevance. In patients with newly diagnosed high-risk neuroblastoma, detection of tumor cells by TLDA in a bone marrow sample negative by immunocytology at the end of therapy was associated with significantly worse event-free survival than bone marrow samples negative for tumor cells by NB5 assay.

The current study includes a secondary aim to evaluate neuroblastoma cell detection in blood and bone marrow by NB5 assay. These samples will be obtained as part of the companion NANT biology study (N04-05), such that only patients who have co-enrolled on that study will contribute samples for this secondary aim.

2.6.3 γ-H2AX as a Marker of Radiation Exposure

Ionizing radiation introduces double strand DNA breaks. In response to double strand DNA breaks, histone H2AX near these DNA breaks becomes phosphorylated to γ-H2AX, which facilitates DNA repair.[91] Recently, multiple groups have evaluated γ-H2AX levels in peripheral blood cells as a marker of double strand DNA breaks and, therefore, as a marker of radiation exposure. In one of the earliest studies of this approach, peripheral blood lymphocytes from patients undergoing diagnostic CT scans were evaluated using immunofluorescence for γ-H2AX.[92] As early as 30 minutes following CT scan, an increase in the number of γ-H2AX foci could be detected, with levels returning to baseline by 24 hours post-radiation exposure. This marker has also been utilized to estimate radiation exposure in children undergoing cardiac catheterization.[93]

Quantification of γ-H2AX has also been used as a biomarker of radiation exposure in patients with cancer. In one study, patients receiving external beam radiotherapy to a range of tumor sites had lymphocyte γ-H2AX levels quantified.[94] γ-H2AX showed a linear correlation with estimated whole body radiation exposure. The slope of the correlation varied both with tumor site and with time from start of radiotherapy, with later time points showing a less steep slope.

Another study evaluated γ-H2AX foci in patients receiving cisplatin and external beam radiotherapy.[95] Cisplatin reduced the formation of γ-H2AX foci in response to radiation, suggesting that cisplatin may compromise DNA repair following radiation-induced double strand DNA breaks. These findings have direct relevance for the current study evaluating two putative radiation sensitizers (vorinostat and irinotecan) in combination with ^{131}I-MIBG.

One study has evaluated γ-H2AX foci by immunofluorescence in patients with thyroid carcinoma receiving therapeutic ^{131}I (mean dose 92 mCi).[96] They noted that γ-H2AX levels peaked by 2 hours post-infusion, but remained elevated above baseline even by 120 hours post-infusion. These findings have direct relevance to the current study utilizing another ^{131}I radionuclide, ^{131}I-MIBG.

Preliminary studies from UCSF demonstrate that γ-H2AX foci increase after ^{131}I-MIBG, with a peak at hour 72-96 before decreasing at hour 120. In the current study, we will evaluate γ-H2AX levels as a biomarker of radiation exposure and as a pharmacodynamic tool to assess whether the addition of either irinotecan or vorinostat to ^{131}I-MIBG impairs DNA repair pathways. Patients who consent to this optional correlative biology study will have peripheral blood obtained at study entry for baseline quantification of γ-H2AX foci by flow cytometry. Due to radiation safety concerns about handling peripheral blood within the first 72 hours following ^{131}I-MIBG infusion, follow-up peripheral blood samples will be obtained at hours 72 and 96 after ^{131}I-MIBG infusion.

2.6.4 Expression of DNA Damage Response Genes

Therapeutic radiation exerts its antitumor effect by inducing DNA damage. Specific cellular pathways are initiated in response to this DNA damage. Expression of genes involved in these pathways can be quantified using quantitative RT-PCR.[97, 98]

The Coleman Laboratory at Lawrence Livermore National Laboratory has developed a panel of 16 genes whose peripheral blood expression changes in response to external beam radiotherapy, with extent of change correlated to radiation exposure. Preliminary data from 11 patients treated with ^{131}I-MIBG at UCSF demonstrate 5 transcripts (*CDKN1A, FDXR, GADD45A, BCLXI,* and *XPC*) with significant changes in gene expression detectable in peripheral blood mononuclear cells between 0 to 72 hours ($p<0.05$) and 7 transcripts (*CDKN1A, FDXR, GADD45A, STAT5B, BCLXI, XPC,* and *BIM*) with significant changes in gene expression between 0 to 96 hours ($p<0.05$). Only 2 transcripts (*CDKN1A* and *FDXR*) had significant differential gene expression across all time points ($p<0.05$). Using multiple regression analysis, 7 transcripts (*CDKN1A, FDXR, GADD45A, STAT5B, BCLXI, XPC,* and *BIM*) showed elevated responses that were dependent on time. Two transcripts (*GADD45A* and *BCLXI*) showed elevated responses that were dependent on chemotherapeutic treatment (^{131}I-MIBG combination protocol vs. single-agent ^{131}I-MIBG).

The current study will include an optional correlative biology study in which mRNA from peripheral blood mononuclear cells will be collected at baseline and at hours 72 and 96 following ^{131}I-MIBG infusion. Quantitative RT-PCR will be used to quantify each gene of interest and the change in expression will be determined for each patient. We will compare change in gene expression between randomized treatment groups. We will also compare changes in these markers based on groups defined by treatment response and treatment-associated toxicity.

2.6.5 Plasma Flt3 Ligand and Serum Amylase as Biomarkers of Radiation Exposure

Hematologic toxicity is the main dose-limiting toxicity of ^{131}I-MIBG.[12] Recent evidence suggests that plasma levels of Flt3 ligand may predict hematologic toxicity of other forms of radiotherapy. In one study of non-human primates, plasma Flt3 ligand levels increased in response to radiation, with the level correlated to radiation dose.[99] Another study demonstrated that patients treated with radioimmunotherapy who had elevated plasma Flt3 ligand levels at baseline were more likely to develop grade $\geq$ 3 thrombocytopenia.[100] Another group evaluated serial plasma Flt3 ligand levels in patients receiving fractionated external beam radiotherapy.[101] Measures of plasma Flt3 ligand during radiotherapy correlated with the proportion of bone marrow radiated.

Evaluation of serum amylase levels 24 hours after total body radiation have indicated a correlation between amylase level and whole body radiation dose.[102] A previous group evaluated changes in serum amylase in 8 patients treated with ^{131}I-MIBG therapy. All 8 patients demonstrated peak increases in serum amylase at a median of 24 hours after ^{131}I-MIBG infusion, likely due to known salivary gland uptake by ^{131}I-MIBG.[103]

Preliminary data from 26 patients treated at UCSF demonstrate robust modulation of plasma Flt3 ligand by 72 hours after ^{131}I-MIBG infusion (median fold increase from baseline 3.6; p=0.0005), with levels continuing to increase in some patients by hour 96. Patients receiving ^{131}I-MIBG as part of a combination protocol (with either vorinostat or irinotecan) showed greater degree of Flt3 modulation at hour 72. Serum amylase was also significantly modulated by 72 hours after ^{131}I-MIBG infusion (median fold increase from baseline 3.1; p <0.0001), with levels decreasing by hour 96.

Given our limited ability to predict toxicity and response following ^{131}I-MIBG therapy, the current study will include an optional correlative study in which patients will have plasma Flt3 ligand and serum amylase measured at study entry and again at hours 72 and 96 following ^{131}I-MIBG infusion. We will compare changes in these markers between randomized treatment groups. We will also compare changes in these markers based on groups defined by treatment response and treatment-associated toxicity.

2.6.6 Assistive MIBG Scan Scoring System

^{123}I-MIBG scans are typically interpreted qualitatively or semi-quantitatively by radiologists, often with poor inter-reader reliability. The Center for Research Informatics and the Human Imaging Research Office at the University of Chicago have developed a computerized method for assistive scoring of MIBG scans to overcome this inter-reader variability and to improve longitudinal comparisons. In a pilot study, 25 scans from 11 patients treated at the University of Chicago were evaluated. Planar images were scored by two experienced radiologists, according to the currently accepted standard of assigning each of 9 skeletal segments and one segment for any soft tissue disease with an uptake score of 0-3 per region. A semi-automated, computerized segmentation algorithm was developed to divide the scan image into 9 segments and assign an extension score by relative MIBG signal intensity when compared to physiologic MIBG uptake in the liver. Of the 25 scans, there was complete agreement between computer and the radiologists for 13 (52%). The two radiologists agreed on 21 scans (84%). There were six false positives (24%) and no false negatives, illustrating the high sensitivity of this method.

Recently, the group has extended the efficiency of the segmentation algorithm. The current scoring system is an assistive platform, designed to provide a consistent and standardized method for recording and tallying MIBG-avid lesions. The University of Chicago group is currently piloting this scoring platform by evaluating fifty scans obtained both from the University of Chicago and the Quality Assurance Review Center (QARC).

The current trial will include a correlative radiology study to prospectively evaluate this technique in a group of patients with relapsed MIBG-avid neuroblastoma treated with ^{131}I-MIBG therapy.

2.6.7 NET Protein Expression and MIBG Avidity

The norepinephrine transporter (NET) appears to be the main mediator of active MIBG uptake by neuroblastoma cells. In cell lines, extent of MIBG correlates with norepinephrine transporter mRNA expression.[5] Uptake of MIBG by neuroblastoma cells *in vitro* can be blocked using desipramine, a norepinephrine transporter inhibitor.[104] In archival neuroblastoma tumor samples, norepinephrine transporter mRNA and protein expression correlate with MIBG avidity on diagnostic scans.[3, 105] However, the correlation between NET protein expression and response to ^{131}I-MIBG therapy remains unknown.

In the current trial, archival tumor material will be used to quantify NET protein expression by immunohistochemistry and results correlated with response to ^{131}I-MIBG therapy.

2.6.8 Institutional Assignment of Curie Score

The Curie score is a semi-quantitative scoring system to describe the burden of ^{123}I-MIBG-avid disease in patients with metastatic neuroblastoma.[106] Curie scoring has largely been used as part of research studies and as part of response assessment in clinical trials, including centrally-reviewed assignment of Curie score in NANT trials. Recently, data have emerged that response as assessed by Curie score is prognostic in patients with newly-diagnosed high-risk metastatic neuroblastoma.[107, 108] Based on these results, clinical assignment of Curie score has become more critical. However, it is not known to what extent institutional assignment of Curie score will correlate with assignment of Curie score by experienced central review radiologists. Efforts are underway to study this issue in patients with newly-diagnosed metastatic neuroblastoma (in Children's Oncology Group protocol ANBL12P1). In the current study, we will assess this issue in patients with relapsed or refractory neuroblastoma.

2.6.9 Sinusoidal Obstruction Syndrome (SOS) after ^{131}I-MIBG therapy

The risk of SOS in patients who receive myeloablative chemotherapy following ^{131}I-MIBG therapy is not well understood. A previous NANT trial (NANT01-02) evaluated a block of ^{131}I-MIBG therapy two weeks prior to conditioning with carboplatin, etoposide, and melphalan and determined that this regimen was tolerable, though the dose of ^{131}I-MIBG was 12 mCi/kg. More recent efforts have focused on increasing the dose of ^{131}I-MIBG and allowing a longer period of recovery between ^{131}I-MIBG and the start of conditioning. Anecdotal cases of SOS have been reported, though the exact risk is not known.[109] Moreover, the impact of a radiation sensitizer given together with ^{131}I-MIBG on the risk of subsequent SOS is not known.

It is anticipated that only a minority of patients will receive subsequent myeloablative therapy after completing therapy on the current protocol. Nevertheless, these patients provide an opportunity to track and describe the incidence of SOS in these patients after subsequent myeloablative therapy.

2.6.10 Circulating Tumor DNA

Recently, new technology has emerged that enables detection of tumor-specific genetic aberrations in cell free DNA collected from the plasma. This technology enables detection of a range of potential mutations, amplifications, copy number alterations, and translocations of interest. For example, Dr. Brian Crompton has used this approach on baseline plasma from five children with newly diagnosed high-risk neuroblastoma and was able to quantify circulating tumor DNA (ctDNA) burden from all five patients based upon copy number alterations and *MYCN* amplification (personal communication). This testing will be explored on a research basis to describe baseline levels of ctDNA and changes in ctDNA levels in response to therapy.

2.7 Rationale for Amendments

2.7.1 Amendment 4

Amendment 4 includes three main changes. First, the platelet criterion for proceeding to course 2 of therapy has been lowered from $\geq$ 50,000/µl (no transfusions for one week) to $\geq$ 25,000/µl (no transfusions for one week). This change is made as patients may have stable, low level thrombocytopenia after ^{131}I-MIBG therapy and stem cell rescue without clinical consequence. As stem cell support is mandated after the second course of therapy, proceeding to a second course without having to wait for full platelet recovery is expected to be in the best interest of patients enrolling to study. This approach has been shown to be safe.[25] Second, patients who might otherwise have enrolled on study have declined participation as they would have had to travel to return to a NANT center to receive stem cell support. Since stem cells are considered supportive care and not anticancer therapy, Amendment 4 explicitly

allows receipt of stem cells at a non-NANT COG designated transplant center. This change also reduces expense of shipping stem cells and potential risk of damage to stem cell product during shipment. Third, selected patients previously treated with ^{131}I-MIBG will now be eligible to participate. This change is based upon experience from a prior NANT trial (NANT07-01) and a trial of ^{131}I-MIBG with vincristine and irinotecan. Both trials allowed prior ^{131}I-MIBG therapy on other protocols and the toxicity profile of both trials was acceptable. Compassionate access ^{131}I-MIBG protocols typically allow up to a cumulative lifetime exposure of 54 mCi/kg (three infusions of 18 mCi/kg). To account for potential disparate response rates between patients previously treated with and without ^{131}I-MIBG, randomization will now also be stratified based upon this prior therapy.

2.7.2 Amendment 5

This amendment reflects the change of institution for Steven DuBois, MD, Study Chairperson and IND holder, from University of California, San Francisco to Dana-Farber Cancer Institute. An additional update to NANT participating sites includes a change in Principal Investigator for Hospital for Sick Children, Toronto from Sylvain Baruchel, MD to Meredith Irwin, MD. The shipping address for correlative study specimens was revised. There are also minor editorial and informed consent changes associated with the change in shipping addresses.

2.7.3 Amendment 6

This amendment adds a late blood draw (protocol Day 15 at time of stem cell infusion) to extend the correlative radiobiology studies. Since it is possible that the markers under investigation as part of Aim 1.6 remain elevated at the 72 and 96 hour time point, it is of interest to determine whether these markers return to baseline by Day 15 and whether failure to return to baseline by Day 15 correlates with increased risk of toxicity. Changes were made to Sections 10.1 and 10.3.1. Section 10.1 clarifies how protocol enrollment will be handled during performance of scheduled interim analyses and a change to Section 10.3.1 clarifies the definition of evaluable for response in a secondary per-protocol analysis. Clarifying language has been added to Section 6.3 to accommodate local needs at one MIBG treating center. Additional updates include changes in the site investigator at Stanford, change in protocol coordinator, updated address for shipment of correlative radiobiology samples, and minor editorial changes.

2.7.4 Amendment 7A

This amendment changes the preferred ^{131}I-MIBG supplier from Jubilant Draximage to NDP, adds evaluation of circulating tumor DNA as a correlative biomarker to existing Aim 1.6, and modifies the statistical plan to add a rule for stopping the trial early if a winner can be identified at the time of planned interim analysis. Moreover, for patients enrolled with Amendment 7A and later, only a single course of therapy will be allowed due to financial limitations. Additional updates include changes in the site investigator at Stanford and minor editorial changes. Required observations for anatomical imaging were clarified and the response criteria v1.2 was revised to clarify intent of these criteria for morphologic bone marrow disease and overall response. Editorial revisions were made to update the TLDA assay to NB5 TLDA assay within the protocol and the informed consent document.

2.7.5 Amendment 8

This amendment includes the change in NANT Site Principal Investigator for University of Chicago, Comer Children's Hospital from Susan Cohn, MD to Ami Desai, MD as well as the change in NANT Site Principal Investigator for Seattle Children's Hospital from Julie Park, MD to Navin Pinto, MD. It also includes the removal of Memorial Sloan Kettering Cancer Center as a site and the addition of University of North Carolina, Chapel Hill as a new site.

3.0 PATIENT ELIGIBILITY CRITERIA AND REGISTRATION

3.1 Patient Preparation for Study Entry and Registration

The NANT Operations Center will accept reservations for this study from MIBG and non-MIBG institutions via email to nantrsvp@chla.usc.edu. Reservations for a treatment slot made by a non-MIBG institution must be confirmed by a NANT MIBG institution within 7 calendar days of reservation.

Notification of MIBG Treatment Center and Submission of MIBG Scans for Review:
The referring physician is expected to contact the MIBG treating institution to discuss the patient. The most recent MIBG scan and report must be sent to the MIBG treatment center as part of this review. The MIBG institution will confirm they have accepted the patient as a possible candidate for this study with the NANT Operations Center.

Co-Enrollment on NANT04-05
Co-enrollment on NANT04-05, the NANT Biology Study, is required for all patients enrolling on this trial. Patients are strongly encouraged to submit bone marrow as well as blood prior to starting therapy on NANT 11-01.

Confirmation of an Adequate Stem Cell Product for Study Entry:
Written documentation of a stem cell product that meets eligibility criteria (section 3.2.6) must be received and reviewed in the NANT Operations Center to confirm stem cell eligibility. **This documentation must be sent to the Operations Center once consent is obtained and well in advance of patient registration in order to allow time to confirm this aspect of study eligibility.**

Documentation of Bone Marrow Disease Status for Study Entry:
On study bone marrow aspirate and biopsy report is required prior to randomization on this study. Bone marrow status at entry will be used for stratification.

Timing of Registration and Treatment Initiation:
It is recommended that patients be registered no later than the Friday prior to therapy initiation. Initiation of protocol therapy is required within 7 days of registration on study.

Patient Registration On Study:
NANT trials have two components for data entry workflow: 1. Paper collection and data entry at the NANT Operations Center through CAFÉ database; 2. Remote data entry via Medidata Rave clinical data management system.

Sites will initiate the enrollment process by completing the subject screening form in Medidata Rave web-based application. The subject screen form alerts the NANT Operations Center to all prospective enrollments and timing. Sites will continue to send patient demographic information, signed informed consent and all documentation confirming eligibility to NANT Operations Center at Childrens Hospital Los Angeles by FAX at (323) 361-1803 or emailed to nantcrf@chla.usc.edu Monday through Friday, 8:30am – 5:00 pm Pacific Time at (323) 361-5687 except holidays.

Receipt of the signed consent form from only the referral center is acceptable documentation for eligibility confirmation purposes. However, consent from the MIBG center must be received prior to any protocol therapy administration at the MIBG institution.

Once all necessary documentation is received in the NANT Operations Center, sites can complete the enrollment process by submitting the eligibility form in Medidata RAVE. The NANT Operations Center will validate eligibility and assign a unique NANT registration and study subject number in Medidata RAVE. The treatment arm will be assigned by randomization by the NANT Operations Center at the time of study registration. Once eligibility is verified, the NANT Operations center will send an email confirming registration and randomized treatment arm. This email must be received prior to starting any protocol therapy or the patient will be declared ineligible. The registration email will be sent to the treating facilities, Study Chair, Study Vice-Chair, and relevant committee members. The registration steps are summarized in the Table below.

Table: Summary of Registration Steps		
#		**Comment**
1	Complete subject screening form in Medidata RAVE	
2	Send patient demographic information forms, signed informed consent and all documentation confirming eligibility to NANT Operations Center *(available on the web site (www.NANT.org))*	FAX at (323) 361-1803 or emailed to nantcrf@chla.usc.edu
3	Complete eligibility form in Medidata RAVE	
4	NANT Operations Office verifies eligibility and assigns unique NANT registration and study subject number in Medidata RAVE	
	Treatment arm will be assigned by randomization by the NANT Operations Center	
5	NANT Operations Center will send an email confirming registration and assigning NANT registration/study number and randomized treatment arm.	Confirmation sent to the treating facilities, Study Chair, Study Vice-Chair, and relevant committee members.
6	Patient can begin treatment	Receipt of signed consent from MIBG treatment site in the NANT Ops Center is required before starting treatment

A registration worksheet is available on the web site (www.NANT.org) in the data forms packet to assist institutions with registration requirements for this protocol.

Contact Person:

Research Coordinator
NANT Operations Center
Children's Hospital Los Angeles
4650 Sunset Blvd, MS #54
Los Angeles, CA 90027
Phone: (323) 361-5687
FAX: (323) 361- 1803

To allow non-English speaking patients to participate in this study, bilingual health services will be provided in the appropriate language when feasible.

Important note: The eligibility criteria listed below are interpreted literally and cannot be waived.

3.2 Inclusion Criteria

3.2.1 Age
Patients must be ≥ 12 months and ≤ 30 years of age when registered on study.

3.2.2 Diagnosis
Patients must have a diagnosis of neuroblastoma either by histologic verification of neuroblastoma and/or demonstration of tumor cells in the bone marrow with increased urinary catecholamines.

3.2.3 Disease Stage/Risk Group
Patients must have a history of high-risk neuroblastoma according to COG risk classification at the time of study enrollment. Patients who were initially considered low or intermediate-risk, but then reclassified as high-risk are also eligible.

3.2.4 Response to Prior Therapy
Patients must have at least ONE of the following:

a. **Recurrent/progressive disease** at any time prior to enrollment (regardless of overall response to frontline therapy)

b. **Refractory disease**: persistent sites of disease after achieving a best overall response of stable disease to frontline therapy after a minimum of 4 cycles of induction therapy AND patient has never had recurrent/progressive disease

c. **Persistent disease:** persistent sites of disease after achieving a best overall response of partial response to frontline therapy after a minimum of 4 cycles of induction therapy AND patient has never had recurrent/progressive disease.

1) If patient has 3 or more MIBG avid sites OR a Curie Score of $\geq$ 3, then no biopsy is required for eligibility.

2) If patient has only 1 or 2 MIBG avid sites AND Curie Score of 1-2, then a biopsy confirmation of neuroblastoma and/or ganglioneuroblastoma in at least one site (bone marrow, bone, or soft tissue) is required to be obtained at least 2 weeks after last day of prior radiotherapy to that site or at least 2 weeks after last day of prior chemotherapy/biotherapy.

3.2.5 Sites of Disease: MIBG Uptake
Patients must have evidence of MIBG uptake into tumor at ≥ one site (bone or soft tissue) within 3 weeks prior to entry on study and subsequent to any intervening therapy.

3.2.6 Autologous peripheral blood hematopoietic stem cells

The minimum dose for peripheral blood stem cells is 1.5×10^6 CD34+ cells/kg.

Patients planning to receive a stem cell dose of $1.5 - 1.9 \times 10^6$ CD34+/kg must have a back-up aliquot of $1.5 - 1.9 \times 10^6$ CD34+/kg available until a minimum of 6 patients have been rescued with stem cell dose of $1.5 - 1.9 \times 10^6$ CD34+/kg without delayed engraftment. If the first 6 patients receiving this reduced stem cell dose do not have delayed engraftment, then future patients may enroll with only a minimum of 1.5×10^6 CD34+ cells/kg and no back-up aliquot. If any of the first 6 patients have delayed engraftment, then the required stem cell dose will be changed to 2.0×10^6 CD34+ cells/kg. Participating centers will be notified of this change by a study-specific memo.

Patients planning to receive a stem cells dose of $\geq 2.0 \times 10^6$ CD34+/kg are not required to have a back-up aliquot at any time during the study.

Only un-purged stem cells are allowed unless a center has separate FDA approval for infusion of purged stem cells.

For patients whose body weight exceeds ideal body weight (IBW) by more than 20%, adjusted body weight may be used for calculation of PBSC dose (Reference: Bone Marrow Transplant. 40(7):665-9; Appendix VI).

3.2.7 Performance status
Patients must have a Lansky ($\leq$ 10 years) or Karnofsky (> 10 years) score of at least 50 (Appendix I).

3.2.8 Prior Therapy
Patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study.

Myelosuppressive chemotherapy: Last dose of any myelosuppressive chemotherapy was given at least 2 weeks before study enrollment.

Prior therapy with irinotecan or vincristine is allowed as long as neither were given in combination with ^{131}I-MIBG.

Biologic (anti-neoplastic agent; includes retinoids): must have received last dose at least 7 days prior to study enrollment.

Prior therapy with vorinostat is allowed as long as it was not given in combination with ^{131}I-MIBG.

Monoclonal antibodies: must have received last dose at least 7 days or 3 half-lives, whichever is longer, prior to study enrollment. Please refer to table posted at www.nant.org for definition of half lives for specific monoclonal antibodies.

Radiation: Patients must not have received radiation for a minimum of two weeks prior to study enrollment. Patients whose only site(s) of disease have been radiated are eligible as long patient has MIBG avidity 2 weeks after completion of radiation.

A minimum of 12 weeks prior to study enrollment is required following prior large field radiation therapy (i.e. craniospinal, whole abdominal, total lung, > 50% marrow space).

Patients are excluded if they have received total body irradiation (TBI).

Prior ^{131}I-MIBG: With Amendment 4, patients previously treated with ^{131}I-MIBG are now also eligible if they meet other entry requirements as well as ALL of the following requirements:

a. At least 6 months from the date of last ^{131}I-MIBG;
b. Response other than progressive disease on first restaging after ^{131}I-MIBG;
c. ^{131}I-MIBG given as monotherapy and not in combination with systemic anticancer agents;
d. Cumulative lifetime dose of ^{131}I-MIBG at enrollment does not exceed 18 mCi/kg (based upon assigned dose during that prior therapy; to account for unavoidable dose rounding, maximum delivered dose must not exceed 19 mCi/kg).

Stem Cell Transplant (SCT): Patients are eligible 6 weeks after myeloablative therapy with autologous stem cell transplant (timed from study enrollment). Patients status post-allogeneic stem cell transplant are excluded. Patients must meet adequate bone marrow function definition (see organ function requirements, below) post-myeloablative therapy. Patients who received stem cell reinfusion following non-myeloablative therapy are eligible once they meet peripheral blood count criteria in 3.2.10.1.

Growth factor(s): See section 3.2.10.1 for restrictions on hematopoietic growth factors.

3.2.9 Concomitant Therapy Restrictions

a. Patients must not be receiving any other anti-cancer agents or radiotherapy at the time of study entry or while on study.
b. Since valproic acid has HDAC inhibitory activity, patients must not have received valproic acid within 30 days of study entry.
c. Since vorinostat may prolong the QT interval, patients must not be receiving other medications known to prolong the QT interval at the time of study entry (see first column of table in Appendix II). Pentamidine must not have been received within 1 week of study enrollment.
d. Due to drug-drug interaction with irinotecan, patients must not be receiving enzyme-inducing anticonvulsants (phenobarbital, phenytoin, or carbamazepine) at the time of study entry.
e. No medications that interfere with ^{131}I-MIBG uptake should be given within 7 days of planned start date for ^{131}I-MIBG (see Appendix V). These same medications are prohibited within 7 days after ^{131}I-MIBG infusion.

3.2.10 Organ Function Requirements

3.2.10.1 Hematologic Function:

a. ANC: $\geq$ 750/uL (no short-acting hematopoietic growth factors within 7 days of blood draw documenting eligibility and no long-acting hematopoietic growth factors within 14 days of blood draw documenting eligibility)
b. Platelet count: $\geq$ 50,000/µl, transfusion independent (no platelet transfusions within 7 days of blood draw documenting eligibility)
c. These criteria must be met by all patients, regardless of bone marrow involvement with tumor.
d. There is not an eligibility criterion for hemoglobin.
Patients must have hemoglobin ≥ 10 g/dL on the day of or prior to each ^{131}I-MIBG infusion.

3.2.10.2 Renal Function:

Patients must have adequate renal function defined as age-adjusted serum creatinine ≤ 1.5 x normal for age (see below):

Age	Maximum Allowable Serum Creatinine
≤ 5 years	0.8 mg/dL
> 5 and ≤ 10 years	1.0 mg/dL
> 10 and ≤ 15 years	1.2 mg/dL
> 15 years	1.5 mg/dL

3.2.10.3 Liver Function:
a. Total bilirubin ≤ 1.5 x normal for age, and
b. SGPT (ALT) < 3 x upper limit of normal (note that for ALT, the upper limit of normal for all NANT sites is defined as 45 U/L).

3.2.10.4 Cardiac Function:
a. Normal ejection fraction (≥ 55%) documented by either echocardiogram or radionuclide MUGA evaluation OR normal fractional shortening (≥ 27%) documented by echocardiogram, and
b. Corrected QT (QT_c) interval ≤ 450 msec.

3.2.10.5 Lung Function:
Normal lung function with no dyspnea at rest or oxygen requirement.

3.2.10.6 Coagulation Function:
a. International Normalized Ratio (INR) ≤ 1.5
b. Partial thromboplastin time (PTT) ≤ 1.5 times upper limit of normal.

For patients having labs drawn via heparinized catheters, it is important to request heparin-adsorbed values.

3.2.10.7 Reproductive Function:
All post-menarchal females must have a negative beta-HCG. Males and females of reproductive age and function must use effective contraception for the duration of their participation or for 3 months after last dose of protocol therapy, whichever is longer.

3.2.11 Coexisting Medical Conditions
Patients with other ongoing serious medical issues must be approved by the study chair prior to registration.

3.3 Exclusion Criteria

3.3.1 Pregnancy, breast feeding, or unwillingness to use effective contraception during the study.

3.3.2 Patients status post-allogeneic stem cell transplant are not eligible.

3.3.3 Patients who, in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study.

3.3.4 Obese patients whose calculated dose of ^{131}I-MIBG exceeds 1200 mCi must receive a treatment dose of at least 85% of protocol maximum dose of 1200 mCi to be evaluable for the primary endpoint of the study. If such a dose cannot be administered at the planned treating center and the patient is not able to travel to another MIBG center that can accommodate a higher dose, then the patient will be ineligible.

3.3.5 Patients with disease of any major organ system that would compromise their ability to withstand therapy.

3.3.6 Patients who are on hemodialysis.

3.3.7 Patients with an active or uncontrolled infection. Patients on prolonged antifungal therapy are still eligible if they are culture negative, afebrile, and meet other organ function criteria.

3.3.8 Patients and/or families who are physically and psychologically unable to cooperate with the radiation safety isolation.

3.3.9 Patients with a history of deep venous thrombosis that was not associated with the presence of a central venous catheter.

3.3.10 Patients must not have active diarrhea (defined as $\geq$ Grade 2 per CTCAE v4 [Grade 2 = increase of 4-6 stools/day over baseline])

3.3.11 Known active infection with HIV, hepatitis B, or hepatitis C (testing of patients not known to be infected with these viruses is not required prior to study entry).

3.3.12 Patients who are receiving Coumadin.

3.3.13 Patient declines participation in NANT04-05.

3.4 Regulatory

3.4.1 Informed Consent

The patient and/or the patient's legally authorized guardian must acknowledge in writing that consent to become a study subject has been obtained, in accordance with institutional policies approved by the US Department of Health and Human Services.

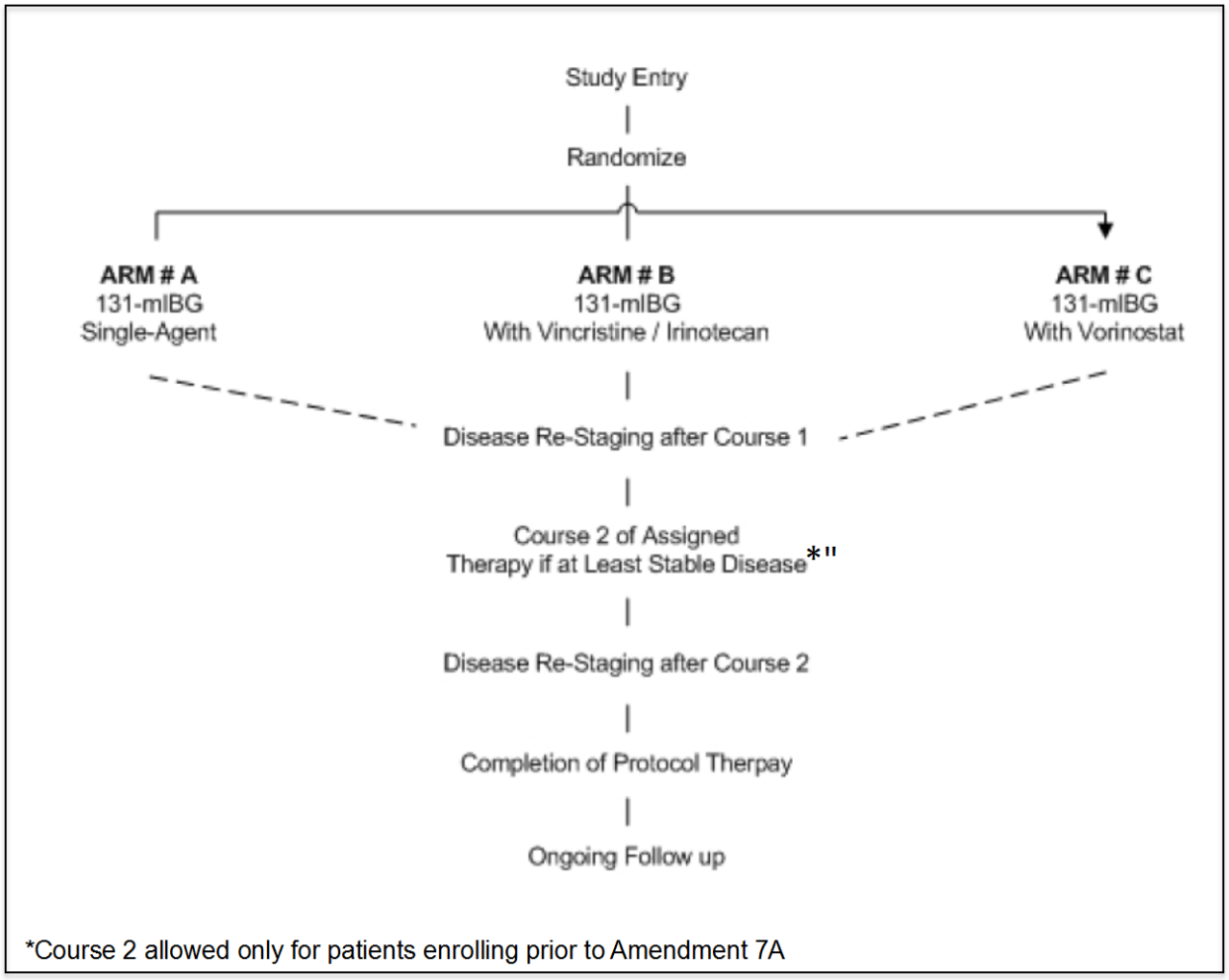
3.4.2 Protocol Approval

All institutional, FDA, and NCI requirements for human studies must be met.

4.0 TREATMENT PROGRAM

4.1 Treatment Overview

Patients will be randomized at study entry to one of three treatment arms.



All patients will undergo disease restaging between Day +43 and +50, 6-7 weeks after ^{131}I-MIBG infusion. Patients enrolling prior to Amendment 7A with at least stable disease to the first cycle can receive a second cycle of therapy, provided they meet stem cell and organ function criteria to proceed to a second cycle (Section 4.6). Patients enrolling with Amendment 7A and later may receive a single cycle.

4.1.1 Single-Agent ^{131}I-MIBG (Arm A)
Patients assigned to single-agent ^{131}I-MIBG will receive 18 mCi/kg ^{131}I-MIBG on Day 1 (Section 4.3) and autologous stem cell infusion on Day 15 (Section 4.4).

Days	1	15	43-50
Therapy	M	HSC	Eval

^{131}I-MIBG	M	Day 1
Stem Cell Infusion	HSC	Day 15
Evaluation	Eval	Day 43-50

4.1.2 ^{131}I-MIBG with Vincristine and Irinotecan (Arm B)

Patients randomized to receive vincristine / irinotecan / ^{131}I-MIBG will receive vincristine 2 mg/m^2 (maximum dose 2 mg; see section 4.2.2.1 for dosing if <3 years old) intravenously on Day 0. They will receive irinotecan 50 mg/m^2 (maximum dose 100 mg) intravenously on Days 0 to 4. Patients will also receive diarrhea prophylaxis with cefixime 8 mg/kg/day orally on Days -1 to +6. Patients will receive 18 mCi/kg ^{131}I-MIBG on Day 1 (Section 4.3) and autologous stem cell infusion on Day 15 (Section 4.4).

Days	-1	0	1	2	3	4	5	6	15	43-50
Therapy	C	C VCR I	C I M	C I	C I	C I	C	C	HSC	Eval

Cefixime	C	Days -1 through Day 6
^{131}I-MIBG	M	Day 1
Irinotecan	I	Days 0 through 4
Vincristine	VCR	Day 0
Stem Cell Infusion	HSC	Day 15
Evaluation	Eval	Day 43-50

4.1.3 ^{131}I-MIBG with Vorinostat (Arm C)

Patients randomized to receive vorinostat / ^{131}I-MIBG will receive vorinostat orally once daily on Days -1 to +12 (14 total days) at a dose of 180 mg/m^2/dose (maximum dose 400 mg). Patients will receive 18 mCi/kg ^{131}I-MIBG on Day 1 (Section 4.3) and autologous stem cell infusion on Day 15 (Section 4.4).

Days	-1	0	1	2	3	4	5-12	15	43-50
Therapy	V	V	V M	V	V	V	V	HSC	EVAL

Vorinostat	V	Days -1 through +12 (14 total days)
^{131}I-MIBG	M	Day 1
Stem Cells	HSC	Day 15
Evaluation	Eval	Day 43-50

4.2 Non-MIBG Drug Administration by Assigned Treatment Arm

4.2.1 Single-Agent ^{131}I-MIBG Arm

Patients randomized to receive single-agent ^{131}I-MIBG will receive only ^{131}I-MIBG on protocol Day 1 per Section 4.3. They will receive stem cell infusion on protocol Day 15 per Section 4.4. No other systemic anticancer agents will be administered.

4.2.2 Vincristine / Irinotecan / ^{131}I-MIBG Arm

Patients randomized to receive vincristine / irinotecan / ^{131}I-MIBG will receive ^{131}I-MIBG on protocol Day 1 per Section 4.3. They will receive stem cell infusion on protocol Day 15 per Section 4.4. In addition, they will receive:

4.2.2.1 Vincristine

Vincristine will be dosed according to the age and size of the patient at the time of scheduled treatment according to the following table. Vincristine should be administered according to institutional guidelines (by IV push or via mini-bag over 15 minutes) prior to irinotecan on day 0 of each course.

Age at time of treatment:	Vincristine dose on Day 0 before irinotecan.
≥ 3 years	2.0 mg/m² IV Push (max dose 2.0 mg)
≥1 year and < 3 years	0.067 mg/kg IV Push (max dose 2.0 mg)

4.2.2.2 Irinotecan

Patients will receive irinotecan 50 mg/m^2 (max dose 100 mg), given by IV infusion over 60-90 minutes on days 0 to + 4.

On the day of ^{131}I-MIBG administration (protocol Day 1), irinotecan is administered 6 hours (+/- 3 hours) after the completion of the ^{131}I-MIBG infusion.

If there is a delay in ^{131}I-MIBG administration due to production or logistical reasons and the patient has already started Day 0 irinotecan, the remaining irinotecan doses should be held and re-dated to resume on the day of ^{131}I-MIBG administration. This strategy is intended to maximize the degree of overlap between the radiation exposure and remaining doses of irinotecan. In this situation, cefixime or cefpodoxime should be extended to continue for two days after the last dose of irinotecan.

See section 5.4 for supportive care guidelines for irinotecan-associated diarrhea.

4.2.2.3 Cefixime or Cefpodoxime Diarrhea Prophylaxis

Cefixime will be given at a dose of 8 mg/kg/day (maximum daily dose 400 mg) given once daily by mouth on days -1 to +6.

If cefixime is unavailable in suitable oral form for adequate dosing, cefpodoxime may be substituted at 10 mg/kg/day (maximum dose 800 mg) divided twice daily on days -1 to +6.

If patients have a documented allergy to third generation cephalosporins, cefixime or cefpodoxime may be omitted. If desired, another broad-spectrum oral antibiotic not listed as a prohibited concomitant medication may be substituted.

4.2.3 Vorinostat / ^{131}I-MIBG Arm

Patients randomized to receive vorinostat / ^{131}I-MIBG will receive ^{131}I-MIBG on protocol Day 1 per Section 4.3. They will receive stem cell infusion on protocol Day 15 per Section 4.4. In addition, they will receive:

4.2.3.1 Vorinostat

Patients will receive vorinostat 180 mg/m^2/dose (maximum dose 400 mg) once daily by mouth, NG, or G-tube on days -1 to +12 (14 total doses).

Vorinostat will be given for 14 days continuously, even if the ^{131}I-MIBG portion of therapy is delayed due to production or other logistical reasons.

For patients unable to swallow capsules, vorinostat will be given as a 50 mg/mL extemporaneous liquid suspension (see section 6.1 for preparation instructions) with dosing according to the nomogram in Appendix III. The maximum absolute vorinostat dose is 400 mg.

Patients who are able to swallow capsules may receive vorinostat capsules instead of suspension if their calculated vorinostat dose is within +/- 10% of a 100 mg increment (eg. 100 mg, 200 mg, 300 mg, or 400 mg). Otherwise, they will need to receive vorinostat as suspension and dosed according to the nomogram in Appendix III. The maximum absolute vorinostat dose is 400 mg.

Vorinostat should be taken with food or within 30 minutes after a meal. The dose should be taken in the morning whenever possible.

On day 1 of therapy, vorinostat should be taken 1 hour prior to the start of the ^{131}I-MIBG infusion. Note that patients on this arm of the trial are to have a BUN and Cr obtained on day 1 prior to the ^{131}I-MIBG infusion to confirm that Cr still meets eligibility criteria in 3.2.10.2. See 4.8.1.5 for guidelines.

4.3 ^{131}I-MIBG Administration for All Treatment Arms

The primary ^{131}I-MIBG supplier for this study is Nuclear Diagnostics Products NDP. If a supply of ^{131}I-MIBG is not available from NDP for a specified date, Jubilant DraxImage, Canada is a secondary ^{131}I-MIBG supplier for this study.

4.3.1 ^{131}I-MIBG Dose and Administration

^{131}I-MIBG 18 mCi/kg is given on day 1 of therapy to all patients regardless of randomized treatment arm.

The maximum absolute dose of ^{131}I-MIBG on this protocol is 1200 mCi, though individual MIBG treating centers may have lower maximum allowed doses due to radiation safety considerations. The dose administered should be within 15% of the prescribed dose (see section 10.3.1 for definition of evaluable for response based upon administered ^{131}I-MIBG dose). There will be no adjustment for obesity except to cap the absolute dose at 1200 mCi.

^{131}I-MIBG is provided by both suppliers with a specific activity not less than 30 mCi/mg MIBG at the calibration date.

Radiopurity is checked by both suppliers prior to shipment. Free radioiodine content is to be re-checked at the treating center prior to infusion according to the methods in Appendix IV and should be < 5% for ^{131}I-MIBG from DraxImage and < 7% for ^{131}I-MIBG from NDP.

The therapeutic dose of ^{131}I-MIBG will be infused intravenously over 1.5-2 hours on day 1 of therapy with appropriate hydration, radiation isolation, thyroid blocking with potassium iodide and bladder protection with a Foley catheter.

The patient will remain in a radiation protected isolation room until radiation emissions meet institutional/state guidelines. This usually takes 3-5 days and then the patient can be discharged.

Patients should be transfused as needed to achieve a documented hemoglobin on the day of or one day prior to ^{131}I-MIBG administration of $\geq$ 10 g/dL.

4.3.2 Hydration

In order to ensure adequate hydration, patients will receive a minimum of 1.5-times maintenance rate IV fluids starting the night prior to ^{131}I-MIBG infusion and continuing until radiation dose rate is < 7 mrem/hr at 1 meter. Once < 7 mrem/hr, IV hydration is at the discretion of the treating institution.

For patients assigned to the vorinostat / ^{131}I-MIBG arm, the total IV plus oral fluid intake must be a minimum of maintenance rate until the patient has completed vorinostat therapy.

4.3.3 Bladder protection

A Foley catheter is strongly recommended for all patients prior to ^{131}I-MIBG infusion and required of all patients < 12 years of age. Patients > 12 years of age may decline Foley catheter placement at investigator discretion if they agree to void every 2 hours throughout the hospital admission. Catheters, if placed, should remain in place for at least 48 hours. For continent

patients, the catheter may be removed after 72 hours or after the patient's dose rate at 1 meter is < 7 mrem/hr, whichever occurs earlier. For incontinent (diapered) patients, the catheter is recommended to remain in place until the patient is released from radiation isolation.

If for physical/anatomic reasons it is not possible to successfully place or retain a catheter, then the treatment may proceed for continent patients who are able to comply with voiding every 2 hours throughout the first 72 hours following the infusion.

4.3.4 Thyroid protection

Potassium iodide (KI) solution will be given in a loading dose of 6 mg/kg by mouth 8-12 hours prior to infusion of MIBG on Day 1, and then 1 mg/kg/dose by mouth starting 4-6 hours after completion of MIBG infusion and continuing every 4 hours on protocol Days 1 to 7 and then 1 mg/kg/dose by mouth once daily on protocol Days 8 to 43.

If ^{131}I-MIBG infusion is delayed due to shipment or other issues and the patient has already received their loading dose, a suggested practice is to start the subsequent KI doses on schedule as though the ^{131}I-MIBG infusion had been completed on schedule.

4.3.5 Radiation isolation

Following the ^{131}I-MIBG infusion, patients must remain in radiation isolation until the patient's radiation emissions meet state/institutional guidelines for release.

4.3.6 Imaging

An MIBG imaging study similar to the diagnostic scan will be done at the time of release from radiation isolation and will not require an additional injection. This is to confirm tumor MIBG uptake and to survey for occult sites of disease.

4.3.7 Whole body dosimetry

While in radiation isolation, whole-body monitoring will be performed using a ceiling-mounted meter at consistent patient geometry. The person obtaining the measurement should be certain the patient is centered under the monitor and that the height of the bed has not been changed.

All times are noted from the beginning of the ^{131}I-MIBG infusion and assumes a 2-hour infusion.

The minimum whole body measurements will be taken as follows, and the exact dates and times marked on the recording data form. All times are measured from the beginning of the MIBG infusion.

Infusion	Hour 0, 1, 2 (including end infusion, with time marked)
Post-Infusion Hours 2-8	Every 30 minutes hours 2-8
Post-Infusion Hours 8-24	Hours 8, 12, 16, 20, 24
Subsequent Days	Every 8 hours until hospital discharge or hour 120, whichever occurs first

If the ceiling mounted monitor is not functional for any reason, then measurements will be taken with a handheld ion chamber at 1 meter from the umbilicus with consistent geometry and distance beginning at the completion of the infusion (hour 2) and at hours 3, 4, 5, 6, 7, 8, then every 8 hours thereafter.

4.4 Autologous Peripheral Stem Cell Infusion for All Treatment Arms

4.4.1 Stem Cell Timing/Dosage

Peripheral blood stem cells will be infused on Day 15 of each course for all patients. In situations in which the DMSO concentration in the stem cell product would exceed an accepted level for infusion within a 24 hour period, stem cell products may be infused over two days to meet this standard.

Stem cell infusion may occur sooner if indicated (i.e. due to an infectious or bleeding complication) after consultation with the Study Chair, Study Vice Chair, or designee.

Stem cell infusion is planned for 2 weeks after ^{131}I-MIBG infusion (Day 15). However, stem cells may be infused in the window +/- 2 days from planned date to avoid weekend or holiday stem cell infusions. If the ^{131}I-MIBG infusion is delayed due to production or logistical reasons, the date of stem cell reinfusion should be re-dated for 14 days after ^{131}I-MIBG infusion.

The minimum dose for peripheral blood stem cells is 1.5 x 10^6 viable CD34+ cells/kg. Patients planning to receive a stem cell dose of 1.5 - 1.9 x 10^6 CD34+/kg must have a back-up aliquot of 1.5 - 1.9 x 10^6 CD34+/kg available until a minimum of 6 patients have been rescued with stem cell dose of 1.5 - 1.9 x 10^6 CD34+/kg without delayed engraftment (section 4.7). If the first 6 patients receiving this reduced stem cell dose do not have delayed engraftment, then future patients may enroll with only a minimum of 1.5 x 10^6 CD34+ cells/kg and no back-up aliquot. If any of the first 6 patients have delayed engraftment, then the required stem cell dose will be changed to 2.0 x 10^6 CD34+ cells/kg and sites alerted to this change via consortium memo.

Patients planning to receive a stem cells dose of $\geq$ 2.0 x 10^6 CD34+/kg are not required to have a back-up aliquot at any time during the study.

For patients whose body weight exceeds ideal body weight (IBW) by more than 20%, adjusted body weight may be used for calculation of PBSC dose (Reference: Bone Marrow Transplant. 40(7):665-9; Appendix VI).

4.4.2 Pre-medications/Stem Cell infusion

Stem cells will be infused following institutional guidelines for prophylaxis of hypersensitivity reactions and monitoring.

4.4.3 Location of Stem Cell Infusion

Stem cell infusion is considered a supportive care measure on this protocol. Stem cells may be infused at a NANT institution or at a COG-designated transplant center (typically closer to a patient's residence). If stem cells are infused at a non-NANT institution, the treating NANT center is responsible for collecting and submitting documentation of stem cell infusion, including dose of infused stem cells, date of infusion, and any AE. According to standard clinical practice, any non-NANT institution infusing stem cells will obtain clinical consent for the stem cell infusion.

4.5 Myeloid Growth Factor Support for All Patients

Patients may receive prophylactic myeloid growth factor support following stem cell infusion at the discretion of the treating investigator according to institutional practice. Myeloid growth factor support is strongly recommended for any patient who develops neutropenia (ANC < 500/µL). A suggested practice is to use filgrastim at a dose of 5 micrograms/kg subcutaneously once daily starting when ANC < 500/µL and continuing until ANC > 2000/µL. A single dose of pegfilgrastim (100 micrograms/kg; maximum dose 6 mg) given subcutaneously may be substituted for filgrastim according to institutional standard practice following ^{131}I-MIBG therapy.

If the ANC falls below 500/µL before stem cell infusion, myeloid growth factor may be initiated prior to stem cell infusion as long as both of the following criteria are met:

- Off vorinostat and/or irinotecan for a minimum of 24 hours prior to start of growth factor;

- Measured or estimated radiation level of < 1 mrem/hr at 1 meter prior to start of growth factor.

4.6 Criteria to Receive a Second Course of Therapy

Patients who meet all of the following criteria at the end of the first course of therapy may proceed to a second course of assigned therapy:

- Enrolled prior to Amendment 7A (patients enrolling with Amendment 7A and later may receive a single course only);
- Adequate stem cells to support a second course per section 3.2.6;
- Organ function meets criteria in section 3.2.10 except:
  - EKG only needs to repeated prior to course 2 for patients randomized to vorinostat arm; all arms require repeat echocardiogram); and
  - Platelets only need to recover to ≥ 25,000/µl (no transfusions for one week prior to CBC documenting platelet recovery to this level);
- No intervening anti-cancer therapy between first course and planned second course;
- Day 1 of planned second course no earlier than Day 43 of first course (minimum 6 weeks between ^{131}I-MIBG infusions);
- No delayed engraftment (section 4.7) or need for dose modification (section 4.8) in first course of therapy;
- Disease status at end of first course of stable disease or better (section 11)
- Cumulative lifetime assigned dose of ^{131}I-MIBG does not exceed 36 mCi/kg at time of planned second infusion on this protocol (based upon assigned dose during that prior therapy; to account for unavoidable dose rounding, maximum previously delivered dose must not exceed 38 mCi/kg).

4.7 Toxicity Assessment and Definition of Engraftment

Toxicity will be graded using the CTCAE criteria, version 4. 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).**

Neutrophil engraftment will be defined as ANC ≥500/µL for a minimum of 3 days following neutrophil nadir, with or without myeloid growth factor support. Date of neutrophil engraftment will be the first day of 3 days with ANC ≥500/µL.

Platelet engraftment will be defined as platelets ≥ 20,000/µL for a minimum of 3 days following platelet nadir without platelet transfusion for one week. Date of platelet engraftment will be the first day of 3 days with platelets ≥ 20,000/µL. If a patient has a platelet transfusion and then does not have another platelet count < 20,000/µL, the date of platelet engraftment will be the first date with platelet count ≥ 20,000 following platelet transfusion.

Delayed engraftment will be defined as failure to engraft neutrophils by Day 43 of protocol therapy (28 days from stem cell infusion) and/or failure to engraft platelets by Day 71 of protocol therapy (56 days from stem cell infusion), in the absence of progressive bone marrow metastatic disease. Any case of delayed engraftment should be reported to the study chair and to the NANT Operations Center.

4.8 Dose Modification

Any dose modification should be reported to the study chair and to the NANT Operations Center

4.8.1 Vorinostat Dose Modifications

4.8.1.1 Patients who develop grade 4 neutropenia on protocol days -1 to +5 (first 7 days) will have vorinostat discontinued. Myeloid growth factor can be initiated early per Section 4.5, recognizing that patients must have been off vorinostat for at least 24 hours and the measured or estimated radiation level must be < 1 mrem/hr at 1 meter prior to start of growth factor.

4.8.1.2 Patients who develop grade 4 neutropenia AND one of the following complications of neutropenia on protocol days 6 to 12 (second 7 days) will have vorinostat discontinued and myeloid growth factor started per section 4.5:

- Documented bacteremia with an organism other than Staphylococcus epidermidis
- Clinical culture-negative sepsis
- Documented systemic fungal infection

Patients who develop grade 4 neutropenia without one of these complications on protocol days 6 to 12 will continue to receive vorinostat at the prescribed dose and schedule. Myeloid growth factor may be initiated starting on protocol day 13 according to Section 4.5 and investigator preference.

4.8.1.3 Patients who develop grade 4 thrombocytopenia on protocol days -1 to +5 (first 7 days) will have vorinostat discontinued.

4.8.1.4 Patients who develop grade 4 thrombocytopenia who become platelet transfusion refractory on protocol days 6 to 12 (second 7 days) will have vorinostat discontinued.

Patients who develop grade 4 thrombocytopenia on protocol days 6 to 12 who can safely be supported with platelet transfusions will continue to receive vorinostat at the prescribed dose and schedule.

4.8.1.5 Patients on Arm C with vorinostat who have a required creatinine on day 1 that exceeds eligibility criteria in 3.2.10.2 should have vorinostat discontinued permanently, hydration increased, and ^{131}I-MIBG infusion postponed until creatinine again within eligibility criteria. If creatinine not back to eligibility levels by the time the dose of ^{131}I-MIBG expires, then the ^{131}I-MIBG should not be administered and the patient will be off protocol therapy.

4.8.2 Irinotecan Dose Modifications

For patients who develop grade 3 diarrhea persisting more than 72 hours with appropriate therapy or any grade 4 diarrhea during the five-day course of irinotecan, the remaining doses of irinotecan should be omitted for the course.

4.9 Concomitant Therapy

4.9.1 No other cancer chemotherapy, radiotherapy, biologic therapy, or immunomodulating agents will be used.

4.9.2 Appropriate antibiotics, blood products, anti-emetics, fluids, electrolytes and general supportive care are to be used as necessary (see also Section 5.0).

4.9.3 No medications that interfere with ^{131}I-MIBG uptake should be given during the week prior or after ^{131}I-MIBG therapy (see Appendix V).

4.9.4 Patients randomized to receive vorinostat may not receive valproic acid until disease re-evaluation following the last planned course of therapy has been completed.

4.9.5 Patients receiving vorinostat have rarely had QT prolongation.[110] A formal QT study was performed in which patients with advanced cancer received a single supratherapeutic dose of vorinostat of 800 mg or placebo.[110] No patients had QT_c prolongation of more than 30 msec and no patients had QT_c > 480 msec. The effect of vorinostat with other drugs that prolong the QT interval is not clear, though no issues were noted on NANT07-03 with vorinostat, ^{131}I-MIBG, and allowed use of ondansetron. Patients assigned to the vorinostat arm may not receive other medications known to prolong the QT_c interval within 1 week prior to or 1 week following vorinostat (Appendix II; note that this list prohibits the use of pentamidine and cautions investigators with the use of ondansetron).

4.9.6 Vorinostat treatment may result in mild increases in serum creatinine. Caution should be exercised with the use of nephrotoxic medications while patients are receiving vorinostat.

4.9.7 The following agents that may interfere with irinotecan metabolism may not be administered to patients assigned to the irinotecan arm until Day 15 of the second course of protocol therapy:

- High-dose dexamethasone (other systemic corticosteroids allowed only for management of asthma, allergy, premedication for blood transfusions, or management of CNS lesions);
- Aprepitant (Emend);
- Ketoconazole and St. John's Wort;
- Phenobarbital, carbamazepine, phenytoin.

5.0 SUPPORTIVE CARE

5.1 Prophylaxis for Pneumocystis Pneumonia

All patients should receive PCP prophylaxis according to institutional guidelines. Pentamidine is prohibited during the week prior to and week following vorinostat therapy due to QT_c prolongation.

5.2 Use of Myeloid Growth Factors

See sections 4.5 and 4.8.1.

5.3 Hydration status

Dose limiting toxicities in previous studies of vorinostat included anorexia, dehydration, diarrhea, and fatigue. The combination of irinotecan and ^{131}I-MIBG is associated with an increased risk of diarrhea. Patients should maintain adequate fluid intake while receiving these therapies.

5.4 Management of Irinotecan-Associated Diarrhea

Diarrhea can be profuse and can occur either early (minutes to hours) or late (days) in the treatment schedule. Early diarrhea results from cholinergic stimulation, while late diarrhea relates to bacterial processing of irinotecan metabolites.

Early diarrhea: Patients who have the onset of diarrhea during the irinotecan infusion or in the hours following the completion of an irinotecan infusion should receive atropine (suggested dose 0.01mg/kg IV, maximum dose 0.4 mg). Prolongation of the irinotecan infusion to 90 minutes may be beneficial. Early onset diarrhea is usually accompanied by cholinergic manifestations (diaphoresis and abdominal cramping). Since irinotecan is being administered on a prolonged schedule (daily x 5) it may become difficult to distinguish early vs. late diarrhea. If a patient with presumed early diarrhea does not improve with administration of atropine, they should be instructed to begin treatment for late diarrhea.

Late diarrhea (more than 24 hours after irinotecan administration): Patients will be given Loperamide for late diarrhea based on body weight (according to the chart below). If late diarrhea occurs at home after the last dose of irinotecan is given, each family will be instructed to have antidiarrheal medication available and begin treatment at the first episode of poorly formed or loose stools or the earliest onset of bowel movements more frequent than normally expected for the patient. Patients will also be instructed to contact their physician if any diarrhea occurs. Patients and families will be given the instruction sheet in Appendix VII.

Loperamide dosing recommendations for late diarrhea

Loperamide 1 teaspoon = 1 mg; 1 caplet = 2 mg

Maximum dose of loperamide per day: < 6 years: 4 mg/day; 6-11 years: 6 mg/day; >11 years: 16 mg/day, using the non silicone-containing product.

Weight: > 43 kg:	4 teaspoons or 2 caplets (4 mg) after the first loose bowel movement, followed by 2 teaspoons or 1 caplet (2 mg) every 2 hours. During the night the patient may take, 2 caplets (4 mg) every 4 hours rather than1 caplet (2 mg) every 2 hours.
Weight: 30kg – 43kg:	2 teaspoons or 1 caplet (2 mg) after the first loose bowel movement followed by 1 teaspoon or one-half caplet (1 mg) every 2 hours. During the night the patient may take, 1 caplet (2 mg) every 4 hours rather than one-half caplet every 2 hours.
Weight: 20kg – < 30kg:	2 teaspoons or 1 caplet (2 mg) after the first loose bowel movement followed by 1 teaspoon or one-half caplet (1 mg) every 3 hours. During the night, the patient may take 1 caplet (2 mg) every 4 hours rather than one-half caplet every 3 hours.
Weight: 13kg - < 20kg:	1 teaspoon (1 mg) after the first loose bowel movement followed by 1 teaspoon (1 mg) every 3 hours. During the night, the patient may take 1 teaspoon (1 mg) every 4 hours rather than every 3 hours.
Weight: < 13kg:	Half teaspoon (0.5 mg) after the first loose bowel movement followed by half teaspoon every 3 hours. During the night, the patient may take half teaspoon (0.5mg) every 4 hours rather than every 3 hours.

If there is failure of loperamide to control diarrhea after 24 hours of use, patients should receive **octreotide** (Sandostatin™) at the dose of **10 micrograms/kg/dose** subcutaneously every 12 hours x 3 days.

Because patients will be receiving prolonged exposure to antibiotics (i.e., cefixime or cefpodoxime), patients having significant diarrhea should have stool samples evaluated for *C difficile* toxin, as this is a potentially treatable cause of diarrhea. If patients have fever with diarrhea, or bloody diarrhea, evaluation of stools for bacterial culture should also be performed.

6.0 DRUG INFORMATION

6.1 Vorinostat

Vorinostat (also known as Zolinza™)
CAS Registry Number: 149647-78-9
NSC# 701852 IND# 71976
6.1.1 Structure and Molecular Weight
The chemical names for vorinostat are N-hydroxy-N'-phenyl-octane-1,8-diotic acid diamide or N-hydroxyl-N'-phenyl (9CI) octanediamide. The molecular formula is*:* C14H20N203. The molecular weight is 264.32.

6.1.2 Description
Vorinostat is a histone deacetylase (HDAC) inhibitor. Histone deacetylases (HDACs) are a group of proteins that regulate gene transcription by affecting the acetylation status of histones. Vorinostat binds to the catalytic pocket of HDAC enzymes and inhibits their activity.

6.1.3 How Supplied
Vorinostat is supplied by Merck and Co., Inc. The 100 mg dose of vorinostat is provided in white opaque gelatin capsules (size 3). The capsules are supplied in HDPE (high-density polyethylene) bottles. Each bottle contains 120 of the 100 mg capsules.

The inactive ingredients contained in each capsule are microcrystalline cellulose, sodium croscarmellose, and magnesium stearate.

6.1.4 **Additional Information on Storage and Disposal of Vorinostat**

6.1.4.1 Investigational clinical supplies must be received by a designated person at the study site, handled and stored safely and properly, and kept in a secured location to which only the investigator and designated assistants have access. Clinical supplies are to be dispensed only in accordance with the protocol. The investigator is responsible for keeping accurate records of the clinical supplies received from the manufacturer, the amount dispensed to and returned by the subjects/patients, and the amount remaining at the conclusion of the study.

6.1.4.2 In accordance with Good Pharmacy Practices, gloves should always be worn by study personnel if directly handling tablets or capsules that are returned (i.e., when counting returns). The Clinical Monitor should be contacted with any questions concerning investigational products where special or protective handling is indicated.

6.1.4.3 Vorinostat should be stored at 20-25°C (68-77°F), with excursions permitted between 15-30°C (59-86°F). Vorinostat capsules should be stored at room temperature (do not store above 30°C) in a dry, limited-access area. Care will be taken to maintain acceptable storage temperature. The clinical supplies storage area at the site will be monitored by the site staff for temperature consistency with the acceptable storage temperature range specified in this protocol or in the product label attached to the protocol. Documentation of temperature monitoring will be maintained on site.

6.1.4.4 Vorinostat capsules should not be opened or crushed by patients or caregivers. Vorinostat capsules will be opened by pharmacists for preparation of the liquid suspension (see below). Spills of powder from vorinostat capsules due to damaged or broken capsules should be cleaned up carefully to minimize inhalation of vorinostat, and direct contact of the powder in vorinostat capsules with the skin or mucous membranes should be avoided. If such contact occurs, wash thoroughly. The affected area must be washed at least 3 times with ethyl alcohol, followed by water.

6.1.4.5 Due to biohazard concerns unused study drug will be destroyed locally. Drug will be destroyed according to standard operating procedures of the institutional pharmacy.

6.1.4.6 Drug accountability
The investigator/pharmacist must maintain accurate records of the disposition of all vorinostat received, issued to the subject, and any drug accidentally destroyed. At the end of the study, information describing vorinostat supplies (e.g. lot numbers) and disposition of supplies for each subject must be

provided, signed by the investigator, and stored by study personnel. Vorinostat may not be used outside the scope of this protocol, nor can vorinostat be transferred or licensed to any party not participating in this clinical study. Opened bottles returned by patients should be documented in the patient-specific investigational Agent Accountability Record (i.e. logged in as "returned by patients" and logged out as "for destruction") and destroyed on-site in accordance with institutional policy.

6.1.5 Guidelines for Administration and Compounding
Vorinostat is administered orally or via NG tube or G-tube. Vorinostat should be taken with food or within 30 minutes after a meal. The dose should be taken in the morning whenever possible. Altered taste and decreased food and liquid intake are associated with vorinostat administration. These toxicities can be actively managed with fluid management and nutritional consult. During the period of vorinostat administration, patients are recommended to consume adequate fluid daily to prevent dehydration. Patient may require electrolyte replacement. Early use of anti-emetics is encouraged. If patients experience dysgeusia, popsicles or Gatorade may be successful in maintaining oral intake. Missed doses will not be made up and patients should not double-up on missed doses during treatment

Patients will receive vorinostat as a liquid suspension or as capsules. Capsules can be used only for patients with a calculated dose within +/- 10% of a 100 mg dosing increment (eg. 100 mg, 200 mg, 300 mg, or 400 mg).

The COG phase I study of vorinostat and NANT07-03 both used the following formula for preparing a liquid suspension with a final vorinostat concentration of 50 mg/mL. This suspension should be prepared by the institutional investigational pharmacy.

Required components
Vorinostat 100 mg capsules (20)
OraPlus or Suspensol S 20 mL
OraSweet 20 mL

Instructions
Add 20 mL of Suspensol S or OraPlus into an amber or clear glass 4 ounce bottle. Place the contents of 20 capsules of vorinostat 100 mg into the same bottle and shake to disperse. Shaking may take up to 3 minutes. Once dispersed, add 20 mL of OraSweet to achieve a total volume of 40 mL. Shake again to disperse. Final concentration is 50 mg/mL. Store at room temperature. The suspension is stable for 4 weeks when stored at room temperature, away from excessive heat and humidity. The suspension should not be mixed with food or beverages.

The maximum daily dose of vorinostat on this protocol is 400 mg.

6.1.6 Potential Drug Interactions
The major pathways of vorinostat metabolism involve glucuronidation and β-oxidation. As vorinostat is not eliminated via CYP450 pathways, no drug-drug interactions are expected with known CYP450 inhibitors or inducers.

Although vorinostat is not a potent reversible CYP450 inhibitor, studies performed to monitor gene expression changes indicated some potential for CYP2C9 and CYP3A4 activity suppression. However, these changes were observed at concentrations higher than the pharmacologically relevant concentration.

6.1.7 Special Handling
Vorinostat is an anticancer drug. Clean powder spills from broken or damaged vorinostat capsules carefully minimizing inhalation. Wash spill area at least 3 times with ethyl alcohol, followed by water.

6.1.8 Patient Care Implications
Because vorinostat's dose limiting toxicities in previous studies included anorexia, dehydration, diarrhea, and fatigue, patients should maintain adequate fluid and food intake.

6.1.9 Toxicities

Likely (happens to 21-100 children out every 100 children)	Less Likely (happens to 5-20 children out every 100 children)	Rare (happens to < 5 children out every 100 children)
• Anemia • Thrombocytopenia • Fatigue • Anorexia • Diarrhea • Nausea • Vomiting	• Lymphopenia • Weight loss • Fever with and without neutropenia • Neutropenia • Increased creatinine • Leukopenia • Hyperglycemia • Rigors/Chills • Alopecia • Constipation • Dehydration • Dry Mouth • Dyspepsia • Dysgeusia • Infection • Hypoalbuminemia • Elevated liver function tests (AST, ALT, Alk. Phos and bilirubin) • Electrolyte abnormalities (hypocalcemia; hypophosphatemia; hypokalemia; hyponatremia) • Muscle spasm and/or weakness • Dizziness • Abdominal pain • Cough • Dyspnea • Thrombosis	• Skin necrosis • Prolonged QTc (Low-grade prolonged QTc interval has rarely been reported in patients receiving vorinostat, though the relationship to vorinostat remains undetermined)

Also reported on vorinostat trials but with the relationship to vorinostat undetermined:
BLOOD AND LYMPHATIC SYSTEM DISORDERS - Febrile neutropenia
CARDIAC DISORDERS - Atrial fibrillation; Atrial flutter; Chest pain - cardiac; Left ventricular systolic dysfunction; Myocardial infarction; Palpitations; Pericardial effusion; Sinus bradycardia; Sinus tachycardia; Ventricular fibrillation
EAR AND LABYRINTH DISORDERS - Tinnitus; Vertigo
EYE DISORDERS - Blurred vision
GASTROINTESTINAL DISORDERS - Abdominal distension; Anal hemorrhage; Bloating; Cheilitis; Colitis; Dysphagia; Esophageal hemorrhage; Esophagitis; Flatulence; Gastric hemorrhage; Gastritis; Gingival pain; Lower gastrointestinal hemorrhage; Mucositis oral; Oral hemorrhage; Small intestinal obstruction; Stomach pain; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Death NOS; Edema limbs; Gait disturbance; General disorders and administration site conditions - Other (angioedema); General disorders and administration site conditions - Other (failure to thrive); Malaise; Multi-organ failure; Non-cardiac chest pain; Pain
HEPATOBILIARY DISORDERS - Hepatic failure
INFECTIONS AND INFESTATIONS - Infections and infestations - Other (Herpes zoster)

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising; Vascular access complication; Wound dehiscence
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Cardiac troponin I increased; Electrocardiogram QT corrected interval prolonged; GGT increased; INR increased[4]; Investigations - Other (elevated LDH); Lipase increased
METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hypoglycemia; Hypomagnesemia; Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Chest wall pain; Myalgia; Neck pain; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (tumor hemorrhage); Tumor pain
NERVOUS SYSTEM DISORDERS - Ataxia; Cognitive disturbance; Depressed level of consciousness; Dysphasia; Encephalopathy; Facial muscle weakness; Facial nerve disorder; Headache; Intracranial hemorrhage; Ischemia cerebrovascular; Lethargy; Memory impairment; Nervous system disorders - Other (Guillain-Barre syndrome); Nervous system disorders - Other (head injury); Nervous system disorders - Other (polyneuropathy); Paresthesia; Peripheral motor neuropathy; Peripheral sensory neuropathy; Seizure; Syncope; Tremor
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression; Insomnia; Personality change; Psychosis
RENAL AND URINARY DISORDERS - Acute kidney injury; Hematuria; Proteinuria; Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Pelvic pain; Uterine hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchopulmonary hemorrhage; Epistaxis; Hypoxia; Nasal congestion; Pharyngeal mucositis; Pharyngolaryngeal pain; Pleural effusion; Pleuritic pain; Pneumonitis
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Dry skin; Hyperhidrosis; Nail loss; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Purpura; Rash maculo-papular

Note: Vorinostat 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.

6.1.10 Vorinostat availability and ordering
Vorinostat will be supplied by Merck & Co. Vorinostat will be shipped to treating institutions through the contract pharmacy Almac. Instructions and forms for ordering vorinostat from Almac are posted to the NANT website.

6.2 ^{131}I-METAIODOBENZYLGUANIDINE (^{131}I-MIBG)
[Nuclear Diagnostics Products (NDP) IND # 32,147]

NOTE: The primary ^{131}I-MIBG supplier for this study is NDP. If a supply of ^{131}I-MIBG is not available from NDP for a specified date or a site cannot receive ^{131}I-MIBG from NDP, Jubilant Draximage is a secondary ^{131}I-MIBG supplier for this study. Before a secondary supplier is authorized for use for this study, approval from the protocol chair/NANT Operations Center is required.

Common name Iobenguane sulfate (m-Iodobenzylguanidine sulfate, MIBG)

Active ingredient: 3-[^{131}I]-iodobenzylguanidine Sulfate

Pharmacologic class: Radiopharmaceutical therapeutic agent

Molecular formula: $[C_8H_{10}IN_3]\bullet 1/2\ H_2SO_4$

Molecular weight: 324.13 g/mol

Physical form: Colorless crystals

Solubility: Soluble in water

Formulation: The MIBG sulfate is synthesized by Nuclear Diagnostic Products (Rockaway, NJ) using the following materials:

- MIBG (Iodophenyl-methly-guanidine hemisulfate)
- Cupric Sulfate Powder, USP
- Ammonium Sulfate Powder, FCC
- Ascorbic Acid Injection, USP
- Sodium Hydroxide, NF
- Sterile Water for Injection, USP
- 131I as Sodium Iodide Solution
- AG 1-X2 Resin

Specific activity: Not less than 30 mCi/mg (1110 MBq/mg) MIBG at the calibration date.

Radiochemical purity: Not less than 95% of the ^{131}I must be as MIBG upon initial measurement at NDP. Not less than 93% of the ^{131}I must be as MIBG upon re-check at the treating center.

For sites other than Memorial Sloan-Kettering (MSKCC), thin layer chromatography and/or high pressure liquid chromatography (HPLC), or other standardized institutional method must be performed after the product is thawed and prior to infusion (Appendix IV). For MSKCC, NDP will perform same day quality control for free iodine. NDP will send a copy of the quality control report to the MSKCC Nuclear Pharmacy who will perform a confirmatory quality control assay per Appendix IV prior to infusion.

Radiolytic decay: ^{131}I decays by beta emission and associated gamma emission with a physical half-life of 8.04 days.

How supplied: For sites other than MSKCC, ^{131}I-MIBG from NDP is supplied as a clear, colorless liquid in a single-dose 30 mL sterile vial. Product is shipped frozen. For MSKCC, NDP will prepare the dose in 60 mL syringes for patient use and will be ready for administration.

Storage: ^{131}I-MIBG from NDP is to remain frozen until ready for use, then thawed at room temperature prior to administration.

Stability: Product is stable for up to 45 hours at -70 °C. Once thawed, product must be infused within 4 hours.

Distribution

NDP ^{131}I-MIBG will be provided by Nuclear Diagnostic Products, Rockaway, NJ. Call 3 weeks prior to infusion to place an order:
Phone: (973) 664-9696
Web: www.ndprx.com

**Sites will need to inform NDP that orders for this study will be billed directly to the NANT Operations Center. When ordering please refer to "NANT 11-01."

Toxicity

Likely (happens to 21-100 children out every 100 children)	Less Likely (happens to 5-20 children out every 100 children)	Rare (happens to < 5 children out every 100 children)
• Myelosuppression (anemia, thrombocytopenia, leukopenia, neutropenia) • Nausea • Dry mouth • Hyperamylasemia	• Hypothyroidism • Sterility • Hair thinning • Vomiting • Fatigue • Infection • Bleeding/bruising • Anorexia • Changes in blood pressure during and after MIBG infusion (hypotension & hypertension)	• Pain in salivary glands or mouth • Decreased function of adrenal gland • Decreased heart function • Hepatotoxicity • Secondary leukemia • Pneumocystis pneumonia • Bronchiolitis obliterans with organizing pneumonia (BOOP) • Hyperthyroidism

6.3 ^{131}I-METAIODOBENZYLGUANIDINE (Draximage® Therapeutic ^{131}I-MIBG)

(Jubilant Draximage® IND # 76,227)

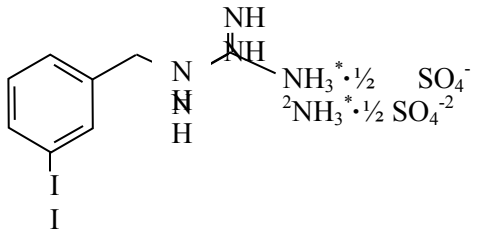
NOTE: Jubilant Draximage will serve as a secondary supplier for ^{131}I-MIBG if the drug is not available from NDP for the desired treatment date or site. **Before a secondary supplier is authorized for use for this study, approval from the protocol chair/NANT Operations Center is required.**

Common name Iobenguane sulfate (m-Iodobenzylguanidine sulfate, MIBG)

Active ingredient: 3-[^{131}I]-iodobenzylguanidine Sulfate

Pharmacologic class: Radiopharmaceutical therapeutic agent

Structural formulae:



Molecular formula: $[C_8H_{10}IN_3]\cdot 1/2\ H_2SO_4$

Molecular weight: 324.13 g/mol

Physical form: Colorless crystals

Solubility: Soluble in water

Melting point: Decomposition occurs between 166-167°C.

Purity: MIBG (cold raw material): not less than 99%

Formulation: The MIBG sulfate is synthesized by Draximage (Kirkland, Quebec, Canada), with the following formulation:

3-Iodobenzylguanidine Sulfate < 0.83 mg

Niacinamide	16 mg
Sodium Chloride	9 mg
Benzyl Alcohol	9 µL
Sodium Acetate Trihydrate	0.68 mg
Cupric Nitrate (II)	< 0.02 mg
Hydrochloric Acid	Trace
Sodium Hydroxide Pellets	Trace
Water for Injection	q.s. to 1.0 mL

Specific activity: Not less than 30 mCi/mg (1110 MBq/mg) MIBG at the calibration date.

Radiochemical purity: Not less than 95% of the ^{131}I must be as MIBG upon re-check at the treating center.

Thin layer chromatography and /or high pressure liquid chromatography (HPLC), or other standardized institutional method must be performed after the product is thawed and prior to infusion (Appendix IV).

Radiolytic decay: ^{131}I decays by beta emission and associated gamma emission with a physical half-life of 8.04 days.

How supplied: ^{131}I-MIBG from DraxImage is supplied in a single-dose 10 mL or 30 mL glass vial.

Storage: The product will be shipped frozen and should be stored at 2 °C to 8 °C upon arrival.

Stability: The product is stable at 2 °C to 8 °C for 2 days with excursions permitted at room temperature.

Distribution

Draximage® Therapeutic ^{131}I-MIBG will be provided by Draximage®, Canada. Call 3 weeks prior to infusion to Draximage Customer Service at Order Department and Customer Service
Phone: 1-888-633-5343; 8am – 5pm Eastern Time
Fax: 1-866-431-4288
Fax: 1-514-694-3865
Email: customerservice@draximage.com
Web: www.draximage.com

**Sites will need to inform Draximage Customer Service that orders for this study will be billed directly to the NANT Operations Center. When ordering please refer to “NANT 11-01.”

Toxicity

Likely (happens to 21-100 children out every 100 children)	Less Likely (happens to 5-20 children out every 100 children)	Rare (happens to < 5 children out every 100 children)
• Myelosuppression (anemia, thrombocytopenia, leukopenia, neutropenia) • Nausea • Dry mouth • Hyperamylasemia	• Hypothyroidism • Sterility • Hair thinning • Vomiting • Fatigue • Infection • Bleeding/bruising • Anorexia • Changes in blood pressure during and after MIBG infusion (hypotension & hypertension)	• Pain in salivary glands or mouth • Decreased function of adrenal gland • Decreased heart function • Hepatotoxicity • Secondary leukemia • Pneumocystis pneumonia • Bronchiolitis obliterans with organizing pneumonia (BOOP) • Hyperthyroidism

6.4 VINCRISTINE

Source and Pharmacology: Vincristine is an alkaloid isolated from Vinca rosea Linn (periwinkle). It binds to tubulin, disrupting microtubules and inducing metaphase arrest. Its serum decay pattern is triphasic. The initial, middle, and terminal half-lives are 5 minutes, 2.3 hours, and 85 hours respectively; however, the range of the terminal half-life in humans is from 19 to 155 hours. The liver is the major excretory organ in humans and animals; about 80% of an injected dose of vincristine sulfate appears in the feces and 10% to 20% can be found in the urine. Within 15 to 30 minutes after injection, over 90% of the drug is distributed from the blood into tissue, where it remains tightly, but not irreversibly bound. It is excreted in the bile and feces. There is poor CSF penetration.

Formulation and Stability: Vincristine is supplied in a glass vial each mL of which contains vincristine sulfate, 1 mg (1.08 µmol); mannitol, 100 mg; sterile water for injection; Acetic acid and sodium acetate are added for pH control. The pH of Vincristine Sulfate Injection, USP ranges from 3.5 to 5.5. This product is a sterile solution. Store refrigerated at 2-8°C or 36-46°F. Protect from light and retain in carton until time of use. Do not mix with any IV solutions other than those containing dextrose or saline.

Guidelines for Administration: See the Treatment section of protocol. Vincristine sulfate must be administered via an intact, free-flowing intravenous needle or catheter. Care should be taken to ensure that the needle or catheter is securely within the vein to avoid extravasation during administration. The solution may be injected either directly into a vein or into the tubing of a running intravenous infusion.

When dispensed the container or syringe containing vincristine must be enclosed in an over-wrap bearing the statement “Do not remove covering until moment of injection. Fatal if given intrathecally. For Intravenous use only.”

Vincristine Toxicity:

Likely (happens to 21-100 children out of 100)	**Less Likely** (happens to 5 -20 children out of 100)	**Rare** (happens to less than 5 children out of 100)
• Hair loss • Constipation • Loss of deep tendon reflexes	• Jaw pain • Headache • Muscle Weakness • Abdominal pain and bloating • Bone marrow suppression that is mild and brief (leukopenia,	• Extravasation is rare but if it happens it will cause local ulceration • Shortness of breath and bronchospasm • Paralytic ileus • Ptosis, diplopia, night blindness • Hoarseness • Vocal cord paralysis • SIADH

	thrombycytopenia, anemia) • Peripheral paresthesias including numbness, tingling and pain, clumsiness, wrist drop, foot drop, abnormal gait	• Seizures • Defective sweating • Difficulty walking or inability to walk • Sinusoidal obstruction syndrome (SOS, formerly VOD) (when used in combination with other agents); • Blindness • Optic atrophy • Urinary tract disorders (including bladder atony, dysuria, polyuria, nocturia, urinary retention) • Autonomic neuropathy with postural hypotension • 8th cranial nerve damage with dizziness, nystagmus, vertigo and hearing loss
Risk to unborn child of unknown frequency or timing: Fetal toxicities and teratogenic effects of vincristine (either alone or in combination with other antineoplastic agents) have been noted in humans. The toxicities include: chromosome abnormalities, malformation, pancytopenia, and low birth weight. It is unknown whether the drug is excreted in breast milk.		

Supplier: Commercially available. See package insert for more detailed information.

6.5 IRINOTECAN

Mechanism of Action: Irinotecan is a semisynthetic water-soluble analog of camptothecin that exerts its cytotoxic effect through inhibition of the nuclear enzyme topoisomerase I. Irinotecan is a prodrug that undergoes deesterification to a much more potent topoisomerase-I inhibitor, SN-38. The lactone forms of both irinotecan and SN-38, undergo pH dependent hydrolysis to a hydroxy acid (carboxylate) species. SN-38 is glucuronidated to SN-38G.

Formulation & Stability: Irinotecan is supplied in amber, glass, single-dose vials containing 40mg and 100 mg of irinotecan hydrochloride (on the basis of trihydrate salt) as a 20 mg/ml solution. The intact vials should be stored at room temperature and protected from light.

Guidelines for Administration: Irinotecan must be diluted before infusion. Irinotecan should be diluted in 5% Dextrose Injection USP (preferred) or 0.9% Sodium Chloride Injection, USP, to a final concentration range of 0.12 to 2.8mg/ml. The solution is physically and chemically stable for up to 24 hours at room temperature (approx 25 ^{O}C) and in ambient fluorescent lighting. Solutions diluted in 5% Dextrose Injection, USP, and stored at refrigerated temperatures (approximately 2 ^{O} to 8 ^{O}C), and protected from light are physically and chemically stable for 48 hours. Refrigeration of admixtures using 0.9% Sodium Chloride Injection, USP, is not recommended due to a low and sporadic incidence of visible particulates.

Irinotecan should be administered as an intravenous infusion over 60-90 minutes. For patients with early diarrhea, prolonging the infusion to 90 minutes is allowed. See protocol for supportive care measures.

Irinotecan Toxicity:

Likely (happens to 21-100 children out of 100)	**Less Likely** (happens to 5 -20 children out of 100)	**Rare** (happens to less than 5 children out of 100)
• Diarrhea (can be immediate) • Cholinergic symptoms: rhinitis, increased salivation, miosis, lacrimation,diaphoresis, flushing. • Mucositis • Nausea and vomiting • Stomach pain	• Mildly high liver and kidney function tests • Constipation • Delayed Diarrhea • Headaches • Blood-clots** • Anemia • Thrombocytopenia • Rash	• Skin inflammation • Trembling • Blood in the urine • Mildly increased level of protein and glucose in the urine • Low amount of protein in the blood • Mouth sores • Headache • Dizziness and hypotensiion

• Loss of appetite • Fever • Loss of body water • Loss of strength and energy • Decrease in the number of red and white blood cells and platelets made in the bone marrow • Eosinophila • Hair loss • Elevations in transaminases, alkaline phosphatase, bilirubin,	• Stomatitis Dyspepsia	• Sensation of warmth on face • Confusion and/or disoreintation • Inflammation of the large intestine • Illeus • Anaphylaxis • Dehydration • Bradycardia • Pain at infusion site • Pneumonitis • Inflammation of the lungs with cough and congestion

**this toxicity is seen more commonly when irinotecan is given in combination with fluorouracil and leucovorin, it may rarely be a life threatening event.

Supplier: Irinotecan is commercially available. See package insert for further details.

6.6 CEFIXIME

Source and Pharmacology: Cefixime is a third generation cephalosporin antibiotic for oral administration that inhibits bacterial cell wall synthesis by binding to one or more of the penicillin-binding proteins and interfering with the final transpeptidation step of peptidoglycan synthesis. Its spectrum of activity is similar to other third-generation agents, including Enterobacteriaceae, and β-lactamase producing *H. influenzae* and *N. gonorrhea*, and *Staph. aureus*. It is excreted primarily by the kidney. It has a serum half-life of approximately 3-4 hours.

Formulation: Cefixime is available in a powder for oral suspension, which when reconstituted, provides 100 mg/5ml. The powder for oral suspension is strawberry flavored and contains sodium benzoate, sucrose, and xanthan gum (available as 50 ml, 75 ml & 100 ml bottle with powder for reconstitution).

Toxicity:

Likely (happens to 21-100 children out of 100)	**Less Likely** (happens to 5 -20 children out of 100)	**Rare** (happens to less than 5 children out of 100)
	• Diarrhea • Abdominal pain • Nausea and vomiting, • Dyspepsia	• Headache • Dizziness • Seizures • Anaphylaxis • Hypersensitivity reactions • Thrombocytopenia, • Leucopenia, • Neutropenia, • Eosinophilia, • Pseudomembranous colitis, • Increase in BUN/SCr and ALKP, • Hepatitis, jaundice • Stevens Johnson / toxic epidermal necrolysis

Supplier: Commercially available through Lupin, Inc. See package insert for further information.

6.7 CEFPODOXIME PROXETIL

Source and Pharmacology: Cefpodoxime proxetil is a third generation cephalosporin antibiotic for oral administration that inhibits bacterial cell wall synthesis by binding to one or more of the penicillin-binding proteins and interfering with the final transpeptidation step of peptidoglycan synthesis. Its spectrum of activity is similar to other third-generation agents, including Enterobacteriaceae, and β-lactamase producing *H. influenzae* and *N. gonorrhea*, and *Staph. aureus*. It is excreted primarily by the kidney. It has a serum half-life of approximately 2-4 hours.

Formulation: Cefpodoxime proxetil is available as 100 mg & 200 mg oblong tablets & powder for oral suspension, which when reconstituted, provides either 50 mg/5 ml OR 100 mg/5 ml. The powder for oral suspension is lemon crème flavored (available as 50 ml, 75 ml & 100 ml bottle with powder for reconstitution).

Cefpodoxime Proxetil Toxicity:

Likely (happens to 21-100 children out of 100)	**Less Likely** (happens to 5 -20 children out of 100)	**Rare** (happens to less than 5 children out of 100)
	• Diarrhea • Diaper Rash	• Abdominal pain • Nausea and vomiting, • Headache • Seizures • Anaphylaxis • Chest pain • Hypersensitivity reactions • Thrombocytopenia, • Leucopenia, • Neutropenia, • Eosinophilia, • Pseudomembranous colitis, • Increase in BUN/SCr and ALKPhos • Prolonged PT / PTT • Chest pain • Vaginal candidiasis • Aplastic Anemia • Stevens Johnson, • Toxic Epidermal Necrolysis

Supplier: Commercially available through a variety of manufacturers (generically available). See package insert for further information.

6.8 POTASSIUM IODIDE (KI, SSKI)

Formulation: Oral solution 1 gram / milliliter.

Diagnostic MIBG Scan: Dose 1 –3 drops / dose, administered daily beginning 30 minutes prior to radionuclide injection, and continuing for 3 days post-injection for ^{123}I-MIBG and for 7 days for ^{131}I-MIBG.

Therapeutic MIBG Treatment: Loading dose of 6 mg/kg by mouth 8-12 hours prior to infusion of MIBG on day 1, and then 1 mg/kg/dose by mouth starting 4-6 hours after completion of MIBG infusion and continuing every 4 hours on protocol days 1 to 7 and then 1 mg/kg/dose by mouth once daily on protocol days 8-43.

Storage: Room temperature

Toxicity:

Likely (happens to 21-100 children out every 100 children)	**Less Likely** (happens to 5-20 children out every 100 children)	**Rare** (happens to < 5 children out every 100 children)
	• Gastrointestinal distress (nausea / vomiting / diarrhea / stomach pain)	• Vasculitis • Flare up of adolescent acne • Irregular heartbeat • Confusion • Tiredness • Fever • Hypersensitivity (hives) • Burning of mouth / throat • Metallic taste • Rash • Hypothyroidism with overuse • Swelling of lymph glands

6.9 GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) (Filgrastim, Neupogen).

Source and Pharmacology:

Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. Filgrastim is a 175 amino acid protein with a molecular weight of 18,800 daltons manufactured by recombinant DNA technology utilizing E.coli bacteria into which has been inserted the human granulocyte colony stimulating factor gene. It differs from the natural specific colony-stimulating factor which regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens). The elimination half-life is similar for subcutaneous and intravenous administration, approximately 3.5 hours. The time to peak concentration when administered subcutaneously is 2 to 8 hours.

Formulation and Stability:

Supplied as a clear solution in 300 ug/ml (1 ± 0.6 x 108 U/mg) (1 ml or 1.6 ml) vials. Vials are preservative free and are intended to be single-use vials; do not reuse opened vials. Filgrastim must be stored between 2° and 8°C. Stability has been demonstrated for at least 24 months when stored under these conditions. Do not use if discolored or if there is particulate matter. For IV use, dilute in D5W to concentrations > 15 ug/ml; G-CSF is incompatible with normal saline. At dilutions from *5* ug/ml to 14 ug/ml, add human serum albumin to a final albumin concentration of 2 mg/ml to protect against absorption of the GCSF to container walls (glass or plastic). Filgrastim, when diluted as described above, is compatible with a number of plastics commonly used in the manufacture of syringes, IV bags, infusion sets, and IV pump cassettes. These include polyvinyl chloride, polyolefin, and polypropylene. Diluted filgrastim should be stored at 2° to 8° C and used within 24 hours. **Do not shake or freeze.**

Guidelines for Administration:

Administer once daily, subcutaneously without dilution or if necessary dilute with 5% dextrose in water, preferably to concentrations of 15 ug/ml or greater for IV administration. Dilutions should be prepared as close to the time of administration as possible (up to 24 hours), since the product is preservative-free. When diluting Filgrastim to *5*-14 ug/ml in D5W, it is necessary at all times to add human serum albumin, to reach a final albumin concentration of 2 mg/ml.

Likely (happens to 21-100 children out every 100 children)	**Less Likely** (happens to 5-20 children out every 100 children)	**Rare** (happens to < 5 children out every 100 children)

• Mild to moderate medullary bone pain	• Local pain or irritation at injection site • Increased alkaline phosphatase, LDH and uric acid • Thrombocytopenia • Fever	• Allergic reactions (more common with IV than subcutaneous administration) • Skin rash, urticaria and/or facial edema • Respiratory wheezing and/or dyspnea • Hypotension and/or tachycardia • Low grade fever • Splenomegaly • Splenic rupture • Worsening of existing skin rashes • Sickle cell crises in patients with sickle cell disease • Excessive leukocytosis • Cutaneous vasculitis • Adult respiratory distress syndrome • MDS or AML (in patients with severe chronic neutropenia and long term administration

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

6.10 PEGFILGRASTIM (NEULASTA)

Source and Pharmacology:
Pegfilgrastim is the pegylated form of recombinant methionyl human G-CSF (filgrastim). Pegfilgrastim is produced by covalently binding a 20-kilodalton (kD) monomethoxypolyethylene glycol molecule to the N-terminal methionyl residue of filgrastim. The molecular weight of pegfilgrastim is 39 kD. G-CSF is a lineage specific colony-stimulating factor which regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens).After subcutaneous injection the elimination half-life of pegfilgrastim ranges from 15 to 80 hours and the time to peak concentration ranges from 24 to 72 hours. Serum levels are sustained in most patients during the neutropenic period postchemotherapy, and begin to decline after the start of neutrophil recovery, consistent with neutrophil-dependent elimination. After subcutaneous administration at 100 mcg/kg in 37 pediatric patients with sarcoma, the terminal elimination half-life was 30.1 (+/- 38.2) hours in patients 0 to 5 years-old, 20.2 (+/- 11.3) hours in patients 6 to 11 years-old, and 21.2 (+/- 16) hours in children 12 to 21 years-old.

Pegfilgrastim Toxicity:

Likely (happens to 21-100 children out every 100 children)	**Less Likely** (happens to 5-20 children out every 100 children)	**Rare** (happens to < 5 children out every 100 children)
• Mild to moderate medullary bone pain	• Local pain or irritation at injection site • Headache • Increased alkaline phosphaste, lactate dehydrogenase and uric acid. • Thrombocytopenia	• Low grade fever • Allergic reactions (anaphylaxis, angioedema, or urticaria) • Generalized erythema and flushing • Splenomegaly • Splenic rupture • Sickle cell crises in patients with sickle cell disease (SCD) • Excessive leukocytosis • Sweet's syndrome (acute febrile neutrophilic dermatosis) • Adult respiratory distress syndrome

Unknown frequency and timing: Fetal toxicities and teratogenic effects of pegfilgrastim in humans are unknown. Conflicting data exist in animal studies. It is unknown whether the drug is excreted in breast milk.

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

Formulation and Stability:
Supplied as a preservative-free solution containing 6 mg (0.6 mL) of pegfilgrastim (10 mg/mL) in a single-dose syringe with 27 g, ½ inch needle with an UltraSafe® Needle Guard. The needle cover of the prefilled syringe contains drug natural rubber (a derivative of latex). Store refrigerated at 2º-8ºC (36º-46ºF) and in the carton to protect from light. Prior to injection, pegfilgrastim may be allowed to reach room temperature protected from light for a maximum of 48 hours. Avoid freezing.

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

6.11 LOPERAMIDE

Formulation and Stability: Supplied as a clear, cherry flavored syrup (1 mg/5 mL) or as a scored green caplet (2 mg).

Mechanism of Action: Loperamide HCl acts by slowing intestinal motility and by affecting water and electrolyte movement through the bowel

Toxicities: Overdoses of loperamide HCl may result in CNS depression, constipation, and nausea. Children may be more sensitive to CNS effects than adults.

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

6.12 Drug Inventory Records

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all drugs.

7.0 REQUIRED OBSERVATIONS/MATERIAL AND DATA TO BE ACCESSIONED

7.1 Clinical and Laboratory Studies

Blood and urine studies must be performed within 2 weeks of study enrollment. Tumor disease evaluation (including appropriate imaging studies, bilateral bone marrow aspirate and biopsy for standard histology and urine catecholamines) are required within 3 weeks of study entry and subsequent to any prior therapy. Echocardiogram and ECG are required within 4 weeks of study entry and subsequent to any prior therapy. Initiation of protocol therapy is required within 1 week of study enrollment.

References to second course of therapy in the following table are only relevant to patients enrolling prior to Amendment 7A.

OBTAIN OTHER STUDIES AS NEEDED FOR GOOD PATIENT CARE.

Observation	Before Entry	Days -1 to +7 of Each Course[1]	Days 8 to 43 of each Course	Day 43 to 50 of each Course
Physical Exam[5] (Ht, Wt, BSA, VS) Performance status only at entry	X	Day 4 (+/- 1 day)	Every other week	X
CBC, Diff, Platelets[5]	X	Day 4 (+/- 1 day)	Twice weekly[2]	X
AST, ALT[5], Total[5] + Direct Bilirubin, Albumin	X	Day 4 (+/- 1 day)	Weekly	X
BUN, Serum Creatinine[5]	X	Day 1 prior to MIBG infusion for Vorinostat Arm Only and Day 4 (+/- 1 day) for All Patients	Weekly	X
PT/INR and PTT[5]	X	Weekly on Vorinostat Arm	Weekly on Vorinostat Arm	X
Electrolytes, Calcium, Magnesium, Phosphorus	X	Day 4 (+/- 1 day)	Weekly	X
T_4, TSH	X			X
Serum β-HCG[3,5]	X			X
MUGA or Echocardiogram[5]	X			X
Electrocardiogram[5]	X	Vorinostat Arm Only: Day 4 (+/- 2 days)		X (Vorinostat Arm Only)
Urinalysis	X			X
Urine catecholamines VMA/HVA	X			X
Bilateral BM Aspirate + Biopsy for morphology[4, 7]	X			X
Blood and Marrow for N04-05	X			X
Anatomic imaging (CT or MRI scan) of chest/abdomen/pelvis plus any other known sites of measurable disease[4]	X			X
MIBG diagnostic scan (REQUIRED)[4, 5] Use same isotope with each scan	X			X
MIBG whole body scan		On release from radiation isolation		
Whole body dosimetry		Days 1 to 4[6]		
Blood for biology studies (if consent)		See Section 8	See Section 8	
Archival slides for biology studies (if consent)		See Section 8		

1. No other routine bloodwork/radiology studies are required while patient is hospitalized unless clinically indicated. Patients with prior electrolyte imbalance should be monitored during the hydration.
2. Continue until ANC > =500 cells/μL and platelet count > = 20,000 cells/μL x 3 days without transfusion then weekly until Day +43.
3. Obtain for females 10 years of age and older or post-pubertal.
4. Tumor imaging = CT and/or MRI (Chest abdomen pelvis) plus CT/MRI imaging of any other sites with MIBG update, MIBG for optimum visualization of all areas of bulk tumor (primary & metastasis) as well as chest/abdomen/pelvis. CT or MRI of chest/abdomen/pelvis + any other known lesions of tumor (repeat of chest/abdomen/pelvis CT or MRI is required even if scans are negative for tumor at study entry).If patient has a history of tumor lesions in the skull, orbits or brain, OR if MIBG scan shows uptake in these same areas, then a CT or MRI of the brain/orbits is strongly suggested. For patients with epidural or hepatic tumor lesions, MRI is the recommended imaging technique. All disease status tests must be performed ≤ 3 weeks prior to study entry and subsequent to any intervening therapy
5. Required for verification of eligibility. All results should be faxed to NANT Operations Center with study registration eligibility. An eligibility worksheet is available in the data forms packet on NANT website (www.nant.org).
6. See section 4.3.7 for dosimetry guidelines.
7. Bilateral bone marrow aspirates and biopsies to be performed at each disease evaluation, even if negative at study entry.

7.2 Recommended Follow-Up Observations

The following are recommendations only, but may be altered at discretion of treating physician. Repeat the following if abnormal on a monthly basis until stable or normalized after the end of therapy. If normal at the end of therapy then repeat only as clinically indicated:

- History, physical exam (Ht, Wt, VS)
- CBC/Differential, Platelets, AST, ALT, Bilirubin, BUN, Creatinine
- T_4, TSH and MIBG scan every 3 months until 1 year post treatment, then every 6 months for 3 years or until progression, death or other therapy.

Patients will be followed for life for any delayed toxicities related to protocol therapy and for the development of second malignancies.

Data on patients who undergo subsequent myeloablative therapy within 6 months of completing protocol therapy will be collected to assess the incidence and severity of SOS in these patients (Aim 1.10).

After completion of protocol therapy, the disease status, sites of relapse, and last alive date will be recorded until first relapse/progression or until patient receives another non-protocol therapy, after which only last alive date will be reported, as well as date of death and cause of death (if applicable). In addition, those patients who undergo subsequent myeloablative therapy within 6 months of completing protocol therapy will be followed for the development of SOS within 21 days following the infusion of stem cells post-myeloablative treatment.

7.3 Documentation of Tumor Response

Patients will undergo complete disease re-staging between Day 43 and Day 50 of each course of therapy (only a single course of therapy allowed for patients enrolling with Amendment 7A and later). It is recommended that all scans and tests previously done to document tumor lesions be performed in subsequent evaluations of disease status.

All patients will have diagnostic bone marrows performed with each disease evaluation, regardless of involvement at study entry. To confirm a bone marrow response of CR, an additional bone marrow examination for morphology (for a total of 2 bone marrow exams) must be done a minimum of three weeks apart (section 11.2.1).

Once a patient receives therapy other than prescribed on this protocol, no further scans or bone marrow evaluations will be required for this protocol since the patient will no longer be evaluable for response to protocol therapy.

8.0 CORRELATIVE BIOLOGY STUDIES

Collection of samples for correlative biology studies is optional and not required for study entry. Although these studies cannot be mandated, all institutions are strongly urged to submit specimens for all consenting patients.

Aside from submission of archival tissue to quantify NET expression (section 8.3), these studies are to be performed during both courses of therapy for patients enrolling prior to Amendment 7A and during the single allowed course of therapy for patients enrolling with Amendment 7A and later.

8.1 Evaluation of Minimal Residual Disease by 5 Gene NB5 assay TaqMan low density array (TLDA) Assay

All patients are required to co-enroll onto the NANT Biology Study, N04-05. Patients should submit blood and/or bone marrow at study entry and at the time of day 43-50 restaging studies. Submission of blood and bone marrow for NB5 TLDA assay will be according to the instructions in N04-05. Patients can submit only bone marrow for NB5 TLDA assay if blood volumes for research do not allow for blood submission.

8.2 Evaluation of Blood Markers of Radiation Exposure

8.2.1 Sample requirements

The following samples will be obtained at each time point (see section 8.2.2):

- 2.5 mL of peripheral blood in PAXgene RNA blood tube (provided by the study)
- 5 mL of peripheral blood in EDTA (usually purple top) tube
- 5 mL of peripheral blood in serum separator (usually red top) tube

8.2.2 Sampling scheduling

Samples will be obtained according to the following schedule. **Note that the schedule differs slightly based upon randomized arm of the study to which a specific patient has been assigned.**

Sample Timepoint (Obtain in Each Course)	Samples to be Obtained
Baseline Draw #1 • For ^{131}I-MIBG only arm: Anytime on the day prior to ^{131}I-MIBG infusion (Protocol Day 0) • For ^{131}I-MIBG/vorinostat arm: Prior to first dose of vorinostat (Protocol Day -1) • For ^{131}I-MIBG/irinotecan/vincristine arm: Prior to first dose of vincristine/irinotecan (Protocol Day 0)	2.5 mL in PAXgene RNA blood tube 5 mL in EDTA (usually purple top) 5 mL in serum separator (usually red top) tube
Baseline Draw # 2 Day of ^{131}I-MIBG infusion, anytime prior to start of infusion (all patients) = Protocol Day 1	2.5 mL in PAXgene RNA blood tube 5 mL in EDTA (usually purple top) 5 mL in serum separator (usually red top) tube
72 hours (+/- 2 hours) from start of ^{131}I-MIBG infusion (all patients) = Protocol Day 4	2.5 mL in PAXgene RNA blood tube 5 mL in EDTA (usually purple top) 5 mL in serum separator (usually red top) tube
96 hours (+/- 2 hours) from start of ^{131}I-MIBG infusion if patient still admitted to hospital (all patients) = Protocol Day 5	2.5 mL in PAXgene RNA blood tube 5 mL in EDTA (usually purple top) 5 mL in serum separator (usually red top) tube
Protocol Day 15 (or whenever patient receives stem cell infusion, if stem cell infusion does not occur on Day 15)	2.5 mL in PAXgene RNA blood tube 5 mL in EDTA (usually purple top) 5 mL in serum separator (usually red top) tube

8.2.3 Sample processing

- **PAXgene tubes**: After collecting blood into a PAXgene tube, the tube should be gently inverted 8-10 times. Keep the tube at room temperature for 2 - 72 hours and then freeze upright in a wire rack (not Styrofoam holder) for 24 hours at -20°C and then transfer to -70 or -80°C freezer until batch shipment.

- **EDTA tubes**: Blood from EDTA tubes will be used to obtain fixed cells for γ-H2AX testing (using the SmartTube system) and plasma for flt3 ligand testing. Once collected, EDTA tubes should be gently inverted 6-8 times and transferred to the treating center's specimen processing lab immediately. Upon arrival in the specimen processing lab, remove 1 mL of whole blood, place the 1 mL of whole blood into a SmartTube (provided by the study), cap tightly, and for process as described below. Reserve the remaining 4 mL of whole blood for plasma isolation as described below.

    - SmartTube Processing: Once 1 mL of whole blood has been added to the SmartTube, activate the tube manually. To activate a SmartTube manually,

make sure the cap is screwed on securely and then bend the SmartTube in the middle until you feel the ampoule inside break, then invert the SmartTube 10 times to ensure good mixing. Incubate the activated SmartTubes at room temperature for 8 minutes.

After the 8 minute incubation, immediately transfer the SmartTubes to a -80°C freezer or place in direct contact with dry ice. The SmartTubes should be stored at -80°C until ready for batch shipment. Samples frozen in SmartTubes should not be stored at temperatures warmer than -80°C. Smart Tubes have not been validated for storage in liquid nitrogen. Retain frozen until ready for batch shipment.

- Plasma Isolation: Centrifuge the samples in a refrigerated centrifuge (4°C) for 10 minutes at 1500 g or greater. Next, remove the plasma using a transfer pipette and transfer the plasma into at least two cryovials with a minimum of 0.5 mL plasma per cryovial. Cap the vials securely and label as described below. Immediately freeze the plasma in an upright position in a -70°C to -80°C freezer. Retain frozen until ready for batch shipment.

- **Serum separator tubes**: Allow the blood to clot at 4°C (or in a bucket with ice) for at least 30 minutes but no longer than 2 hours. Centrifuge the blood at 1000g for 15 minutes at 4ºC (preferred) or room temperature to separate the serum (top, straw-colored layer) from the red blood cells (bottom, red layer). Aliquot the serum into at least two cryovials with a minimum of 0.5 mL serum per cryovial. Cap the vials securely and label as described below. Immediately freeze the serum in an upright position in a -70°C to -80°C freezer. Retain frozen until ready for batch shipment.

8.2.4 Sample labeling

PAXgene tubes, SmartTubes, plasma aliquot tubes, and serum aliquot tubes should be labeled with the patient's NANT ID number, the protocol number N11-01, the date of blood draw, "Course 1" or "Course 2", and one of the following indicators of the sample timing:
--Baseline draw 1
--Baseline draw 2
--Hour 72
--Hour 96
--Day 15 (label as "Day 15" even if drawn on a different day due to timing of stem cell infusion)

No other identifiers should be included on the label.

8.2.5 Sample shipment

Frozen plasma aliquots, serum aliquots, SmartTubes, and PAXgene tubes from all four draws should be batch shipped after all samples from that course have been obtained. These samples should be shipped frozen on dry ice. Ensure adequate dry ice to keep the samples frozen.

Sample shipment must include a generic NANT specimen transmittal form and the study-specific specimen transmittal form included in the study-specific data forms packet.

Samples should be shipped by Federal Express Priority Overnight to the following address:

ATTN: Myriam Armant, TransLab
Boston Children's Hospital
61 Binney Street
ENDERS Room 428
Boston, MA 02115
(617) 713-8085

FedEx account number 361615042 should be used for all shipments and be sure to also provide reference number 9618307.

Samples should only be shipped on Mondays-Thursdays to allow for weekday delivery. Email both myriam.armant@childrens.harvard.edu and Dr. DuBois at steven_dubois@dfci.harvard.edu with the tracking number at the time of sample shipment.

8.2.6 Laboratory Methodology

PBMCs will be isolated from SmartTubes. γ-H2AX foci will be quantified in fixed PBMCs using flow cytometry. This work will be completed by the Haas-Kogan Laboratory at Dana-Farber.

Total mRNA will be isolated from PAXgene tubes, using the commercial PAXgene Blood RNA kit. RT-PCR will be used to quantify expression levels of the 9 genes previously shown to be modulated by radiation exposure: *GADD45A*; *CDKN1A*; *PCNA*; *SGK*; *HSP132*; *DDB2*; *TNRFSF6*; *XPC*; and *SESN1*. This work will be completed by the Coleman Laboratory at Lawrence Livermore National Laboratory.

Plasma flt3 ligand and serum amylase will be quantified using commercial kits. This work will be completed by the TransLab at Dana-Farber/Boston Children's.

8.3 Evaluation of NET Protein Expression

8.3.1 Sample requirements

Five unstained slides will be submitted once. Material obtained from biopsy to diagnose or treat recurrent disease will be submitted. If not available, material from biopsy/resection to obtain initial diagnosis of neuroblastoma will be submitted. If not available, material from resection of primary tumor during initial induction will be submitted.

Materials obtained during bone marrow biopsy or fine needle aspirate procedure are not acceptable to support this evaluation.

8.3.2 Sampling scheduling

Samples will be submitted once, after study enrollment.

8.3.3 Sample processing

No additional processing is required at the treating institution.

8.3.4 Sample labeling

Slides or slide box should be labeled with the patient's NANT ID number, the protocol number N11-01, and the date of biopsy/resection.

No other identifiers should be included on the label.

8.3.5 Sample shipment

Slides should be shipped by Federal Express overnight to the following address:

Steven DuBois, MD
Dana-Farber Cancer Institute
450 Brookline Avenue, Dana 3, Room 3-109
Boston, MA 02215
Phone: 617-632-5460

FedEx account number 361615042 should be used for all shipments and be sure to also provide reference number 9618307.

Samples should only be shipped on Mondays-Thursdays to allow for weekday delivery. Email Dr. DuBois at steven_dubois@dfci.harvard.edu with the tracking number at the time of sample shipment.

8.3.6 Laboratory Methodology

NET protein expression will be assessed by immunohistochemistry and scored semi-quantitatively as previously described.[105] If other promising proteins involved in MIBG transport

emerge, such as vesicular monoamine transporters (VMATs), then these proteins will also be quantified.

8.4 Evaluation of Automated MIBG Scoring System

^{123}I-MIBG scans from study entry and following course 1 of therapy will be uploaded to the NANT PACS system for all patients as part of planned central review. These images will also be forwarded to Dr. Volchenboum at the University of Chicago for evaluation of computer-assisted MIBG scoring. The imaging group at the University of Chicago will not have access to MIBG scan reports from treating institutions or results of central review of scans. Following the study, the scores obtained using the computer-assisted platform will be compared to those assigned by treating institutions and/or by central review.

8.5 Evaluation of Circulating Tumor DNA

Patients who provided consent for optional evaluation of blood markers of radiation exposure and have remaining aliquots of plasma leftover after quantification of plasma flt3 ligand will be included in this evaluation of circulating tumor DNA. Aliquots will be transferred to the Crompton laboratory at Dana-Farber Cancer Institute, cell free DNA extracted using standard techniques, and then sequenced using next generation sequencing techniques. As appropriate, droplet digital PCR (ddPCR) may also be used to obtain comparative pilot data for these two approaches. No additional samples need to be collected from patients to support this work.

9.0 CRITERIA FOR REMOVAL FROM PROTOCOL AND OFF STUDY CRITERIA

9.1 Criteria for Removal from Protocol Therapy

a. Progressive disease
b. Completion of planned therapy (maximum of two courses of therapy on this protocol for patients enrolling prior to Amendment 7A and a maximum of one course of therapy on this protocol for patients enrolling with Amendment 7A or later)
c. Patient/parent withdrawal from therapy
d. Unacceptable adverse events by protocol criteria or physician judgment
e. Entry onto another therapeutic study and/or another anti-cancer therapy.
f. Pregnancy

Patients who are off protocol therapy are to be followed until they meet the criteria for off study.

9.2 Off Study Criteria

a. Death
b. Lost to follow-up
c. Patient/Parent withdrawal of consent.

10.0 STATISTICAL CONSIDERATIONS

10.1 Pick-the-Winner Design

Up to a total of 105 eligible and evaluable patients will be randomly assigned to one of the 3 MIBG arms. At completion of the study, the regimen with the highest observed response rate (by central review of response) after one course of therapy will be selected as the "winner" – i.e. the regimen that will be recommended as the MIBG backbone for future studies – provided that toxicity, tolerability, and other considerations confirm the feasibility of the regimen.

Two interim analyses for futility (to drop an inferior arm) will be undertaken: after 60 patients (approximately 20/arm) and after 81 patients (approximately 27/arm) have been randomized and completed at least one course and deemed evaluable for response. This analysis will be performed initially using site report of response. The following rule will be applied: if one of the arms is clearly inferior to the other two, then consideration will be given to dropping that single regimen. Study accrual will not pause while awaiting the results of any interim analysis (see below for modification per Amendment 7A). However, study accrual will be halted if needed to complete central review of response prior to dropping any regimen. The criterion to be used is based on Bayesian posterior probabilities (see Appendix VIII).

In addition, if one arm is dropped at the time of the 1st interim analysis, then after 35 patients have been randomized to each of the remaining arms and evaluated after the 1st course, we will compare these 2 remaining arms based on site report of response. If one arm is not clearly superior according to Bayesian posterior probabilities, accrual will continue until 42 evaluable patients per remaining arm have been randomized (yielding total sample of 104 patients in this scenario).

If neither futility rule is met, accrual will continue until 35 patients/arm are eligible and evaluable, for a total of 105 patients. With Amendment 7A, the design was modified to apply stochastic curtailment principles to permit early selection of a winner (see below). In order to account for potentially ineligible or inevaluable patients, a maximum of 115 patients are anticipated to enroll to yield 105 eligible and evaluable patients.

10.1.1 Amendment 7A – Dated 6-8-2017

The results of the 1st interim analysis were submitted to the independent DSMB on March 15, 2017, with the recommendation not to drop any arm at that time and to continue as planned - with one modification to the study design (addition of rules to permit early selection of a winner). This recommendation was accepted by the DSMB.

The proposed modification to the design involves applying "stochastic curtailment" at the time of the next interim analysis (after 81 patients). Specifically, after 81 patients have been evaluated, the Arm with the greatest observed response rate will be identified (designated here as Arm X). Under a variety of scenarios, exact calculations will be done to determine the probability (conditional on the observed data of the 1st 81 patients) that the observed response rate for Arm X will still be greater than the observed response rates for other 2 Arms after 105 patients have been enrolled and evaluated. If this probability (or more precisely, the probability across a variety of scenarios) is greater than 0.90, then accrual will be terminated with the conclusion that the true response rate for Arm X is higher than the true response rates for the other 2 Arms. See the Table below for skeleton of the table that will be created.

Table: Calculations* for the Probability that Arm X will Still have the Highest Observed Response Rate at the End of the Trial with 35 Patients per Arm (given the Results of the 1st 81 Patients)**

p_X = True Probability of Response on Arm X	p=0.10=True Probability of Response on the 2 Other Arms	p=0.15=True Probability of Response on the 2 Other Arms	p=0.20=True Probability of Response on the 2 Other Arms	p=0.25=True Probability of Response on the 2 Other Arms
0.10				
0.15				
0.20				
0.25				
0.30				
0.35				

**i.e. that the Arm X observed response rate would be greater than the observed response rates of other 2 arms
*Calculations are based on exact binomial probabilities

If this conditional probability is less than 0.90 for any one of the scenarios in which all 3 arms have the same true response rate (highlighted in the Table above), then the study will continue as originally planned – with the possibility that one arm will be dropped because its response rate is inferior to the response rates of the other 2 arms.

The table below summarizes the minimal impact of incorporating stochastic curtailment at this stage, on the overall probability of correct selection, for a variety of scenarios (in none of these scenarios is the probability of correct selection decreased).

Table: Probability* of Selecting the Correct Arm

True Probability of Response for the 2 Inferior Arms	Without Stochastic Curtailment Difference in True Probability of Response between Best Arm and the Other 2 (least favorable configuration)			With Stochastic Curtailment Difference in True Probability of Response between Best Arm and the Other 2 (least favorable configuration)		
	+0.0	+0.05	+0.10	+0.0	+0.05	+0.10
0.10	1.00	0.676	0.855	1.00	0.705	0.875
0.15	1.00	0.627	0.818	1.00	0.658	0.827
0.20	1.00	0.599	0.780	1.00	0.622	0.814
0.25	1.00	0.592	0.770	1.00	0.608	0.788
0.30	1.00	0.583	0.736	1.00	0.607	0.777

*Probabilities of correct selection estimated by simulation, using same methods a results in the Appendix

Logistic Considerations for 2nd Interim Analysis: Study accrual will pause while awaiting the results of the 2nd interim analysis, until it is clear that 81 patients are "evaluable for response". In addition, no final decision will be made to terminate accrual or drop an arm, until central review of response is completed.

10.2 Randomization

Patients will be randomized at study entry. In order to increase the likelihood of good balance between groups, patients will be stratified prior to randomization according to: disease status (relapsed/progressive vs. refractory disease vs. partial response during induction); bone marrow involvement by routine morphology (bone marrow involved at study entry vs. bone marrow free of disease at study entry); age (< 18 years and ≥ 18 years); and receipt of prior ^{131}I-MIBG (received or not received). The dynamic allocation method of Simon and Pocock will be used to assign patients to one of the 3 MIBG regimens, while balancing across the margins of each of the four stratification variables.[111]

10.3 Response Evaluation

10.3.1 Definition of Evaluable for Response

All patients who are registered onto the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories (see Section 11):

a. Complete response
b. Complete response-Minimal Residual Disease (MRD)
c. Partial response
d. Stable disease
e. Minor response
f. Progressive disease
g. Early death from malignant disease
h Early death from toxicity
i. Early death because of other cause
j. Inevaluable (not assessable, insufficient data, patients off therapy early due to toxicity)
k. Never received any of the study drugs: ^{131}I-MIBG, vorinostat, vincristine, or irinotecan.

All eligible, randomized patients will be included in the intent-to-treat analysis of response, according to the randomization outcome. Patients in groups a through c will be classified as responders; patients in groups d through k will be classified as non-responders. In a second analysis, the proportion of responders (patients in groups a through c) will be calculated from the subset of patients who are evaluable for response (as defined in the next paragraph).

In the secondary analysis (per protocol), patients will be considered evaluable for response if they receive ^{131}I-MIBG that is at least 85% of their prescribed dose (or at least 85% of 1200 mCi protocol maximum dose for patients whose calculated dose exceeds 1200 mCi) plus at least 80% of any prescribed systemic agents (at least 4 days of irinotecan or at least 11 days of vorinostat), or withdraw early due to tumor progression, clinical progression/deterioration, or toxicity. In addition, patients who do not withdraw early must meet the criteria to be adequately evaluated for each of the 3 disease parameters (if they had disease at baseline for that parameter) as described in Section 11, in order to be considered evaluable for response. For each patient not evaluable for response, an additional patient will be enrolled to meet the accrual goals in Section 10.5.

Per Amendment 7A (for clarification): The primary analysis will be a modified intent-to-treat analysis: all eligible, randomized patients who receive any amount of ^{131}I-MIBG will be included. There will be 2 secondary, additional analyses: an intent-to-treat analysis (all eligible, randomized patients) and a "per protocol" analysis based on all patients who are "evaluable for response" (described above). These will serve as "sensitivity" analyses.

10.3.2 Primary Response Endpoint and Additional Assessments of Treatment Effect

The primary endpoint and analysis will be objective tumor response after one course of therapy based on central review, for all eligible randomized patients who received any amount of ^{131}I-MIBG (modified intent-to-treat analysis). Two secondary analyses will (a) include all eligible randomized patients and (b) only patients evaluable for response as defined in the paragraph above in section 10.3.1. Patients will undergo complete disease staging (^{123}I-MIBG diagnostic scan, bilateral bone marrow aspirate and biopsy, anatomic imaging with CT or MRI scan, and urine catecholamines) at baseline and then approximately 6 weeks following ^{131}I-MIBG infusion. Response will be assessed according to NANT response criteria v1.1. These criteria are modified from the International Neuroblastoma Response Criteria[112] in that they utilize RECIST 1.1 criteria for measurable tumors[113] and Curie score for MIBG scan response.[106] Patients with an institutional report of complete response, complete response-MRD, partial response, or stable disease will have their response centrally reviewed. An exact logistic regression analysis will be used to compare the 3 arms in terms of response, while controlling for the stratification variables.

For the subset of patients receiving a second course of therapy (enrolled prior to Amendment 7A), the response to the second course of therapy will be reported as well as the overall response to two courses of protocol therapy. Response to the first course will nevertheless be the primary endpoint for study.

Additional endpoints for response to treatment will include response at each site of disease: bone marrow; soft-tissue sites; and ^{123}I-MIBG using NANT response criteria. Progression-free survival and overall survival (summarized with Kaplan-Meier plots) will be additional secondary endpoints.

10.4 Toxicity Evaluation

Toxicity is the secondary endpoint of this trial. All patients who begin treatment with vincristine, irinotecan, vorinostat, or ^{131}I-MIBG will be evaluable for toxicity. Toxicity will be graded using the CTCAE criteria, version 4.0 which can be downloaded from the CTEP home page (http://ctep.cancer.gov).

Patients will undergo standard screening for toxicity used in previous NANT ^{131}I-MIBG studies. All patients who begin study therapy will be evaluable for toxicity. All toxicities will be tabulated and reported according to received treatment arm. Differences in the incidence of toxicities between treatment arms will be assessed using the Fisher's exact test.

10.5 Sample Size and Study Duration

While we expect that both the vincristine/irinotecan and the vorinostat arms will demonstrate higher response rates compared to the MIBG-alone arm – and that the selection therefore will only involve those

two arms – we have included the MIBG-alone arm to calibrate the improvement and better understand the impact of these chemotherapy additions. However, for purposes of determining the number of required patients, we will consider that all three arms are candidates of selection (i.e. could result in the highest observed response rate). The number of patients (35 per MIBG regimen, if no arms are dropped as inferior) was calculated to ensure an 80% chance that the regimen with the highest response rate is selected – if in fact that response rate is at least 15% better than the other two (using the methods described by Sobel and Huyett [114] and summarized by Bechhofer, Santner and Goldsman [115]). Because we have incorporated Bayesian criteria for dropping an inferior arm or stopping early, the power of correct selection and other design characteristics were determined by simulation (see Appendix VIII for more details).

Table A in Appendix VIII summarizes the probability of correct selection, the probability of dropping one arm for futility, the probability of dropping the superior arm for futility, and the average number of patients in the inferior arm.

Estimates of the number of available patients for this study are derived from previous ^{131}I-MIBG studies at NANT ^{131}I-MIBG institutions. Specifically, NANT01-02 accrued 50 patients with refractory disease over approximately 40 months of active patient recruitment. NANT04-06 accrued 26 patients with relapsed or refractory disease over the approximately 40 months during which that study was open. This accrual rate underestimates the number of available patients given that NANT04-06 was a phase I study that utilized a 3+3 dose escalation strategy and accrual was closed for a minimum of 6 weeks after the third patient at each dose level was treated. CHOP, Cincinnati Children's, UCSF, and the University of Michigan treat 25, 12, 12, and 7 patients per year on their respective expanded access ^{131}I-MIBG protocols. Newer NANT MIBG centers now include Children's Hospital Boston, Cook Children's Hospital, Children's Hospital of Colorado, Hospital for Sick Children, and Seattle Children's Hospital. These newer centers will increase access to MIBG treatment slots.

We therefore conservatively estimate that 4 patients per month will be available for enrollment in the current study. This accrual rate will allow completion of the study within 30 months, assuming all three regimens accrue a total of 35 patients/arm (i.e. interim futility analysis does not result in early closure of one of the arms). In order to account for potentially ineligible or inevaluable patients, a maximum of 115 patients are anticipated to enroll to yield 105 eligible and evaluable patients.

10.6 Monitoring Plan for Toxicity

Although we expect all three regimens to be generally well tolerated, several steps and procedures are in place in this trial, to monitor toxicities and safety. These are summarized briefly here and described more fully in the subsections below. All toxicities observed will be reported and summarized in the final report. The steps described below were developed for ongoing monitoring.

(1) Delayed engraftment after reduced stem cell dose. Until the 1st 6 patients who receive a stem cell dose of only 1.5 - 1.9 x 10^6 CD34+/kg, demonstrate timely engraftment without problems (Section 10.6.1), all patients must have a stem cell dose of at least 2.0 x 10^6 CD34+/kg, or must have a back-up aliquot.
(2) Monitoring delayed engraftment. Throughout the conduct of the trial, the details of any patient who fails to achieve timely engraftment will be formally reviewed (Section 10.6.2).
(3) Monitoring for toxic death. Each death during treatment or within 2 months of stem cell infusion will be formally reviewed (Section 10.6.3).
(4) Monitoring plan for other toxicities. Summaries are created every 6 months for review by the study PI and by the NANT DSMB. In addition, at the time of the 1st interim futility analysis, toxicities will be formally compared (Section 10.6.4).

10.6.1 Monitoring plan for delayed engraftment after reduced stem cell dose

Previous NANT ^{131}I-MIBG trials (NANT N04-06 and N07-03) utilized a slightly higher minimum stem cell dose (2.0 x 10^6 CD34+ cells/kg) than planned for the current trial (1.5 x 10^6 CD34+ cells/kg). In order to evaluate the safety of this lower stem cell dose within this context, patients planning to receive a stem cell dose of 1.5 - 1.9 x 10^6 CD34+/kg must have a back-up aliquot of 1.5 - 1.9 x 10^6 CD34+/kg available until a minimum of 6 patients have been rescued with a stem cell dose of 1.5 - 1.9 x 10^6 CD34+/kg without delayed engraftment. If the first 6 patients receiving this reduced stem cell dose do not have delayed

engraftment, then future patients may enroll with only a minimum of 1.5 x 10^6 CD34+ cells/kg and no back-up aliquot. If any of the first 6 patients have delayed engraftment, then the required stem cell dose will be changed to 2.0 x 10^6 CD34+ cells/kg.

Patients planning to receive a stem cells dose of 2.0 x 10^6 CD34+/kg are not required to have a back-up aliquot at any time during the study.

10.6.2 Monitoring plan for delayed engraftment
Any time a patient fails to engraft (as defined in Section 4.7), the status of the study will be examined. Information reviewed will include the specific details of the patient whose engraftment was delayed, as well as the engraftment patterns of the other patients to date. A decision will be made by the NANT Study Management Committee, whether to report immediately to the DSMC or to include the information in the next DSMC report (within 6 months).

10.6.3 Monitoring plan for toxic death
Any time that a patient dies while undergoing treatment prior to a stem cell infusion, or within two months of stem cell infusion, the status of the study will be examined. Information reviewed will include the specific details of the patient who died, the timing of the death, the specifics of all patients who experienced the same type of toxicity that did not lead to death, and all toxicities observed to date.

Each death on study not due to tumor will be reviewed by the NANT Study Management Committee, reported to NANT DSMB and a decision in consultation with NANT DSMB and IND sponsor will be made to close the trial, modify the trial, or continue unchanged. In addition, it will be determined if the event requires that new information be added to the informed consent. Each death occurring within 30 days of completing the last dose of either ^{131}I-MIBG or vorinostat, whichever occurs last, regardless of cause will be reviewed and reported to the NANT DSMB and to the FDA according to standard procedure.

10.6.4 Monitoring plan for other toxicities
In addition to monitoring for delayed engraftments and toxic deaths, we will formally summarize the toxicities experienced at the time of the first interim futility analysis (20 patients per arm). The vincristine/irinotecan and the vorinostat arms will each be compared to the single-agent MIBG arm. Differences in the occurrence of Grade 3+ non-hematologic toxicities that are significant at the 0.10 level (two-sided Fisher's exact test) at the time of the first interim futility analysis will be flagged for further evaluation. The results of the interim analyses will be reviewed by the NANT Study Management Committee, reported to NANT DSMB and a decision in consultation with NANT DSMB will be made to close the trial, modify the trial, or continue unchanged.

Every 6 months, reports are prepared for DSMB review. These reports will summarize toxicities experienced which will allow an assessment of the safety for the trial overall, as well as each of the 3 arms individually. We do not plan to calculate p-values for every single type of toxicity, but the data summaries will permit identification of unexpected patterns in terms of severity or frequency.

10.7 Analysis of Correlative and Exploratory Studies
The correlative biology studies are considered descriptive. Based on prior NANT studies, we anticipate that a minimum of 80% of patients will agree to participate in studies that require additional blood sampling.

10.7.1 Whole Body Radiation Exposure
In the current study, all patients will have whole body radiation doses calculated based on serial measurements of radiation exposure rate with an ionization chamber set at a fixed distance and geometry. Our primary analyses will compare whole body radiation dose between randomized treatment groups and also between responders vs. non-responders using analysis of variance. Secondary analyses will compare whole body radiation dose between patients with and without grade 4 neutropenia, grade 4 thrombocytopenia, and grade $\geq$ 3 non-hematologic toxicity.

10.7.2 Minimal Residual Disease by NB5 Assay
We will use TLDA to determine the ability of each the three ^{131}I-MIBG regimens to reduce peripheral blood and bone marrow disease burden. Peripheral blood and bone marrow samples will be obtained at study entry and at the time of subsequent bone marrow evaluations. For each patient, we will calculate

their change in TLDA DG score from baseline to end of course 1 of therapy. The primary analysis will be an analysis of variance to compare this change in TLDA DG score in the bone marrow between randomized treatment groups. Secondary analyses will include evaluations of change in peripheral blood TLDA DG score as well as baseline level of blood and bone marrow disease burden by TLDA as predictors of response to ^{131}I-MIBG therapy.

10.7.3 Evaluation of γ-H2AX Foci, Flt3 Ligand, Amylase, and DNA Damage Response Genes

For each radiation biomarker of interest, we will focus on comparing changes in each biomarker across the three randomized treatment arms. Each biomarker is a continuous variable, with values measured at 5 time points (two pre-^{131}I-MIBG and one each at hours 72, 96, and Day 15 after start of ^{131}I-MIBG infusion). We will determine the relative (percent) change of the post-^{131}I-MIBG values from 2nd evaluation for each biomarker for each patient. The changes from the 1st to the 2nd value in the ^{131}I-MIBG only arm will be used to estimate the within patient variability, while changes from the 1st to 2nd value in the other two arms will reflect the impact of each radiation sensitizer alone on each marker. Changes post ^{131}I-MIBG will be presented in context with the estimated intrapatient variation.

To determine the effect of radiation sensitizers on each biomarker, we will use an analysis of variance (comparing treatment arms) with repeated measures (change at 72 hours, at 96 hours, and at Day 15) to model the relative change of each biomarker as a function of randomized treatment arm and time. We will introduce a linear contrast to assess the general impact of the presence or absence of a radiation sensitizer (combining the irinotecan and vorinostat arms). Additional models will evaluate calculated whole body radiation exposure as a covariate in the model and separately as the outcome variable as a function of randomized treatment arm.

A series of secondary analyses will evaluate associations between baseline biomarker values and changes in biomarker values with toxicity and response to therapy. We will test for a univariate association between the baseline value or the degree of change in each biomarker and clinical response using two-sample t-tests, with patients separated into groups of responders (partial response or better after one course) and non-responders (less than partial response). We will evaluate an association with toxicity in a similar way, separating patients based on the presence or absence of grade $\geq$ 3 non-hematologic toxicity and also based on the presence or absence of grade $\geq$ 4 neutropenia or grade ≥ 4 thrombocytopenia in that course. Although testing will be two-sided, our expectation is that greater changes post ^{131}I-MIBG in each of the biomarkers will be associated with higher response rates and greater toxicity. For each outcome, there will be 24 tests of association (6 biomarkers and 4 times). Nominal p-values will be reported without correction for multiple testing. Resampling will be used to assess robustness and internal validity of the univariate associations.

We will next construct a series of logistic regression models with response and toxicity (separately) as the dependent variables. Key covariates will include the relative change of the biomarker of interest, the baseline value, randomized treatment arm, the disease status (recurrent vs. primary refractory) and calculated whole body radiation exposure. If multiple biomarkers show promise, additional models will include covariates for each of these covariates or a composite biomarker score obtained using principal component analysis.

We will also evaluate whether patterns observed in the first course of therapy are seen during the second course of therapy, though the primary analyses will be based on data from the first course of therapy.

The sample size for the radiation biomarker studies is based on an estimated 80% participation rate, yielding 84 patients, or 28 patients per randomized arm. For the primary analyses, an analysis of variance with repeated measures will be used to establish an effect of either vorinostat or irinotecan on the amount of change in each biomarker. To obtain a (conservative) estimate of the power of the test of the effect of treatment arm, we will consider a one-way analysis of variance comparing the 3 arms at a single time point With 28 patients in each arm, using a 0.05-level F-test for comparing the 3 mean changes, there will be 91% power when the true mean change in the group with the greatest change exceeds the true mean change in the group with the smallest change, by at least one “sigma” in magnitude – where “sigma” is equal to the common between-patient standard deviation (i.e. patient-to-patient variability).

10.7.4 Evaluation of Assistive MIBG Scoring System

The performance characteristics of the assistive MIBG scoring system will be described, using NANT nuclear medicine central review as the gold standard. Scatterplots and a general measure of

concordance (the concordance correlation coefficient [116] or the weighted kappa statistic [117], depending on the distributions of the nuclear medicine central review scores, will be used to summarize the agreement of the two measures. Since the long-range goal would be to replace standard nuclear medicine Curie scoring with the assistive scoring, regression methods will also be used to evaluate how well the assistive score can "predict" the gold standard score.

10.7.5 Evaluation of NET Expression and Clinical Response to ^{131}I-MIBG

NET immunohistochemistry results will be coded on a semi-quantitative intensity scale (0 – 3). We will utilize Fisher's exact test to compare categories of NET intensity between patients with and without response to the first course of therapy (according to 10.3.1).

In a secondary analysis, we will also attempt to validate a previous report of lower NET expression among patients with *MYCN* amplified tumors. We will perform Fisher exact test to compare categories of NET expression between patients with *MYCN* amplified vs. non-amplified tumors.

10.7.6 Concordance of Institutional and Central Assignment of Curie Score

Treating institutions and NANT MIBG scan central reviewers will each assign patients a Curie score at diagnosis, end of course 1, and end of course 2 (if applicable). For the purposes of this exploratory aim (aim 1.9), the analytic cohort will include any of these scans assigned a Curie score by an institution that does not include a designated NANT MIBG scan central reviewer. Descriptive statistics will be provided that include 1) concordance rate between institutional and central review as well as 2) the percent of patients for whom MIBG response category (per section 11.3) would change based upon differences in Curie score between institutional and central review. Analysis of the Curie scores will be the same as described in 10.7.4, with the gold standard being the NANT MIBG scan central reviewer. For the comparisons of overall response, data will be summarized with contingency tables and the weighted kappa statistic.

10.7.7 Sinusoidal Obstruction Syndrome after ^{131}I-MIBG Therapy

For the purposes of assessing Aim 1.10, the number of patients treated with protocol therapy who go on to receive subsequent myeloablative therapy with stem cell rescue within 6 months of completing study therapy will be determined based upon data collected during protocol follow-up. Of this population, the number and percent of patients who develop SOS of any grade within 21 days of stem cell rescue after myeloablative therapy will be reported based upon specific query on protocol follow-up forms.

For the purposes of this protocol, the definition of SOS will mirror the definition used in NANT and COG trials that include myeloablative therapy: serum total bilirubin > 2.0 mg/dL, with at least 2 of the following findings within 21 days of receipt of stem cell infusion after myeloablative therapy:

- hepatomegaly with right upper quadrant pain;
- ascites; or
- weight gain >5% above baseline.

Severe SOS is defined as an episode of SOS in addition to a specific organ failure:

a. Hepatic encephalopathy (CTC Grade 4 hepatic failure), OR
b. Pulmonary dysfunction: Continuous oxygen support (CTC Grade 3 hypoxia) for > 48 hours, ventilatory support not clearly attributable to another cause, OR
c. Renal dysfunction: serum creatinine > 3 times the ULN (CTC Grade 3 creatinine), or the need for dialysis (CTC Grade 4 renal), not clearly attributable to another cause.

All other cases of SOS not meeting the definition of severe SOS will be considered mild-moderate.

Additional details, including time from last ^{131}I-MIBG to start of myeloablative therapy, type of conditioning for myeloablative therapy, severity of SOS, need for defibrotide therapy, and outcome of SOS will be collected and reported descriptively. The number of patients undergoing myeloablative therapy and the number of those patients developing SOS will be reported according to randomized arm assignment.

10.7.8 Circulating Tumor DNA (ctDNA)

Evaluation of ctDNA in the context of this trial is considered a pilot investigation. Descriptive data on percent of patients with detectable ctDNA at each time point will be reported for the entire cohort and then separately for responders and non-responders (as defined in section 10.3). Data using next generation

sequencing method and ddPCR method obtained from the same time point will be presented using descriptive statistics, with no formal statistical testing planned.

10.8 Inclusion of Women and Minorities

The study is open to all participants regardless of gender or ethnicity. Review of accrual to past NANT studies of new agents demonstrates the accrual of both genders and all NIH-identified ethnicities to such studies. Although we will summarize the response rate by gender and race/ethnicity, the relatively small number of patients entered into this trial will not permit formal comparisons.

11.0 NANT RESPONSE CRITERIA v 1.2

Below are the NANT Response Criteria. Since patients on this study must have MIBG avid tumors, any reference to MIBG non-avid tumors is not applicable.

Overall response will incorporate all three parameters: CT/MRI, MIBG (FDG-PET is substituted for MIBG non-avid tumors), and bone marrow response with overall response defined as outlined in Section 11.6. Response for each parameter and overall response will be reported by the treating site using the criteria below. However, the final statistical analysis of response will utilize responses as graded by central review, using the same criteria below.

11.1 Response Criteria for CT/MRI Lesions

For lesions evaluated by CT/MRI, this study will use the definitions of measurable disease from the Response Evaluation Criteria in Solid Tumors (RECIST 1.1; European Journal Cancer 45: 228-247, 2009) modified per the criteria below to define target lesions (lesions which are measurable AND evaluable for response).

11.1.1 Definition of soft tissue TARGET LESIONS on CT/MRI:

Soft tissue target lesions that will be followed for response must meet criteria in a. and b. below:

a. A target lesion must be measurable, defined as a soft tissue lesion that can be accurately measured in at least one dimension with a longest diameter ≥10 mm, or for lymph nodes ≥15 mm in short axis. (The short axis is measured after identifying the longest diameter of a lymph node or nodal mass, and then measuring the longest perpendicular diameter to that as the short axis).

b. A target lesion must also be evaluable for response: To be evaluable, the lesion must also be MIBG or FDG-PET avid (if tumor known to be MIBG non-avid), or have a biopsy as required in the eligibility criteria. If one soft tissue lesion present at enrollment is biopsied showing neuroblastoma or ganglioneuroblastoma at any time point, then all other soft tissues lesions present at enrollment are considered evaluable. Bone marrow positivity does not affect soft tissue evaluability.

   NOTE: Soft tissue components of bone lesions will be considered measurable lesions if > 10mm in at least one dimension, and evaluable for response if MIBG avid (or PET-avid if tumor known to be MIBG non-avid).

   Serial measurements of target lesions are to be done with the same method of assessment (either CT or MRI) used to characterize each lesion reported at baseline. The sum of diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the **sum of diameters**.

11.1.2 Non-Target soft tissue lesions will include:

a. Leptomeningeal tumor and tumor in cerebrospinal fluid cytology will be considered non-target lesions.
b. Lesions that are considered likely to be active tumor by the treating physician based on clinical correlation (for example, hepatic and pulmonary nodules)

11.1.3 The following lesions will NOT be followed to evaluate response either as target lesions or non-target lesions, if they meet the criteria below **AND** the treating physician feels they are unlikely to represent active tumor (an exception for active tumor will be made for c. below):

a. Measurable soft tissue lesions (≥ 10mm) that are not MIBG avid or PET avid (if tumor known to be MIBG non-avid) and if biopsied did not show neuroblastoma or ganglioneuroblastoma.
b. Non-measurable soft tissue lesions < 10mm or non-measurable lymph nodes (defined as lymph nodes >10 to <15mm on short axis).
c. Intramedullary bone lesions will not be followed for CT/MRI response even though they are felt to represent active tumor since they will be evaluated with MIBG scans (or FDG-PET scans if MIBG non-avid), and since bone changes on CT/MRI are known to persist after resolution of active tumor.

11.1.4 The response of the CT/MRI lesions will be defined as outlined below:

11.14.1 Complete Response (CR)
Disappearance of all target CT/MRI lesions.

11.1.4.2 Partial Response (PR)
At least a 30% decrease in sum of diameters of CT/MRI lesions, (using longest diameter for non-nodal lesions and short axis for nodal lesions), taking as reference the measurement of target lesions performed at study enrollment. Non-target CT/MRI lesions must be stable to smaller in size. No new lesions. MIBG (PET-FDG for MIBG non-avid tumors) uptake may still be present in lesions positive at enrollment.

11.1.4.3 Progressive Disease (PD)
At least a 20% increase in sum of diameters of target lesions (using longest diameter for non-nodal lesions and short axis for nodal lesions), taking as reference the smallest sum of diameters while on study (this includes the baseline if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of 5 mm. A new target or non-target tumor lesion seen on CT/MRI without MIBG or PET uptake is considered PD, but may be biopsied to rule out PD. An overall substantial worsening of non-target CT/MRI lesions is also considered as a criteria for PD (guidance for substantial includes a 73% increase in volume, of sufficient worsening of overall non-target disease such that the treating physician feels a change in therapy is indicated).

New uptake of MIBG (or FDG-PET for MIBG non-avid tumors) at target and/or non-target lesions which are stable in size, will be captured in MIBG response (or FDG-PET if tumor is MIBG non-avid) and will NOT be graded as CT/MRI progression. CT/MRI would be graded as SD. Biopsy of such lesions can be done to rule out PD for the MIBG (or FDG-PET) response.

11.1.4.4 Stable disease (SD)
(Applies only to patients with target lesions)
Neither sufficient shrinkage in sum of diameters to qualify for PR, and patient does not meet any criteria for PD. No new lesions. Non-target lesions must be stable to smaller in size.

11.1.4.5 Stable disease-no target lesions (SD-NTL)
(Applies to patients with non-target lesions only)
Non-target lesions are still present; may be smaller or stable in size, and do not meet criteria for PD. No new non-target tumor lesions (new lesions may be biopsied to rule out PD).

11.1.4.6 Not involved (NI)
No target or non-target lesions

11.1.4.7 Non-target lesions only
Patient has no target lesions, but does have non-target lesions

11.1.4.8 Not evaluable (NE)
CT/MRI scans are of inadequate quality as assessed by central reviewer, or scans are not repeated of all lesions with tumor documented at entry. (Note that patients not evaluable at a given time point may be evaluable for response at later time points if all scans done with adequate quality at later time point.) Or if based on Study Management Committee/PI review it is deemed that there is insufficient data to grade response

11.1.4.9 Not done (ND)
No CT/MRI scans were done at the given time point.

11.2 Response Criteria for Morphologic Bone Marrow Disease

Routine morphology (with or without routine immunocytochemistry) will be used for baseline evaluation and all subsequent response evaluations performed while on protocol therapy, and at end of protocol therapy. Central review will be performed on bilateral biopsies only unless tumor is seen only on aspirates.

Patients with ≤5% tumor on all samples of the bilateral bone marrow aspirate and biopsies at study entry will be evaluable for bone marrow response, but defined separately from patients with >5% at study entry.

The percentage of tumor in an aspirate will be calculated as the number of tumor cells divided by the number of total nucleated cells. The percentage of tumor in a biopsy will be calculated as the percent of tumor cells (including neuroblasts, mature and maturing ganglion cells) based on the bone marrow parenchymal surface area examined (excluding Schwannian stroma).

11.2.1 Complete Response (CR)
Greater than 5% tumor cells seen on any one sample of bilateral aspirates/biopsies performed at study entry, with no tumor cells seen on bilateral aspirates and biopsies at one subsequent time point.

11.2.2 Complete Response-Minimal Residual Disease (CR-MRD)
Percentage tumor ≤ 5% on all samples of bilateral aspirates/biopsies at study entry, with no tumor cells seen on bilateral aspirates and biopsies at one subsequent time point after starting protocol therapy If subsequent time points remain negative, then patient is classified as CR–MRD. If subsequent time points are intermittently positive but with ≤ 10% tumor, then response should be reclassified as SD at the negative time points initially reported as CR-MRD.

11.2.3 Progressive Disease (PD)
Patients with any amount of tumor in the bone marrow at study entry will be considered to have PD if one subsequent evaluation shows > 10% tumor on any one bone marrow sample AND there is a doubling in the amount of tumor compared to study entry (baseline).

For example, a patient entering with ≤ 5% tumor in marrow must increase to ≥ 10% tumor to have PD; a patient entering with 30% tumor must increase to ≥60% tumor. If patients have in increase in tumor amount which is less than the amount specified for PD, the response will be classified as SD.

Patients who enter on study with no tumor seen will be considered PD if ONE subsequent evaluation shows >10% tumor. If ≤ 10% tumor is seen on one subsequent evaluation or intermittently, the response will be classified as SD.

11.2.4 Stable Disease (SD)
Stable disease will be defined as persistence of an amount of tumor in the bone marrow that does not meet criteria for progressive disease or CR or CR Minimal Residual Disease. (Note that patients who enter with any amount of tumor and have one subsequent negative bone marrow which is initially graded as a CR or CR-MRD and then on subsequent time points have 1-10% tumor noted would be then considered as SD at all time points, including the time point initially graded as CR/CR-MRD).

11.2.5 Not Evaluable (NE)
Patients for whom follow-up bone marrows do not include an attempt to obtain bilateral aspirates and biopsies and do not have at least one adequate biopsy sample, as assessed by local site's pathology report for that time point or if based on Study Management Committee/PI review it is deemed that there is insufficient data to grade response.

11.2.6 Not involved (NI)
Patients with no evidence of neuroblastoma in the bone marrow at study entry, and remain negative on subsequent evaluations. (Note that such patients may be reclassified as SD if they meet the SD criteria above with intermittent tumor in the bone marrow).

11.2.7 Not done (ND)
Bone marrow evaluation not done at a given time point.

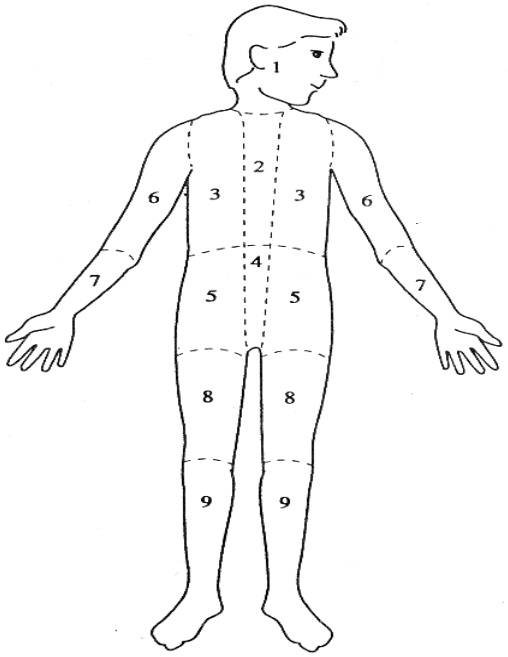
11.3. Response Criteria for MIBG Avid Lesions

MIBG Response will be graded using the Curie scoring scale.[106] The treating site will report the MIBG response using the Curie scoring, however the statistical endpoint of MIBG response will utilize the Curie score from the central reviewer.

MIBG scans will be scored for 10 regions: 9 anatomic regions for skeletal metastases, and a 10th region for any MIBG avid soft tissue disease.

Each of the 10 regions will be given a score of 0-3, as defined below.

Scoring of Skeletal Disease Regions 1 – 9	
Scoring	MIBG uptake
0	No MIBG uptake
1	1 focal lesion
2	> 1 focal lesion
3	> 50% of a region



The **absolute extension score** is obtained by adding the scores of all ten regions. The presence of an MIBG avid lesion, and NOT the "intensity" of MIBG-avidity, determines the scoring within a particular region.

REGIONS 1-9 (SKELETAL DISEASE): Cranio-facial disease is scored in Region 1, cervico-thoracic spine in Region 2, ribs/sternum/clavicles/scapula in Region 3, lumbar-sacral spine in Region 4, pelvis in Region 5, humeri in Region 6, distal upper extremities in Region 7, femurs in Region 8, and distal lower extremities in Region 9 (see figure below).

Region 10 (SOFT TISSUE DISEASE): Soft tissue disease within the neck, chest or abdomen/pelvis is scored within Region 10. Examples of soft tissue lesions may include MIBG avid cervical, paraspinous, adrenal, renal, retroperitoneal, or hepatic masses.

SCORING SOFT TISSUE DISEASE: Score = 1 indicates an isolated soft tissue mass that encompasses < 50% of that region (chest or abdomen/pelvis). Score = 2 indicates > 1 soft tissue lesion in the neck, chest or abdomen/pelvis. A soft tissue score = 3 indicates MIBG avid soft tissue disease that encompasses > 50% of either the chest or abdomen/pelvis. (For example, a large adrenal mass in which the MIBG avidity encompasses > 50% of the abdomen/pelvis is scored a 3 for Region 10). Cervical soft tissue disease is included in the chest region.

If no MIBG-avid soft tissue lesions are present, then score 0 for Region 10.

If a solitary soft tissue lesion extends into both the chest and abdomen/pelvic regions, score 3 for that lesion. For example, a large paraspinous mass that extends along the thoracic and lumbar spine would be scored a 3 for Region 10. Any corresponding metastatic bone disease within the thoracic or lumbar spine would be scored separately for the cervico-thoracic spine (Region 2) and lumbar spine (Region 4).

Conjugate planar imaging will be used to score a given region. SPECT scans may be used as an adjunct, but only to help delineate the location of the MIBG avid lesion.

The **relative score** is calculated by dividing the absolute score at each time by the corresponding pre-treatment overall score. The relative score of each patient is calculated at each response assessment and classified as below:

a. Complete response: all lesions of uptake on MIBG scan completely resolved.
b. Partial response: Relative score $\geq$ 0.1 to $\leq$ 0.5
c. Stable disease: Relative score $>$ 0.5 to $<$ 1.2
d. Progressive disease: New lesions on MIBG scan OR a relative score $\geq$ 1.2. Biopsy of new lesions may be done to rule out progressive disease. If biopsy is negative for tumor (neuroblastoma and/or ganglioneuroblastoma), patient will not meet definition of PD.
e. Not evaluable: MIBG scan of inadequate quality as assessed by central reviewer.
f. Not involved: No MIBG avid lesions at study entry and subsequent response time points.
g. Not done: MIBG scan not done at a given response time point.

11.4 Response Criteria using FDG-PET scans (for MIBG non-avid patients)
This section included as a reference only and will NOT be used in NANT 2011-01 as all patients must be MIBG avid

Patients known to be non-avid for MIBG should have FDG-PET scans performed for monitoring response. FDG-PET avid lesions will be scored by the presence or absence of a lesion that is two times above background.

11.4.1 Complete response (CR)
Resolution of all FDG-PET uptake in all FDG-PET avid lesions identified at baseline and no new FDG-PET avid lesions.

11.4.2 Partial response (PR)
Reduction of number of lesions by FDG-PET by ≥ 50%. No new FDG-PET avid lesions.

11.4.3 Stable disease (SD)
Changes that do not meet the criteria for PR or PD.

11.4.4 Progressive disease (PD)
New lesions on FDG-PET scan. Note: biopsy may be done to exclude causes of FDG-PET uptake other than tumor. If biopsy is negative for tumor (neuroblastoma and/or ganglioneuroblastoma), patient will not meet definition of PD.

11.4.5 Not evaluable (NE)
FDG-PET scan of inadequate quality to evaluate response or if based on Study Management Committee/PI review it is deemed that there is insufficient data to grade response.

11.4.6 Not involved (NI)
No FDG-PET uptake that is two times above background

11.4.7 Not Done (ND)
FDG-PET scan not done at that timepoint.

On central review, FDG-PET avid lesions will be evaluated by both the Curie scoring method and by enumeration of lesions to grade response.

11.5 Urine Catecholamines

Due to variance with diet and concomitant medications, frequently missing dopamine levels, and lack of standardized methodology for this assay, urine catecholamines will not be utilized in grading response. Results of urine catecholamines will still be requested at all response evaluation time points and recorded in database as both actual and as "elevated" or "not elevated".Catecholamines must be at least 3 standard deviations above the mean for age to be classified as elevated.

11.6 Definition of Overall Response for Each Patient

The criteria below will be used to define the overall response for each patient, with consideration of all three individual response parameters: CT/MRI, MIBG (FDG-PET if tumor is not MIBG avid), and Bone Marrow.

11.6.1 Complete Response (CR)
Response of CR or NI for MIBG (FDG-PET if tumor is not MIBG avid), CT/MRI and bone marrow.

11.6.2 Complete Response-MRD (CR-MRD)
Response of CR or NI for MIBG (FDG-PET if MIBG non-avid), CT/MRI response of CR or NI, with BM response of CR-MRD.

11.6.3 Partial Response (PR)
Response of PR in both CT/MRI and MIBG (FDG-PET scan if tumor is not MIBG avid); or response of PR in either CT/MRI or MIBG (FDG-PET if MIBG non-avid) with response of CR, NI, or SD-NTL for other parameter or response of CR for MIBG with SD-NTL for CT response. Bone marrow response of either CR, CR-MRD, NI, or SD for patients with maximum amount of tumor $\leq$5% at that given time point.

11.6.4 Progressive Disease (PD)
Either one of the following will define an overall response of PD:

a. At least one response parameter including CT/MRI, MIBG, and/or bone marrow and/or FDG-PET response is PD. If PD is found by one parameter, the other two parameters are not required to be evaluated to define an overall response of PD.
b. Treating physician grades patient as progressive disease based on clinical assessment without radiographic or bone marrow evaluations.

11.6.5 Stable disease (SD)
Response of stable disease for at least one parameter, with response of SD, NI, or SD-NTL for other parameters.

11.6.6 Minor response (MR)
Complete response, Complete-MRD response, and/or partial response for one parameter (i.e. CT/MRI, MIBG (FDG-PET if MIBG non-avid),bone marrow), with response of stable disease for second parameter and any response other than PD or inevaluable for third parameter.

11.6.7 Not evaluable (NE)
Response of Not evaluable for one or more response parameters including CT/MRI, MIBG (FDG-PET if MIBG non-avid), or bone marrow, unless one parameter that had measurable/evaluable tumor at study enrollment. However, if one parameter is done and demonstrates PD this is defined as an overall response of PD. In addition, response may be declared not evaluable if review by the Study Management Committee/PI deems that there is insufficient data to grade response

11.6.8 No Progression
Baseline status at enrollment was NI or non-target lesions only for CT, NI for bone marrow, NI for MIBG (FDG-PET if MIBG avid) and there has NOT been PD at any site since on protocol therapy.

11.6.9 Not done (ND)
Response was not assessed at this timepoint.

11.6.10 Summary

The overall response as assessed at any particular time point based on consideration of each of the three parameters as defined above is summarized in the following table:

Overall Response Assignment

CT/MRI Response	MIBG Response	Bone Marrow Response	Overall Response
PD for any one parameter with any response (including Not Done or Inevaluable) for the other 2 parameters. PD may also be defined by clinical assessment of the treating physician only.			PD
CR	CR	CR	CR
CR for one parameter with either CR or NI for other parameters			CR
MIBG response= CR or NI CT/MRI response = CR or SD-No Target Lesions or NI		CR-MRD	CR-MRD
CR or PR or SD-No Target Lesions	PR	CR or CR-MRD or NI -OR- SD if ≤ 5% tumor in bone marrow	PR
PR	CR or PR or NI	CR or CR-MRD or NI -OR- SD if ≤ 5% tumor in bone marrow at study entry	PR
SD for one parameter, with SD or NI or SD-NTL for other two parameters			SD
CR or CR-MRD or PR for at least one parameter, SD for a second parameter, and any response other than PD or Not Evaluable for other parameter			Minor Response
Response of Not Evaluable for any one of the 3 parameters that had measurable/evaluable tumor at study enrollment and no PD for any parameter.			Not Evaluable
No response evaluations performed for any of the 3 parameters			Not Done
NI or non-target lesions only at enrollment and no PD on subsequent timepoint	NI at enrollment and no PD at subsequent time point	NI at enrollment and no PD at subsequent time point	No Progression

*For patients who utilize FDG-PET in place of MIBG response, then substitute FDG-PET response for MIBG response in this table to define overall response.

12.0 ADVERSE EVENT REPORTING REQUIREMENTS

12.1 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. Adverse events are reported in a routine manner at scheduled times during a trial. (Please follow directions for routine reporting provided in the data collection packet for this protocol). Additionally, 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 through the use of a written IND safety report (MedWatch) to the Food and Drug Administration (FDA).

An **adverse event** is defined as any unintended or abnormal clinical observation that is not of benefit to the patient. Either the condition was not present prior to exposure to test medication, or it has worsened in intensity or frequency following exposure to test medication. Adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 4. A copy of the CTCAEv4 can be downloaded from the CTEP home page (http://ctep.cancer.gov).

12.2 Expedited Adverse Event Reporting to the FDA

Per CFR 312.32 (c), the sponsor of the IND, Steven DuBois, must notify the FDA and all participating investigators in a written IND safety report of any adverse experience **associated with use of the drug** that is **both serious and unexpected.** Each written notification shall be made as soon as possible, and in no event later than **15 calendar** days after the sponsor's initial receipt of the information. Each written notification may be submitted on FDA Form 3500A (MedWatch) or in a narrative format and must bear prominent identification of its contents, i.e., "IND Safety Report" with an associated 1571 form that will be consecutively numbered. Follow-up information to a safety report should be submitted as soon as the relevant information is available.

The sponsor must also notify FDA **by telephone** or by **facsimile** transmission of any **unexpected fatal or life-threatening experience associated with use of the drug** in the clinical studies conducted under the IND as soon as possible but in no event later than **7 calendar** days after initial receipt of the information.

Each telephone call or facsimile transmission to the FDA shall be transmitted to the FDA division that has responsibility for review of the IND. Following each facsimile transmission of an expedited adverse event report to the FDA, a hard copy version of the report shall be transmitted to the FDA division along with an amended 1571.

12.3 NANT Operations Center Role in Expedited Adverse Event Reporting to the FDA

For purposes of this protocol, the expedited adverse event reports will be submitted to the FDA as above by the NANT Operations Center on behalf of the IND sponsor, Steven DuBois. The NANT Operations Center will also disseminate all expedited adverse event reports with NANT investigators, DraxImage, NDP, and Merck. The IND sponsor, Steven DuBois, has also delegated to the NANT Operations Center the telephone/facsimile FDA notification responsibilities for unexpected fatal or life-threatening experiences.

A cover letter to accompany expedited adverse event reports will be prepared by the NANT Operations Center in collaboration with the IND sponsor, Dr. DuBois. Contents will include:

1. An assessment of the adverse event, its significance/relevance to the study, and impact on the risk/benefit ratio of the study.
2. A statement as to whether this adverse event has been reported previously, and if so, whether the frequency is considered unusually high.
3. A statement as to whether the protocol and/or informed consent should reflect changes in the potential risks involved.

All IND submissions will be maintained in a master file at the NANT Operations Center.

12.4 NANT Site Investigator Role in Expedited Adverse Event Reporting

For any serious adverse event, both expected and unexpected:

- Contact the Study Chairperson and the NANT Operations Center to alert them to the existence of the serious adverse event within 1 working day, or 24 hours, of learning of the event.
- Within 2 working days, or 48 hours, of learning of the event complete the NANT SAE form (see NANT website www.nant.org under protocol data forms/generic forms packet) and email to NANTstaff@chla.usc.edu.
- Follow-up information should be submitted as as an amended NANT SAE report as soon as relevant information is available.

Copies of all serious adverse event reports will be kept on file in the NANT Operations Center

All NANT institutions are to file internal and external AE reports with their Institutional Review Boards according to local institutional policy.

12.5 Additional Adverse Event Reporting Requirements for Merck & Co, Jubilant DraxImage and NDP

12.5.1 Merck & Co

The NANT Operations Office will provide Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) with copies of all serious adverse experiences within two working days for patients randomized to Arm C. Additionally, NANT has agreed to report any pregnancy occurring in association with use of vorinostat to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220). Details of pregnancy outcomes will also be reported. Serious adverse experiences will be reported using MedWatch forms.

NANT will also provide Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) with details of any vorinostat overdoses. For the purposes of this overdose reporting, a vorinostat overdose will be considered any of the following:

- Receipt of a duplicate dose on any protocol day;
- Receipt of more than 14 days of vorinostat therapy;
- Receipt of daily doses more than 30% higher than prescribed;
- Receipt of an absolute daily dose of more than 400 mg.

12.5.2 Jubilant DraxImage

The NANT Operations Office will provide Jubilant DraxImage, Inc with copies of all serious adverse experiences associated with DraxImage's MIBG product within two working days. Serious adverse experiences will be reported using MedWatch forms.

12.5.3 NDP

Dr. Katherine Matthay is the IND holder for the use of NDP's MIBG product. The NANT Operations Office will provide Dr. Matthay with copies of all serious adverse experiences associated with NDP's MIBG product within two working days. Serious adverse experiences will be reported using MedWatch forms.

12.5.4 Additional NANT Obligations

The NANT Operations Office will also provide Merck, Jubilant Draximage and Dr. Matthay with a copy of all FDA Annual Progress Reports and bi-annual study progress reports. FDA Annual Progress Reports will cross-reference the Merck, DraxImage and NDP Investigational Compound Number (IND) at the time of submission.

12.6 Definitions of Adverse Event Terminology

Adverse Event (AE): An adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Reasonable possibility means there is evidence to suggest a causal relationship between the drug and the adverse event.

Unexpected Adverse Event or Unexpected Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not available, is not consistent with the risk information described in the general investigational plan.

Serious Adverse Events (SAE) or Serious Suspected Adverse Reactions: An adverse event or suspected adverse reaction is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

Death of Patient	An event that results in the death of a patient.
Life-Threatening	An event that, in the opinion of the investigator, would have resulted in immediate fatality if medical intervention had not been taken. This does not include an event that would have been fatal if it had occurred in a more severe form.
Hospitalization	An event that results in an admission to the hospital for any length of time. This does not include an emergency room visit or admission to an outpatient facility.
Prolongation of Hospitalization	An event that occurs while the study patient is hospitalized and prolongs the patient's hospital stay.
Congenital Anomaly	An anomaly detected at or after birth or any anomaly that result in fetal loss.
Persistent or Significant Disability/ Incapacity	An event that results in a condition that substantially interferes with the activities of daily living of a study patient. Disability is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (*e.g.,* sprained ankle).
Important Medical Event Requiring Medical or Surgical Intervention to Prevent Serious Outcome	An important medical event that may not be immediately life-threatening or result in death or hospitalization, but based on medical judgment may jeopardize the patient and may require medical or surgical intervention to prevent any of the outcomes listed above (*i.e.,* death of patient, life-threatening, hospitalization, prolongation of hospitalization, congenital anomaly, or persistent or significant disability/incapacity). Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

12.7 Reporting Secondary AML/MDS or Any Other Second Malignancy

Within two working days of a diagnosis of AML/MDS or other secondary malignancy following treatment for cancer, submit the following to the NANT Operations Center:

- A completed NANT SAE Form
- A copy of the pathology report confirming the AML/MDS or other malignancy
- A copy of the cytogenetics report (if applicable)

The NANT Operations Center will report the secondary malignancy to the FDA via MedWatch form and to Steven DuBois (IND Sponsor), Draximage (if their product), NDP (if their product; via Dr. Matthay, IND holder for NDP's MIBG product for use in neuroblastoma), and Merck (if on vorinostat arm of the trial). Reporting to Merck will be within two working days, as for any SAE.

13.0 RECORDS AND REPORTING

See separate Data Forms Packet which includes the data submission schedule in the members section on the NANT web site (www.NANT.org)

The following are required to be submitted for all patients entered:

1. Study case report forms, bone marrow reports (including aspirate and biopsy reports), and radiology reports (CT/MRI/MIBG scans). These forms and reports are faxed to the NANT Operations Center at 323-361-1803 or sent by email as electronic files to nantdata@chla.usc.edu at the end of each course of therapy (day 43-50 evaluation).
2. For **all** patients on study, CT/MRI and MIBG scans done as baseline tumor evaluation at study entry are required to be submitted into the NANT PACS system or on CD for central review (after approval of NANT Operations Center). Bone marrow slides from study entry may also be requested at the discretion of the Operations Center.
3. For all patients the MIBG scans done at day 43-50 will also be submitted for central review. The MIBG scans should be submitted after day 43-50 evaluation. For patients who report a CR/PR/SD on CT/MRI, scans done at day 43-50 should be submitted for central review after day 43-50 evaluation. In questionable cases, the Operations Center may request CT/MRI scans for central review. It is preferable that scans be submitted electronically into the NANT PACS system. However, when this is not possible scans can be submitted on CD to NANT Operations Center.
4. For patients who report a CR or CR-MRD in bone marrow, bone marrow slides will be submitted from study entry and from day 43-50 evaluation, and from the second bone marrow confirming CR if applicable.

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15.0 SAMPLE INFORMED CONSENT

NANT 2011-01: STUDY OF SINGLE-AGENT ^{131}I-MIBG, ^{131}I-MIBG WITH VINCRISTINE AND IRINOTECAN, OR ^{131}I-MIBG WITH VORINOSTAT FOR RESITANT/RELAPSED NEUROBLASTOMA

A New Approaches to Neuroblastoma Therapy (NANT) treatment protocol.

The word "you" used throughout this document refers to you or your child.

WHAT IS THIS STUDY ABOUT?

This study is a clinical trial, a type of research study. Clinical trials include only patients who choose to take part. Please take your time to make your decision about participating. You may discuss your decision with your friends, family, and health care team. If you have any questions, you may ask your study doctor.

You are being asked to participate in this study because you have been diagnosed with neuroblastoma, a type of solid cancer that usually affects children. Your cancer has either grown back (relapsed) or has never gone away (persistent tumor) after standard treatment. Standard treatment may have included chemotherapy, surgery, radiation therapy and/or high-dose chemotherapy with a stem cell transplant.

The purpose of this study is to compare three different treatments aimed at maintaining or improving your response to previous treatments. This study involves the use of an investigational medicine called Metaiodobenzylguanidine (MIBG) alone and in combination with either Irinotecan and Vincristine or Vorinostat. These combinations are also investigational, though the dose and side effects of these combinations have been tested in previous clinical trials.

Once you are registered on this study, you will be randomly assigned to one of three treatments.
You will either receive ^{131}I-MIBG by itself or, ^{131}I-MIBG with Vincristine and Irinotecan, OR ^{131}I-MIBG WITH Vorinostat. Patients on all treatment arms will receive the same dose of ^{131}I-MIBG. Patients on all treatment arms will receive blood stem cells as part of this therapy.

Random assignment means that the treatment to which a patient is assigned is based on chance. It is like a flip of a coin, except assignment is done by a computer. Neither you or your doctor will choose which treatment you will receive. All patients will have an equal chance of being placed in either treatment group. During the study, if fewer patients are responding to one treatment arm, then that treatment arm will close early and only the two best treatment arms will continue.

The three treatment arms are the following:

^{131}I-MIBG Alone:
MIBG is taken up by neuroblastoma tumor cells. MIBG can be combined together with radioactive iodine (^{131}I) in the laboratory to form the radioactive compound ^{131}I-MIBG. The ^{131}I-MIBG compound delivers radiation treatment to the neuroblastoma cancer cells throughout the body.

^{131}I-MIBG with irinotecan and Vincristine:
This study will combine the two chemotherapy drugs, irinotecan and vincristine, that have been used together in some patients with neuroblastoma with the medicine called Metaiodobenzylguanidine (MIBG). The irinotecan / vincristine will be given during the same hospital stay as the ^{131}I-MIBG. Giving the chemotherapy together with the ^{131}I-MIBG may increase the number of patients who respond to ^{131}I-MIBG.

^{131}I-MIBG with Vorinostat:
Vorinostat is a drug that is FDA-approved to treat a certain type of cancer mainly seen in adults. Vorinostat affects the way the DNA that carries our genes is folded in cells. In the laboratory, vorinostat causes neuroblastoma cells to stop growing. This effect is even greater when vorinostat is combined with radiation. Giving vorinostat together with the ^{131}I-MIBG may increase the number of patients who respond to ^{131}I-MIBG.

WHY IS THIS STUDY BEING DONE?

The purposes of this study are:

- To find out which of the three ^{131}I-MIBG therapies have a better tumor response rate after one treatment course.
- To compare the side effects seen by giving ^{131}I-MIBG alone, and ^{131}I-MIBG in combination with Vincristine and Irinotecan and ^{131}I-MIBG with Vorinostat
- To describe the describe which of the three ^{131}I-MIBG treatment regimens has the best tumor effect after two courses, including response in the bone marrow, MIBG lesions, soft tissue lesions and overall survival.
- To describe how many patients who agree to send blood and bone marrow specimens for a special testing are found to have tumor cells by this new more sensitive test (called NB5 assay by TLDA) after the each of the three ^{131}I-MIBG treatment regimens
- To compare the exposure of whole body radiation from ^{131}I-MIBG received on the three regimens using radiation measurements and blood markers of radiation.
- To learn about a new computerized way of reading MIBG scans.
- To learn whether the amount of a certain protein on neuroblastoma cells impacts whether a patient responds to ^{131}I-MIBG therapy or not.

The research is being done because:

Currently there is no known effective treatment for your type of cancer.

This study will compare three ^{131}I-MIBG treatment regimens that have already been used for treatment of neuroblastoma and compare their effects on tumor response and associated side effects. We are trying to see if one therapy is better for people with neuroblastoma.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

A total of 105 patients are expected to enroll on this study. There will be 35 patients on each arm. All patients will be assigned randomly.

WHAT WILL HAPPEN TO ME IF I TAKE PART IN THIS STUDY?

<u>Medical Tests Before You Begin the Study</u>

You will need to have the following exams, tests or procedures to find out if you can be in the study. These exams, tests or procedures are part of regular cancer care and may be done even if you do not join the study. These tests will also be done at various times throughout the study and at the end of the study. The purpose of these tests is to see how well the treatment works and to measure the status of your neuroblastoma. If you have had some of them recently, they may not need to be repeated. This will be up to your study doctor.

Physical exam	Bone marrow tests[#]
Blood tests	Various scans*
Pregnancy test	Echocardiogram and EKG to check the heart function
Urine tests	Test of kidney function (laboratory testing)

[#] Bone marrow tests are done by inserting a needle into the hip bone to remove the marrow which is inside the bone.

* Various scans that are done for diagnosis and checking the response of the tumor to treatment. These may include CT and /or MRI scans and MIBG scans. We will recommend scans specific for your case and we will answer your questions about these scans**.**

During the Study

If the exams, tests and procedures show that you can be in the study, and you choose to take part, then you will need the following tests and procedures during the study. They are part of regular cancer care.

Physical exam	Bone marrow tests
Blood tests and scans	Various tests
Pregnancy test	Echocardiogram and EKG to check the heart function
Urine tests	Test of kidney function (laboratory testing)

You will also be expected to join a companion study to collect blood, bone marrow, and tumor tissue (if available) from patients with neuroblastoma. Your study doctor will talk with you about this study.

Treatment Plan

Before you can get treatment on this study, blood stem cells must be available that meet the study requirements. We will check your child's previously stored stem cells to make sure that they could be used for your child's stem cell infusion.

Patients will then be randomized at study entry to one of three treatment arms. Patients on Arm A will receive a single treatment course with ^{131}I-MIBG alone. Patients on Arm B will receive a single treatment course with ^{131}I-MIBG, vincristine, and irinotecan. Patients on Arm C will receive a single treatment course with ^{131}I-MIBG and vorinostat. After this course of treatment, we will check to see your response and then check to see how you are doing over time.

A map of one treatment course on each treatment arm is shown here:

TREATMENT ARM A: ^{131}I-MIBG ALONE:

Days	1	15	43-50
Therapy	M	HSC	Eval

^{131}I-MIBG	M	Day 1
Stem Cell Infusion	HSC	Day 15
Evaluation	Eval	Day 43-50

TREATMENT ARM B: ^{131}I-MIBG + VINCRISTINE/IRINOTECAN:

Days	-1	0	1	2	3	4	5	6	15	43-50
Therapy	C	C VCR I	C I M	C I	C I	C I	C	C	HSC	Eval

Cefixime	C	Days -1 through Day 6
^{131}I-MIBG	M	Day 1
Irinotecan	I	Days 0 through 4
Vincristine	VCR	Day 0
Stem Cell Infusion	HSC	Day 15
Evaluation	Eval	Day 43-50

TREATMENT ARM C: ^{131}I-MIBG + Vorinostat

Days	-1	0	1	2	3	4	5-12	15	43-50
Therapy	V	V	V M	V	V	V	V	HSC	EVAL

Vorinostat	V	Days -1 through +12 (14 total days)
^{131}I-MIBG	M	Day 1
Stem Cells	HSC	Day 15
Evaluation	Eval	Day 43-50

Treatment with ^{131}I-MIBG

Treatment with ^{131}I-MIBG will be done at a hospital that is set up to take care of patients that are treated with radioactive substances. This means that you may need to travel some distance to another hospital to get this treatment. Your doctor will talk with you about where the different hospitals are that can give the ^{131}I-MIBG treatment. Your nurse and other members of the team that take care of you can help you plan for the trip to get this treatment.

Patients will be admitted to an ^{131}I-MIBG treatment center one day before starting MIBG therapy. On the following day (Day 1), ^{131}I-MIBG is given into a temporary IV or in your central venous catheter over 90-120 minutes. IV fluids for hydration and other medicines will be given through your central venous catheter.

Patients who get ^{131}I-MIBG are considered to be “hot” or radioactive and special precautions are taken to care for you during this time until the radiation level has gone down to a level where these precautions are no longer needed (usually 4 - 5 days). Special care precautions include:

- A single room in a bed surrounded by a lead shield to keep family and the staff who take care of you from being exposed to radiation from the ^{131}I-MIBG treatment. This usually takes about 5 days.
- The length of time family can visit inside the room in front of the protective lead shield that is around your bed will depend on how much radiation is measured in the room each day by the radiation specialist. Usually family can visit for a total of 30-45 minutes on the first day and longer on the days after that because there will be less radiation measured in the room each day.
- Family may visit anytime outside of the room or behind a lead shield. You will be able to see who is visiting over this shield.

- No one will be able to spend the night in this special room with you during this time.

Your urine will be radioactive after treatment with ^{131}I-MIBG. A urinary catheter will be inserted through your urethra into the bladder to drain the radioactive urine from your body. This catheter will be removed 3 –5 days following the treatment.

You will also take a medicine by mouth (potassium iodide) to prevent thyroid damage from the radioactive iodine contained in the ^{131}I-MIBG compound. The medicine will be taken together by mouth beginning before treatment and continuing for a total of 6 weeks.

Treatment with Vincristine and Irinotecan (Treatment Arm B only)

If you are randomized to **treatment arm B**, you will receive vincristine and irinotecan in addition to the ^{131}I-MIBG. All patients will get the same irinotecan and vincristine dose.

Before chemotherapy starts on this arm, you will be given the antibiotic Cefixime (on Day -1) and will continue taking the Cefixime for a total of 8 days (until Day +6). Cefixime can be taken in tablet or liquid form. The purpose of the Cefixime is to reduce your chance of having bad diarrhea while getting the irinotecan. If there is trouble getting Cefixime, another antibiotic called cefpodixime can be used instead. After one day of Cefixime, you will start taking irinotecan and vincristine.

Irinotecan is given daily for 5 days in a row (Day 0 through Day 4). Irinotecan is given each day by IV over 60-90 minutes. Vincristine is given by IV over 5-15 minutes only on the first day irinotecan is given (Day 0). Because the most common side effect of irinotecan is diarrhea, you will be instructed to take Loperamide (Imodium) after the first loose stool. You will be given written instructions on how much and how often this medicine should be taken. Additional medicines will be used if the diarrhea is severe.

The day after vincristine and the first dose of irinotecan are given, you will be treated with ^{131}I-MIBG (Day 1).

Treatment with Vorinostat (Treatment Arm C only)

If you are randomized to **treatment arm C**, you will receive vorinostat in addition to the ^{131}I-MIBG. The vorinostat is given by a pill or a liquid depending upon your size. If you cannot take medicines by mouth, the vorinostat can be given into a tube placed in the nose or stomach. All patients enrolled will get the same Vorinostat dose. You will receive vorinostat orally once daily for 14 days (Days -1 to +12). Two days after starting Vorinostat, ^{131}I-MIBG will be given (Day 1).

All Treatment Arms:
All patients on this study will receive the same dose of ^{131}I-MIBG on Day 1 and autologous blood stem cell infusion on Day 15. You will receive your stored stem cells back by vein two weeks after the ^{131}I-MIBG infusion. This may be done as a day visit to the hospital or may require a brief (one night) stay in the hospital. The stem cells can be given at a NANT hospital, or at a pediatric transplant center that is more convenient for you that has been approved by your NANT doctor.

If you develop a low white blood count, your may receive G-CSF (Neupogen) or pegfilgrastim in order to help your white blood cells recover faster after treatment. G-CSF (or pegfilgrastim) will be given either as an under the skin (subcutaneous) shot or through your IV (intravenous) once a day. Pegfilgrastim, if given, is usually given as a one-time under the skin (subcutaneous) shot. You may also get a dose (or doses) of one of these medicines after you have received your stem cells, even if your blood counts are not low yet. Your doctor will let you know if you will receive GSCF or pegfilgrastim, if needed.

You can receive this therapy once as part of this study.

When you have finished treatment on this study:

After you stop treatment on your assigned treatment arm, you will continue to have tests and scans done (listed below) to measure how much tumor is left. If test results show you have abnormal organ functions,

tests will be repeated monthly until test results are stable or normal. You doctor will tell you how often these tests and evaluations will be done.

Medical Tests after the Study:

	Bone marrow tests#
Physical exam	
Blood tests	Various scans*
Urine tests	Pregnancy Testing
	Echocardiogram and EKG to check the heart function

A table detailing the tests and procedures required before, during and after the study has been attached to the end of this consent.

Optional Tests in this Study

You will be asked if you want to participate in the following tests that are being done to learn more about ^{131}I-MIBG therapy. **This part of the study is voluntary.** The results of these tests would not be told to you or your doctor or become part of your medical record. These results would also not be used to make decisions about your care while enrolled on this study. You can decide not to let the doctors do these tests and will still be able to be treated as part of this clinical study. There are checkboxes on the next to last page of this consent form to mark whether you are willing to participate in these voluntary studies.

- **Evaluation of Blood Markers of Radiation Exposure**

  Part of the research goal for this study is to look at changes in certain proteins and genes in the blood before and after you have been treated with on each arm of this study**.** For this test, 12.5 mL of blood (2 and a half teaspoons) will be taken 4-5 times during the course of therapy to measure certain proteins and genes in the blood cells. You will not need separate clinic visits to participate in this part of the study. The total amount of blood drawn for testing will be about 62.5 mL (about 12.5 teaspoons) over approximately 16-17 days. The blood can be drawn or taken from your central line (or port). This amount of blood drawn over this time period is considered safe to donate. Samples will be sent to Dana-Farber and Boston Children's Hospital in Boston for research testing.

- **Evaluation of Transport Protein on Neuroblastoma Cells**

  Part of the research goal for this study is to look to see if the amount of a specific transport protein on neuroblastoma cells corresponds to response to ^{131}I-MIBG therapy. If you give permission, 5 pathology slides from previous tumor biopsies or tumor surgeries will be sent to Dana-Farber and Boston Children's Hospital in Boston for research testing. Only tumor material already collected will be used and no new biopsies will be performed to help with this research testing.

- **Evaluation of Automated MIBG Scoring System**

  On this study, a new scoring system for MIBG scans will be evaluated using MIBG scans from patients who are willing to participate. You will not need to get extra MIBG scans to participate in this portion of the study. If you agree, your scans will be forwarded to the University of Chicago for this automated MIBG scoring. The researchers in Chicago will not have access to the reports from your treating institution.

HOW LONG WILL I BE ON THIS STUDY?

You will receive a single course of treatment on this study.

After you stop treatment, you will continue to have tests and scans done to measure how much tumor is left. Your doctor will tell you how often these tests will be done. Researchers will continue to collect information about you for a lifetime. Information will be collected about whether you are still alive; whether your tumor has grown back and at what sites in the body; whether you have developed any side effects from the treatment; or whether you have developed any additional cancer. Your oncologist or family doctor will give the researchers this information at regular intervals.

CAN I STOP BEING IN THE STUDY?

Yes. If you are thinking about stopping the study, you should talk to your doctor before making a final decision so he/she can tell you how to do this safely. There are certain time points in the study where it would be strongly recommended that you complete the medical supportive care required to avoid very bad and/or fatal side effects.

- Once you have gotten ^{131}I-MIBG treatment, you will stay in the special room until you are no longer radioactive (usually 5 days), since you could expose others to radiation.

- Once you have gotten ^{131}I-MIBG treatment, it would be strongly recommended that you complete the medical supportive care needed to avoid very bad and/or fatal side effects. This includes the stem cell infusion and the potassium iodide for thyroid protection.

The study doctor may stop you from taking part in this study at any time if he/she believes it is in your best interest; if you do not follow study rules; or if the study is stopped.

WHAT ARE THE RISKS OF THE STUDY?

This study looks at how common and serious side effects can be for each patient at a specific dose of a drug. In this type of study, some patients may have very serious side effects and could die as a result of these side effects. For example, in rare instances, patients treated with ^{131}I-MIBG for neuroblastoma or other diseases have died due to liver or lung damage. You may be one of those patients who has serious side effects as a result of participating in this study.

In this study, researchers will be looking at side effects and tumor responses seen in patients all taking ^{131}I-MIBG with different treatment combinations. Since subjects will be assigned to different treatment arms, consisting of either ^{131}I-MIBG alone or in combination with irinotecan/vincristine or vorinostat subjects will have differing tumor responses and side effects. The goal of this study is to see which treatment arm has the best response for your neuroblastoma while comparing the side effects of the different treatments.

Everyone taking part in the study will be watched carefully for any side effects. However, doctors don't know all the side effects that may happen. Side effects may be mild or very serious. Other drugs may be given to make side effects less serious and more comfortable (such as for nausea, headache or itching). Many side effects go away soon after you stop taking the therapies, but it is always possible that side effects can be serious, long lasting or may never go away. There is also a risk of death, including death from bleeding or infection due to low blood counts. Patients are watched carefully and treatment will be stopped if bad side effects develop. There may also be risks we do not know about. You should talk to your doctor about any side effects that you have while taking part in this study.

While on the study, you are at risk for the side effects listed on the following pages.

Possible side effects of ^{131}I-MIBG

Likely (happens to 21-100 children out every 100 children)	**Less Likely** (happens to 5-20 children out every 100 children)	**Rare** (happens to < 5 children out every 100 children)
• Decrease in the number of red and white blood cells and platelets made in the bone marrow. You may need blood and platelet transfusions and sometimes stem cell infusions are necessary. (Stem cells are required on this trial) The dose of ^{131}I-MIBG used in this study will lower your blood counts. • Nausea • Dry mouth • Increase in blood marker of salivary gland irritation (your serum amylase will increase)	• Decreased function of the thyroid gland. This causes tiredness (fatigue), weight gain, constipation, and lower blood pressure. Treatment for life with a medicine to supplement the thyroid gland (i.e. Synthroid or related thyroid supplement) may be needed. • Not being able to get pregnant or father a child • High or low blood pressure during or after the ^{131}I-MIBG infusion • Thinning of the hair • Vomiting • Infection due to low white blood cells • Fatigue due to low red blood cells • Bleeding/bruising due to low platelets • Loss of appetite	• Pain in salivary glands or mouth • Decreased function of adrenal gland. This affects your activity level and growth. It causes tiredness (fatigue), weight changes and blood pressure changes. You may need to take medicine to supplement the adrenal gland. • Decreased heart function • Irritation of the liver. Because some of the radioactive ^{131}I-MIBG is taken up by the liver, there is a possible risk of future liver damage from the ^{131}I-MIBG alone. • Second cancer (such as leukemia) that is different from the kind of cancer you have now • Trouble breathing due to infection or damage to the lung • Overactive thyroid gland

Possible risks from having a bladder catheter placed
In order to safely receive MIBG therapy, you will need to a have a tube or catheter called a Foley catheter temporarily placed in your bladder. The catheter may cause you some discomfort and may increase your risk for getting a bladder infection.

Possible side effects of Potassium Iodide

Likely (happens to 21-100 children out every 100 children)	**Less Likely** (happens to 5-20 children out every 100 children)	**Rare** (happens to < 5 children out every 100 children)
	• Gastrointestinal distress (nausea / vomiting / diarrhea / stomach pain)	• Tingling, pain or weakness in arms and legs • Flare up of acne in teenagers • Irregular heartbeat • Confusion • Tiredness • Fever • Allergic reaction (hives) • Burning of mouth / throat • Metallic taste • Rash • Decreased function of the thyroid gland with overuse • Swelling of lymph glands

This medication is given for 45 days after the ^{131}I-MIBG infusion to protect your thyroid gland.

Possible side effects of Vorinostat

Likely (happens to 21-100 children out of every 100 children)	Less Likely (happens to 5-20 children out every 100 children)	Rare (happens to < 5 children out every 100 children)
• Low blood counts, including low red blood cells (which can cause fatigue or pale appearance) and platelets (which can cause bruising or bleeding). • Fatigue • Poor appetite • Diarrhea • Nausea • Vomiting	• Decrease in specific types of blood cells–(white blood cells which can cause increased risk of infection • Weight loss • Effects on blood coagulation lab tests • Decreased kidney function • High blood sugar • Fever, with or without low blood counts • Chills • Hair loss • Constipation • Dehydration • Dry mouth • Heartburn • Taste changes • Infection • Low protein in the blood (albumin) • Changes in liver blood tests • Changes in blood salts • Muscle spasm or weakness • Dizziness • Abdominal pain • Cough • Shortness of breath • Blood clot	• Skin breakdown • Changes in a specific part of the heart tracing known as an EKG. (Mild changes in a specific part of the heart tracing known as an EKG has been rarely reported in patients treated with vorinostat, though it is not clear whether vorinostat caused these changes or not.)

Other side effects have been reported in patients receiving vorinostat, but it is unknown whether the vorinostat caused the side effect. These side effects include: irregular heart rhythm; heart attack; blurry vision; trouble swallowing; gassiness; mouth sores; gum pain; swelling of arms/legs; trouble walking; chest pain; low blood sugar; aches and pains; tumor pain; trouble talking; changes in a blood test that measures blood clotting factors; headache; increased pressure in the brain; bleeding into the brain; stroke; confusion and memory changes; decreased skin sensation; fainting; tremor; anxiety; blood and/or protein in the urine; difficulties with urination; bleeding into the lungs; nose bleeds; trouble sleeping; stuffy nose; throat pain; increased sweating; nail changes; itchiness; other rashes; flushing; bruising; changes in blood pressure; and inflammation of blood vessels.

Possible side effects of Irinotecan

Likely (happens to 21-100 children out of 100)	**Less Likely** (happens to 5 -20 children out of 100)	**Rare** (happens to less than 5 children out of 100)
• Diarrhea (can be immediate) and may be associated with abdominal cramping, a runny nose, tearing, salivation, sweating, flushing (feeling of warmth and red cheeks), and difficulty adjusting your eyes to light. • Inflammation and/or sores in the mouth • Nausea and vomiting • Stomach pain • Loss of appetite • Fever • Loss of body water • Loss of strength and energy • An increase in the blood of a type of white blood cell called an eosinophil. These are sometimes associated with allergic reactions. • Elevation of the liver and bone enzymes in the blood and of bilirubin (yellow pigment formed in the liver) • Decrease in the number of red and white blood cells and platelets made in the bone marrow • Hair loss	• Increased blood tests for liver and kidney function • Constipation • Diarrhea that may occur later from 1 day to 2 weeks after irinotecan which could cause excessive loss of water and salts from the body • Inflammation and/or sores in the mouth, throat and/or esophagus • Headache • An upset stomach • Fewer red blood cells and platelets in the blood • Rash • Blood-clots**. This is seen when Irinotecan is given with other drugs not used in this treatment plan.	• Skin irritation and/or inflammation • Headache • Dehydration • Trembling • Blood in the urine • Mildly increased level of protein and glucose in the urine • Low amount of protein in the blood • Mouth sores • Allergic reaction, that may be life threatening. • Dizziness and low blood pressure • Sensation of warmth on face • Confusion and/or disorientation • Pain at the infusion site • Slow heart beat • Inflammation of the large intestine • Intestinal blockage • Inflammation of the lungs with cough and congestion • Risk to the unborn child in pregnant patients *

* Birth defects and other serious abnormalities in the unborn baby have been noted with irinotecan in animal studies at doses similar to or less than those used in humans. The timing and frequency of these effects is as yet unknown. These may include multiple birth defects and abnormalities of bone formation,
** This toxicity is seen more commonly when irinotecan is given in combination with fluorouracil and leucovorin. It may rarely be a life threatening event.
small size of baby at birth and increased risk of death of the unborn baby. Irinotecan is excreted in rat milk but this is unknown for humans.

Possible side effects of Vincristine (Oncovin):

Likely (happens to 21-100 children out of 100)	**Less Likely** (happens to 5 -20 children out of 100)	**Rare** (happens to less than 5 children out of 100)
• Hair loss • Constipation • Reversible nerve problem that may affect the way you walk or the feelings in your fingers and toes	• Jaw pain • Headache • Feeling weak (in muscles) • Belly pain and bloating • Low blood counts that is mild and doesn't last very long • Changes in feeling and sensation in the hands and feet including numbness, tingling and pain, clumsiness, wrist drop, foot drop, abnormal walking with foot slapping	• Local damage to nearby tissue if vincristine leaks out of the vein and into the skin • Difficulty breathing • Your intestine stops working properly which can lead to a blocked intestine. • Drooping eyelids, double vision, and trouble seeing at night • Hoarse voice • Vocal cord paralysis • Low levels of body salts • Seizures • Defective sweating • Trouble walking or not being able to walk • Liver damage when used together with other chemotherapy drugs • Damage to the nerves to the eye leading to decreased vision and possible blindness • Trouble urinating, pain with urination, or having to urinate more often • Low blood pressure when going from sitting down to standing up which can make you dizzy. • Nerve damage with dizziness & a spinning sensation, uncontrolled eye movements and hearing loss

Possible side effects of Cefixime:

Likely (happens to 21-100 children out of 100)	**Less Likely** (happens to 5 -20 children out of 100)	**Rare** (happens to less than 5 children out of 100)

	• Diarrhea
	• Belly pain
	• Nausea and vomiting
	• Indigestion
		• Headache
		• Dizziness
		• Seizures
		• Allergic reaction
		• Hypersensitivity reactions
		  ◦ Low white blood cell, neutrophil and platelet counts,
		  ◦ High eosinophil count (one type of white blood cell),
		• Irritation of the colon
		• Increased blood tests of kidney and liver function
		• Hepatitis, yellowing of skin and whites of eyes
		• Redness and irritation of the skin and sometimes the eyes, lips and mouth. There can also be skin peeling.

Possible side effects of Cefpodoxime (Vantin-R)

Likely (happens to 21-100 children out of 100)	**Less Likely** (happens to 5 -20 children out of 100)	**Rare** (happens to less than 5 children out of 100)
	• Diarrhea • Diaper rash	• Belly pain • Nausea and vomiting • Headache • Seizures • Anaphylaxis • Chest pain • Hypersensitivity reactions • Low white blood cell, neutrophil and platelet counts, • High eosinophil count (one type of white blood cell), • Pseudomembranous colitis: causing abdominal cramping watery diarrhea with bloody stools, fever and a feeling of needing to go to the bathroom • High blood tests for liver and kidney function • Change in blood tests for blood clotting factors (prolonged) • Chest pain • Vaginal infection • Bone marrow failure: bone marrow does not produce enough new cells to replenish blood cells • Redness and irritation of the skin, eyes, lips and mouth. There can also be peeling of skin (a little to a lot)

Possible side effects loperamide (Imodium):
Sleepiness, trouble thinking, constipation, and nausea,

Possible side effects of G-CSF (Neupogen)
G-CSF is not an anti-cancer medicine. It helps the growth of white blood cells that fight infection.

Likely (happens to 21-100 children out every 100 children)	**Less Likely** (happens to 5-20 children out every 100 children)	**Rare** (happens to < 5 children out every 100 children)
• Bone pain	• Pain or irritation at injection site • Increased blood tests for alkaline phosphatase, LDH and uric acid • Low platelet count • Fever	• Allergic reactions (more common with giving the drug IV than as an injection under the skin) • Skin rash, itching, puffiness in the face • Shortness of breath or wheezing • Low blood pressure, fast heart rate • Low grade fever • Enlargement of the spleen. • Rupture of the spleen • Worsening of existing skin rashes • Sickle cell crises in patients with sickle cell disease • High white blood cell count in the blood • Irritation of veins in the skin • Adult respiratory distress syndrome (life-threatening lung condition that prevents enough oxygen from getting to the lungs and into the blood) • Bone marrow dysfunction (MDS) or secondary leukemia in patients with very bad ongoing neutropenia (not as seen in cancer patients) and long term administration.

Possible side effects of Pegfilgrastim:
Pegfilgrastim is not an anti-cancer medicine. It helps the growth of white blood cells that fight infection

Likely (happens to 21-100 children out every 100 children)	**Less Likely** (happens to 5-20 children out every 100 children)	**Rare** (happens to < 5 children out every 100 children)
• Bone pain	• Pain or irritation at injection site • Headache • Increased blood tests for alkaline phosphaste, lactate dehydrogenase and uric acid • Low platelet count	• Low grade fever • Allergic reactions • Generalized redness and flushing • Enlarged spleen or rupture of the spleen splenic rupture • Pain or other crises in patients with sickle cell disease (SCD) • High white blood cell count in the blood • Adult respiratory distress syndrome (life-threatening lung condition that prevents enough oxygen from getting to the lungs and into the blood.) • Sweet's Syndrome

Possible side effects associated with stem cells

- ANY TIME BEFORE STEM CELL INFUSION: The freezer where PBSC are stored could malfunction, the container holding them could break and the stem cells could be damaged so they could not be used. This is expected to be an extremely rare event. However, if this occurs, another stem cell collection may be attempted or the back-up stem cells (if available) may be used if they were not damaged.

- If stem cells needed to be shipped from one location to another, they could be lost or damaged during shipping such that they could not be used. This is expected to be an extremely rare event. If this occurs, another stem cell collection may be attempted or the back-up stem cells, if available, may be used.

- Some patients may need extra fluids given into the vein after getting their stem cells. This is to protect the kidneys from the red blood cells mixed in with the stem cells.

Possible side effects of stem cell infusion

Likely (happens to 21-100 children out every 100 children)	**Less Likely** (happens to 5-20 children out every 100 children)	**Rare** (happens to < 5 children out every 100 children)
	• Fever and chills	• Allergic reaction. Can cause difficulty breathing and low blood pressure. • High blood pressure • Infection • Infusion of tumor cells. Tumor cells may still be present in the harvested stem cells and they could regrow after stem cells are infused. • Cough • Chest Tightness • Flushing • Nausea and/or vomiting • Rash • Irregular Heart Beat

Possible risks to unborn child

Patients who agree to participate in this study should not become pregnant while on this study. This study and the medicines used in this study may be hazardous to an unborn child. Patients and their sexual partners should avoid sex and /or an effective method of contraception that is medically appropriate based on your personal doctor's recommendation at that time.

Possible risks to the caregiver(s) of the patient getting MIBG treatment

Caregivers (example: parent, other family member, guardian, friend, partner) will be exposed to radiation while you are being treated with MIBG. Caregivers who could possibly become pregnant during this time need to avoid contact with the patient because the radiation exposure may increase the unborn baby's risk of developing cancer or other health problems.

If your caregiver is pregnant, then special precautions should be used to avoid contact with you during and for 4 weeks after getting MIBG treatment. Should your caregiver or your caregiver's sexual partner be found to have been pregnant while you were getting MIBG treatment and did not know it at the time, please contact your doctor immediately.

Possible long-term side effects of this treatment

- Recurrence of tumor
- Infection
- Sterility and/or delayed onset of puberty
- Increased risk of a second cancer (such as leukemia) different from the kind of cancer you have now.
- Patients who have more than one ^{131}I-MIBG treatment will have greater doses of radiation to the normal organs than those patients having one treatment. It is possible that there may be later damage to the normal function of the liver or other organs.

Possible risks from having blood drawn

The risks from having your blood taken are minimal, but can include an infection or a blood clot. Experienced doctors or nurses will perform these blood draws to minimize this risk. . You will be asked to sign a separate consent for any procedure that needs sedation.

Unknown risks

The treatment combinations may have side effects that no one knows about yet. The researchers will let you and your child know if they learn anything that might make you change your mind about participating in the study.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

There may or may not be direct medical benefit to you. The information learned from this study may or may not benefit other children or young people with solid cancers in the future.

WHAT OTHER CHOICES DO I HAVE IF I DO NOT TAKE PART IN THIS STUDY?

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

- Treatment with chemotherapy medicines without MIBG.
- Treatment with other experimental agents that may be available.
- No neuroblastoma therapy at this time, with care to help you feel more comfortable.

Please talk about these options with your doctor.

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.

Organizations that may look at and/or copy your medical records for research, quality assurance and data analysis include:

- NANT Consortium
- Independent auditor evaluating quality assurance for the NANT Consortium.
- The National Cancer Institute (NCI) and other governmental agencies, like the Food and Drug Administration (FDA), involved in keeping research safe for people.
- Nuclear Diagnostic Products (NDP; ~~alternate~~ supplier of ^{131}I-MIBG)
- Draximage (alternate supplier of ^{131}I-MIBG), a division of Draxis Specialty Pharmaceuticals, Inc
- Merck (supplier of vorinostat)

NANT 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 at the end of this consent.

WHAT ARE THE COSTS OF TAKING PART IN THIS STUDY?
Taking part in this study may lead to added costs to your insurance company. Your health insurance company will be billed for many expenses associated with the costs of this study. These expenses include medications, treatments, hospital charges, and doctors' fees related to your participation in this study.

Irinotecan, vincristine, cefixime/cefpodoxime, loperamide, and G-CSF/Pegfilgrastim are commercially available agents. The cost of these drugs will be billed to your child and/or your child's insurance company. The cost is normally covered by your child's insurance company.

Vorinostat will be provided by Merck, the company that makes this drug. However they do not cover the cost of getting Vorinostat ready and giving it to you, so you or your insurance company may have to pay for this.

^{131}I-MIBG will be supplied by Draximage or NDP, two companies that makes this drug, and will be paid for by the NANT consortium. Your insurance company will be charged for the cost of the administration of the drug.

The optional studies will be done at no cost to if you agree to participate in this voluntary study. However, you or your health plan may need to pay for the costs of the supplies and personnel who draw the blood from you for these tests.

You may have to pay for other things during this study, such as but not limited to, your time, the cost of food you buy while you are being treated at the hospital, car fare, travel to and from the hospital for MIBG treatment, parking, and baby sitter fees.

Taking part in this study may lead to added costs that may be covered by your insurance company. Please ask about any expected added costs or insurance problems.

You will not be paid for taking part in this study.

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.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

WHAT HAPPENS IF I AM INJURED BECAUSE I TOOK PART IN THIS STUDY?

It is important that you tell your study doctor, _____ *[investigator's name(s)],* if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ *[telephone number].*

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

WHAT ARE MY RIGHTS AS A STUDY PARTICIPANT?

Taking part in this study is your choice. You may choose not to take part or not take part in the study. If you decide to take part in this study, you may remove yourself from the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. If you remove yourself from the study, we will still take care of you. We will explain what stopping the treatment may do and we will offer other treatments if they are available.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

In case of injury resulting from this study, you do not lose any of you legal rights to seek payment by signing this form.

A Data Safety and Monitoring Board, an independent group of experts, will be reviewing data from this research throughout the study. We will tell you about new information from this Board or other studies that may affect your health or willingness to stay in the study.

WHO CAN ANSWER MY QUESTIONS ABOUT THE STUDY?

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor _____ *[name(s)]* at _____ *[telephone number].*

For questions about your rights while taking part in this study, call the _____ *[name of center]* Institutional Review Board (a group of people who review the research to protect your rights) at _____ *(telephone number).*

WHERE CAN I GET MORE INFORMATION?

You may call the NCI's **Cancer Information Service** at

1–800–4–CANCER (1–800–422–6237) or **TTY: 1–800–332–8615**

You may visit the NCI Web sites at http://cancer.gov/
For NCI's clinical trials information, go to **http://cancer.gov/clinicaltrials/**
For NCI's general information about cancer, go to **http://cancer.gov/cancerinfo/**

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

You will get a copy of this consent form. If you want more information about this study, ask your study doctor.

CONSENTS FOR EXTRA STUDIES FOR RESEARCH

The following tests are optional. You may still participate in the study even if you do not agree to these tests.

Evaluation of Blood Markers of Radiation Exposure

Circle YES, if you agree to let researchers take blood to evaluate blood markers of radiation exposure. These are extra blood draws. The results of these tests will be confidential and not made available to you or your treating physician.

Circle NO, if you do not want researchers to take extra blood samples to evaluate blood markers of radiation exposure.

YES NO

Signature

Participant: _____ Date: _____

Evaluation of Transport Protein on Neuroblastoma Cells

Circle YES, if you agree to allow pathology slides from previous neuroblastoma biopsies or surgeries to be sent to the Dana-Farber and Boston Children's Hospital in Boston for research testing.

Circle NO, if you do not want to allow your pathology slides to be sent for research testing.

YES NO

Signature

Participant: _____ Date: _____

Evaluation of Automated MIBG Scoring System

Circle YES, if you agree to allow your MIBG scans to be forwarded to the University of Chicago for automated MIBG scoring.

Circle NO, if you do not want to allow your MIBG scans to be forwarded to the University of Chicago for automated MIBG scoring.

YES NO

Signature

Participant: _____ Date: _____

SIGNATURE OF RESEARCH SUBJECT

Your signature below indicates

- You have read this document and understand its meaning;
- You have had a chance to ask questions and have had these questions answered to your satisfaction;
- You consent/assent to your participation in this research study; and

_____
Name of Subject

_____ _____
Signature of Subject Date

SIGNATURE OF PARENT(S)/LEGAL GUARDIAN(S) (If the subject is a minor)

Your signature(s) below indicates

- You have read this document and understand its meaning;
- You have had a chance to ask questions and have had these questions answered to your satisfaction;
- You agree to your child's participation in this research study; and

_____ _____
Name(s) of Parent(s)/Legal Guardian(s)

_____ _____
Signature of Parent/Legal Guardian Date

_____ _____
Signature of Parent/Legal Guardian Date

SIGNATURE OF INVESTIGATOR/PERSON OBTAINING CONSENT

I have explained the research to the subject and/or the subject's parent(s)/legal guardian(s) and have answered all of their questions. I believe that they understand all of the information described in this document and freely give assent/consent/permission to participate.

_____
Name of Investigator/Person obtaining consent

_____ _____
Signature of Investigator/Person obtaining consent Date

SIGNATURE OF WITNESS (if applicable)

My signature as Witness indicates that the subject and/or the subject's parent(s)/legal guardian(s) voluntarily signed this assent/consent/permission form in my presence.

_____
Name of Witness

_____ _____
Signature of Witness Date

SIGNATURE OF INTERPRETER (if applicable)

_____
Name of Interpreter

_____ _____
Signature of Interpreter Date

Complete if applicable:

Please check appropriate box and sign below.

☐Investigator/person obtaining consent's statement of certification for subjects less than seven years of age (assent):

The undersigned, _____, hereby certifies that he/she has discussed all the information contained in the study consent to the subject, and has explained all the information in the study consent to the participant/patient, including any risks that may reasonably be expected to occur. The undersigned further certifies that the subject was encouraged to ask questions, that all questions were answered, and that assent was obtained.

☐ Assent was not obtained for a subject under 18 years of age. *(Please state the reason. Examples include: child is an infant; child is comatose; child lacks cognitive abilities to understand the information.)*

_____

_____

Date:_____

Time:_____

Signature_____

Consent Addendum I: Tests that will be done on this study.

Observation	Before Entry	Days -1 to Day +7 of Course	Days 8 to 43 of Course	Day 43-50 of Course
Physical Exam	X	Day 4	Every other week	X
Blood Tests	X	Day 1* and Day 4	Up to two times a week	X
Urine tests (including Urine Catecholamines)	X			X
Pregnancy test	X			X
Heart tests (echocardiogram and EKG)	X	Day 4 EKG*		X
Bone Marrow Aspirate/biopsy	X			X
CT, MRI and/or MIBG scans	X			X
MIBG scan after MIBG treatment; no extra MIBG is given to you for this scan.		After you get out of your radiation isolation room		
Blood samples for blood markers of radiation exposure (optional)		Three or four blood draws over 5-6 days	One blood draw on Day 15	
Tumor slides sent for research (optional)		X		

***only for patients on Treatment Arm C (^{131}I-MIBG + Vorinostat)**

Consent Addendum 2

Certificate of Confidentiality Information

NANT 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.

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16.0 SAMPLE ASSENT FORM

STUDY OF SINGLE-AGENT ^{131}I-MIBG, ^{131}I-MIBG WITH VINCRISTINE AND IRINOTECAN, OR ^{131}I-MIBG WITH VORINOSTAT FOR RESITANT/RELAPSED NEUROBLASTOMA

A New Approaches to Neuroblastoma Therapy (NANT) treatment protocol

1. Dr. _____ is doing a research study.

2. You have a kind of cancer called **Neuroblastoma.** We are asking you to take part in a research study because doctors want to learn more about treating neuroblastoma using three different types of treatment choices. You will only receive one of the treatments and which treatment you get will be decided by a computer. You will get one medicine called **MIBG** for sure and then the other choices include 3 other medicines.

Here are the three options:

- **MIBG all by itself**
- **MIBG with two other medicines: VINCRISTINE AND IRINOTECAN**
- **MIBG with one other medicine: VORINOSTAT**

Doctors are trying to see and to see what effects (both good and bad) these medicines have on patients and their cancer, and which medicines given together (or by themselves) are better in treating your neuroblastoma. MIBG is a radioactive medicine that is given into the bloodstream (either through your central line or through a small tube placed in a vein in your hand or arm) Vincristine and Irinotecan are also medicines given into your blood stream. Vorinostat is a medicine that is given by mouth as a liquid or pill. The MIBG goes mostly to where the cancer is in your body to give it radiation. The doctors think that giving MIBG may kill neuroblastoma cancer cells and are trying to see if it works better by itself or when it's given together with either Vincristine and Irinotecan or Vorinostat.

3. If you agree to be in this study this is what will happen:

You will get this therapy one time.

MIBG (Given on all Treatment Arms):

You will get MIBG, which is a radioactive medicine given into an IV over 1 hour. Because of this radiation treatment you will need to stay in your room until you go home. This is usually about 5 days. Your parents cannot sleep in your room but they will be able to stay just outside your room and you will be able to see and talk to them anytime you want. They can visit inside your room for a short time each day.

Because not all hospitals can give the MIBG, you might have to go with your parents to another hospital to get the MIBG part. Your doctor will talk with you and your parents about the different hospitals that can give the MIBG, and which one will be the best for you.

You will need to have a **Urinary Catheter** placed to help drain your urine while you are getting the MIBG treatment. A soft tube will be put inside your urethra (the hole where urine comes out of our bodies), and up into your bladder (the place where urine waits inside our bodies until we go to the bathroom). Because the MIBG will be in your urine, and can cause damage to your bladder, the catheter is necessary to prevent this from happening by keeping your bladder completely empty all the time.

Vincristine and Irinotecan (Treatment Arm B only):

You will get vincristine in your IV once on Day 0. You will get Irinotecan in your IV once a day for 5 days (Day 0 through Day 4**)**

Vorinostat (Treatment Arm C only):

You will take vorinostat liquid by mouth once a day for 14 days.

Stem Cells:

MIBG is a medicine that can lower the numbers of your normal blood cells. Stem cells make all the normal blood cells your body needs to be healthy. This includes white blood cells that fight infection, platelets that stop you from bleeding, and red blood cells that carry oxygen to your body. When MIBG is given at higher doses it can damage stem cells so they don't make enough of the normal blood cells we need to live. Your stem cells will be given back to you like a blood transfusion after the MIBG and other treatment is finished. This is called Stem Cell Infusion or Stem Cell Rescue. We give the stem cells back through your central line either in the hospital or in the clinic. It is a lot like getting a platelet transfusion – the stem cells look like watery blood.

Filgrastim/G-CSF:

The G-CSF helps the blood cells grow back faster after treatment. You will get G-CSF if your blood cells are low after getting MIBG. G-CSF is given through your IV or as an injection (shot) from a very small needle into your leg that your parents will learn how to give you. It is given one time a day until your blood cells have started to grow back. We will know when the blood counts are high enough to stop the G-CSF by doing a blood test.

Coming to See the Doctors:

During and after you have finished the treatment, you will have appointments with the doctors who are taking care of you. This is called "**Follow-Up**". This is to see how well the treatment has worked so far. The doctors will want to do some special tests to find this information out. They will include;

- Blood tests (we will do this twice each week to start with, and then less often)
- MRI, CT, and MIBG Scans (special pictures of your tumor)
- Bone marrow test (to look for tumor in your bone marrow)
- Tests for your heart, and your kidneys
- Check your pee

- Feel your belly, look into your eyes and ears, and listen to your heart and lungs.
- Ask you and your parents a lot of questions about how you are feeling, how you are doing in school, and any problems you might be having.
- You may have to come to the clinic to have blood and platelet transfusions when the blood counts are low or stay in the hospital if you have a fever with low blood counts.

You will come to visit your doctor every week or so to start with, then less often if everything is going well.

4. Sometimes things happen to kids in research studies that may make them feel bad. These are called "risks'. Some of the risks of this study are:
   - You may feel sick to your stomach and you may throw up.
   - You may feel tired.
   - You may have a bad appetite.
   - You might have a fever and maybe an infection where you will need to be in the hospital to get medicines to treat the infection. You may feel tired and weak and need a blood transfusion or you may get bruises or have bleeding (most often a nosebleed) and need a platelet transfusion.
   - During MIBG**,** the urinary catheter may be uncomfortable, and some people feel embarrassed having it – but you will only need it for a few days. It may also be hard to be in a room for a long time by yourself until the radiation levels are low enough that it is safe for your parents and everyone else to be around you all the time.
   - The treatments may not work, and your tumor may grow, or it might come back again after the treatment has finished. If this happens we will try other ways to stop the tumor from growing.
   - You could get a different kind of cancer, this doesn't happen often, but can happen years later.
   - It is possible that you could die from the treatment or cancer.

   Not all of these things may happen to you. Or things may happen that the doctors don't know about yet.

5. People also have good things that happen to them when they are in research studies. These are called "benefits". The benefits to you of being in this research study are that this treatment might make your neuroblastoma tumor stay the same size or get smaller for some time. We hope to learn more about this new treatment which could help other children with neuroblastoma.

6. Please talk this over with your parents before you decide whether or not to be in this study. We will also ask your parents to give their permission for you to take part in this study. But even if your parents say "yes" you can still decide not to do this.

7. Being in this study is up to you. You do not have to be in this study if you don't want to. You may stop being in this study at any time but there are two times where you would be at more risk for being sick or having side effects or being dangerous to other people if you stopped being in the study. 1. If you got MIBG and left the special room before the doctors said it was ok to leave, then you would still be

radioactive and this would be dangerous to everyone who was around you since you would give them radiation from your body. 2. If you decided to stop treatment after getting MIBG but before you were given your stem cells back, the high dose of MIBG could kill your blood cells so they would not grow back on their own without getting back your stem cells. In this case you would not be able to make your own blood cells and could have bad infections or bleeding, and you could die from not having enough normal blood cells.

8. You can ask any questions that you have about the study. If you have a question later that you didn't think of now, you can call me or ask me next time. Study doctor's phone number: _____.

9. Optional Blood Samples

   Please check to show if you do or do not agree to allow us to take the 4 or 5 extra blood samples to learn more about the effect of MIBG on the body. These blood samples are usually drawn from your central line.

   _____Yes, it is okay to take the extra blood samples.

   _____ No, it is not okay to take the extra blood samples.

10. Optional Tissue Samples

    Please check to show if you do or do not agree to allow us to use some of your neuroblastoma tumor tissue already removed during previous surgeries or biopsies. You will not have extra surgeries or biopsies just because you say "Yes" to this part of the study.

    _____Yes, it is okay to take the extra blood samples.

    _____ No, it is not okay to take the extra blood samples.

11. Optional MIBG Scan Study

    Please check to show if you do or do not agree to allow us to send copies of your MIBG scans to a hospital that is testing a new way to read MIBG scans. You will not have extra MIBG scans just because you say "Yes" to this part of the study.

    _____Yes, it is okay to send copies of my MIBG scans.

    _____ No, it is not okay to send copies of my MIBG scans.

Signing your name at the bottom means that you agree to be in this study. You and your parents will be given a copy of this form after you have signed it.

Name of Subject: _____

_____ _____
Signature of Subject: **Date**

_____ _____
Signature of Investigator **Date**

_____ _____
Signature of Person Conducting Discussion **Date**

APPENDIX I: PERFORMANCE STATUS SCALES/SCORES

Performance Status Criteria Karnofsky and Lansky performance scores are intended to be multiples of 10					
ECOG (Zubrod)		**Karnofsky**		**Lansky***	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
		90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	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.	80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

The conversion of the Lansky to ECOG scales is intended for NCI reporting purposes only.

APPENDIX II. MEDICATIONS ASSOCIATED WITH PROLONGED QTC

For the most current list of medications, please refer to the following website:
http://www.azcert.org/medical-pros/drug-lists/drug-lists.cfm .

Drugs that are generally accepted as having a known risk of causing Torsades de pointes **Prohibited within one week of vorinostat on Arm C**	Drugs that are generally accepted as having a possible risk of causing Torsades de pointes **Use with caution in patients on Arm C**
Amiodarone (Cordarone®, Pacerone®)	Alfuzosin (uroxatral®)
Anagrelide (Agrylin®, Xagrid®)	Atazanavir (Reyataz®)
Arsenic trioxide (Trisenox®)	Bedaquiline (Sirutro®)
Astemizole (Hismanal®) (Off US market)	Clazapine (Clozaril®
Azithromycin (Zithromax®, Zmax®)	Dolasetron (Anzemet®)
Bepridil Vascor® (Off US mkt)	Eribulin (Havalen®)
Chloroquine (Aralen®)	Famotidine (Pepcid®)
Chlorpromazine (Thorazine®)	Felbamate (Felbatol®)
Cisapride (Propulsid®) (Off US mkt)	Fingolimod (Gilenya®)
Citalopram (Celexa®, Cipramil®)	Foscarnet (Foscavir®)
Clarithromycin (Biaxin®)	Fosphenytoin (Cerebyx®)
Disopyramide (Norpace®)	Gatifloxacin (Tequin®)
Dofetilide (Tikosyn®)	Femifloxacin (Factive®)
Domperidone (Motilium®) (Not on US mkt)	**Granisetron (Kytril®)**
Dronedarone (Multaq®)	Iloperidone (Fanapt®)
Droperidol (Inapsine®) (not on US mkt)	Indapamide (Lozol®)
Droleptan, (Dridol®)	Isradipine (Dynacirc®)
Erythromycin (E.E.S.®)	Lapatinib (Tykerb®)
Escitalopram (Cipralex®, Lexapro®)	**Levofloxacin (Levaquin®)**
Flecainide (Tambocor®)	Lithium (Eskalith®)
Halofantrine (Halfan®)	Mirtazapine (Remeron®)
Haloperidol (Haldol®)	Moxepril/HCTZ (Uniretic®)
Ibutilide (Corvert®)	Nicardipine (Cardene®)
Levomethadyl (Orlaam®) (Off US mkt)	Nioltinib (Tasigna®)
Mesoridazine (Serentil®) (Off US mkt)	**Ondansetron (Zofran®)**
Methadone (Dolophine®)	Ofloxacin (Floxin®)
Moxifloxacin (Avelox®, Avalox®)	Oxytocin (Pitocin®)
Pentamidine (NebuPent®, Pentam®)	Paliperidone (Invega®)
Pimozide (Orap®)	Pasireotide (Signifor®)
Probucol (Off US mkt) Lorelco®	Promethazine (Phenergan®)
Procainamide (Pronestyl®, Procan®)	Quetiapine (Seroquel®)
Quinidine (Quinaglute®, Duraquin®)	Ranolazine (Ranexa®)
Sevoflurane (Ulane®)	Rilpivirine (Edurant®)
Sotalol (Betapace®)	Risperidone (Risperdal®)
Sparfloxacin (Zagam®) (Off US mkt)	Saquinavir (Invirase®)
Terfenadine (Seldane) (Off US mkt)	Sertindole (Serlect) off US mkt
Thioridazine (Mellaril®)	Sunitinib (Sutent®)
Vandetanib (Caprelsa®)	Tacrolimus (Prograf®)
	Tamoxifen
	Telvancin (Vibativ®)
	Telithromycin (Ketek®)
	Tizanidine (Zanaflex®)
	Tolterodine (Detrol®)
	Vardenafil (Levitra®)
	Venlafaxine (Effexor®)
	Voriconazole (Vfend®)
	Vorinostat (Zolinza®)
	Ziprasidone (Geodon®)

APPENDIX III. VORINOSTAT DOSING NOMOGRAM

For patients unable to swallow capsules, vorinostat will be given as a 50 mg/mL extemporaneous liquid preparation (see section 6.1.5 for compounding instructions) rounded to the nearest 10 mg according to the following dosing nomogram.

Patients who are able to swallow capsules may receive vorinostat capsules instead of suspension if their calculated vorinostat dose is within +/- 10% of a 100 mg increment (eg. 100 mg, 200 mg, 300 mg, or max dose 400 mg). Otherwise, they will need to receive vorinostat as suspension and dosed according to the following dosing nomogram.

Body Surface Area (m^2)	**Vorinostat 180 mg/m^2**
0.3-0.35	60 mg
0.36-0.39	70 mg
0.4-0.45	80 mg
0.46-0.55	90 mg
0.56-0.59	100 mg
0.6-0.65	110 mg
0.66-0.69	120 mg
0.7-0.75	130 mg
0.76-0.79	140 mg
0.8-0.85	150 mg
0.86-0.89	160 mg
0.9-0.95	170 mg
0.96-1.05	180 mg
1.06-1.09	190 mg
1.1-1.15	200 mg
1.16-1.19	210 mg
1.2-1.25	220 mg
1.26-1.29	230 mg
1.3-1.35	240 mg
1.36-1.39	250 mg
1.4-1.45	260 mg
1.46-1.55	270 mg
1.56-1.59	280 mg
1.6-1.65	290 mg
1.66-1.69	300 mg
1.7-1.75	310 mg
1.76-1.79	320 mg
1.8-1.85	330 mg
1.86-1.89	340 mg
1.9-1.95	350 mg
1.96-2.05	360 mg
2.06-2.09	370 mg
2.1-2.15	380 mg
2.16-2.19	390 mg
$\geq$ 2.2	400 mg

APPENDIX IV: QUALITY CONTROL FOR FREE IODINE IN ^{131}I-MIBG (DETECTION OF RADIOLYTIC DECOMPOSITION)

Radiopharmaceutical ^{131}I-MIBG from DraxImage or NDP

Preparation Receipt of therapeutic ^{131}I-MIBG from supplier

Materials
Waters Accell Plus CM Sep-Pac
Ethanol
Sterile water
10 ml test tubes

Instrumentation
Capintec well counter
Calculator

Procedure

1. Using a Waters Accell Plus CM SepPac cartridge, wet the sepPac with 5 ml ethanol. The long tip of the SepPac must be connected to the flushes
2. Rinse with 5 ml sterile water
3. Add one drop to 1/20 mL MIBG to SepPac. Dilute to around 0.5 uCi
4. Flush SepPac with 5 ml sterile water into a 10 ml test tube (flush tube)
5. Place cartridge into a second 10 ml test tube (A)
6. Place the cartridge tube into a well counter and record (A), set counter on 131-I
7. Count both tubes (A and flush) in the well counter, add counts together and record (B), set counter on 131-I
8. (A/A+B x 100 = % tagged. Must be greater than 95% for DraxImage product and greater than 93% for NDP product - compare to TLC

Comments

Procedure must be performed within 24 hours of receipt of dose from supplier. Results of bound iodine should be greater than 95% for DraxImage product and greater than 93% for NDP product. Record results on worksheet. Contact physicians if there is an aberrant or low result.

All centers administering MIBG therapy must perform an assay for radiolytic decomposition. The above is a suggested technique. Other techniques may be used provided they meet institutional radiopharmaceutical guidelines.

APPENDIX V: COMPOUNDS THAT INTERFERE WITH MIBG METABOLISM AND UPTAKE

Drugs Known To Reduce Uptake of MIBG

Drug	Mechanism
Sympathomimetics Phenylephrine Phenylpropanolamine Pseudoephedrine, ephedrine	Depletion of storage vesicle contents. These drugs occur in numerous non-prescription decongestants and diet aids. Their use should be excluded.
Antihypertensive / cardiovascular Labetalol Reserpine	Inhibition of catecholamine uptake. Depletion of storage vesicle contents. Depletion of storage vesicle contents. Inhibition of vesicle active transport.
Calcium-channel blockers	Uncertain (also enhances retention of previously stored norepinephrine and MIBG by blocking Ca^{++} mediated release from vesicles)
Tricyclic antidepressants	Inhibition of catecholamine uptake
Cocaine	Inhibition of catecholamine uptake

Drugs Expected To Reduce Uptake of MIBG

Drug	Mechanism
Sympathomimetics Amphetamine and related compounds Beta-sympathomimetics[#] Dobutamine, Dopamine, Metaraminol[#]: Systemic use. Effect unlikely with aerosol administration	Depletion of storage vesicle contents.
Atypical antidepressants Maprotiline Trazodone	Inhibition of catecholamine uptake.
Antipsychotics (major tranquilizers) Phenothiazines* Thioxanthines Butyrophenones *Occasionally used as antiemetic / antipruritic agents	Inhibition of catecholamine uptake
Antihypertensive / cardiovascular Adrenergic neuron blockers	Depletion of storage vesicle contents. Competition for transport into vesicles.

APPENDIX VI: ADJUSTED BODY WEIGHT CALCULATION

For patients whose actual body weight exceeds Ideal Body Weight (IBW) by more than 20%, the dose of stem cells, ^{131}I-MIBG, vorinostat, and irinotecan may be based on Adjusted Body Weight (ABW) rather than actual body weight.

IBW Formulas:
1-17 yrs: height is in cm, weight is in kg
IBW = (height2 x 1.65)/1000

1-17 yrs and 5 feet or taller:
IBW in kg (male) = 39 + (2.27 x height in inches over 5 feet)
IBW in kg (female) = 45.5 + (2.27 x height in inches over 5 feet)

18 yrs and older:
IBW in kg (male) = 50 + (2.3 x height in inches over 5 feet)
IBW in kg (female) = 45.5 + (2.3 x height in inches over 5 feet)

ABW is determined:
ABW=IBW + 0.4 x (Actual BW-IBW)

(Reference: Bone Marrow Transplant. 2007; 40(7):665-9)

APPENDIX VII. PATIENT INSTRUCTIONS FOR TREATING DIARRHEA

Be aware of your/your child's bowel movements. At the first sign that they are becoming softer than usual or if your child has any increase in the number of bowel movements over what is normal for him/her, start treating with loperamide (Imodium). **If he/she does not start taking the loperamide right away, the diarrhea may become severe and last several days or require hospitalization.**

Please follow these directions carefully, using dosing guidelines below:

- Take _____ loperamide at the first sign of diarrhea.
- Continue taking_____ loperamide every ____ hours until your normal pattern of bowel movements returns. Repeat the same doses and frequency if the diarrhea returns.
- Do not give more than _____ of loperamide in a 24 hour period.
- Please call your doctor if you have any questions about taking loperamide, if your child's diarrhea is not under control after two days, or if he/she is feeling extremely weak, lightheaded, or dizzy.
- Make an extra effort to give your child lots of fluids (several glasses of pedialyte, fruit juices, soda, soup, etc.) while your child is participating in this study.
- Side effects may include tiredness, drowsiness, or dizziness. If your child experiences these side effects, or if your child is urinating less frequently than usual, please contact your child's physician.
- Do not give your child any laxatives without consulting with his/her physician.

Loperamide dosing guidelines:
Loperamide 1 teaspoon = 1mg; 1 caplet = 2mg

Maximum dose of loperamide per day: < 6 years: 4mg/day; 6-11 years: 6mg/day; >11 years: 16 mg/day, using the non silicone-containing product.

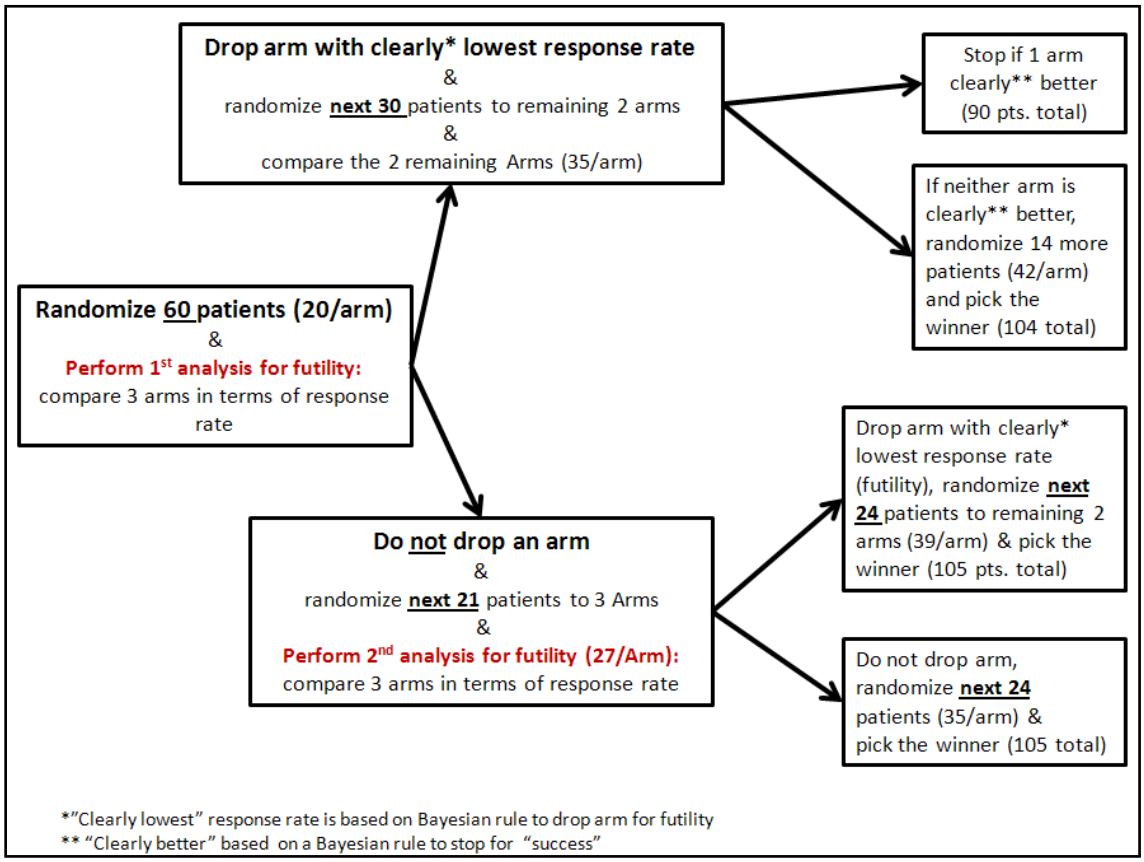
Weight: > 43 kg:	4 teaspoons or 2 caplets (4 mg) after the first loose bowel movement, followed by 2 teaspoons or 1 caplet (2mg) every2 hours. During the night the patient may take, 2 caplets (4mg) every 4 hours rather than1 caplet (2mg) every 2 hours.
Weight: 30kg – 43kg:	2 teaspoons or 1 caplet (2mg) after the first loose bowel movement followed by 1 teaspoon or one-half caplet (1 mg) every 2hours. During the night the patient may take, 1 caplet (2 mg) every 4 hours rather than one-half caplet every 2 hours.
Weight: 20kg – < 30kg:	2 teaspoons or 1 caplet (2mg)after the first loose bowel movement followed by 1 teaspoon or one-half caplet (1mg) every 3 hours. During the night, the patient may take 1 caplet (2mg) every 4 hours rather than one-half caplet every 3 hours.
Weight: 13kg - < 20kg:	1 teaspoon (1mg) after the first loose bowel movement followed by 1 teaspoon (1mg) every 3 hours. During the night, the patient may take 1 teaspoon (1 mg) every 4 hours rather than every 3 hours.
Weight: < 13kg:	Half teaspoon (0.5mg) after the first loose bowel movement followed by half teaspoon every 3 hours. During the night, the patient may take half teaspoon (0.5mg) every 4 hours rather than every 3 hours.

APPENDIX VIII. Interim Analyses using Bayesian Rules

A.1 Overall Trial Design

The pick-the-winner design includes the following three analyses (displayed in schematic below)

- Perform interim analyses after 60 and 81 patients have been treated and evaluated. The goal of these analyses will be to drop an inferior arm, if indicated, as well as to review the toxicities
- Perform the final analysis after 105 patients have been treated and evaluated, with one exception:
  - if an arm is dropped as inferior after 60 patients, then the remaining 2 arms will be analyzed and compared after 35 patients are evaluable in those arms. If the results are clear-cut, and one arm is superior, then the trial will stop; otherwise accrual will continue until 104 patients (42 in the 2 remaining arms) are randomized and evaluated.



A.2 Methods Used for Establishing the (Bayesian) Rules for Dropping the Inferior Arm or Selecting the Superior Arm

A.2.1 Notation and Distributions:

At any given time in the trial,

- let X_A, X_B, and X_C represent the number of responders observed for regimens A (=MIBG alone), B (=MIBG + vincristine + irinotecan), and C (MIBG + Vorinostat), respectively,
- let n_A, n_B, and n_C, represent the total number of patients evaluated for response in arms A, B, and C, respectively,
- and let p_A, p_B, p_C denote the (true) probability of response (i.e. response rates) for regimens A, B, and C.

Thus X_A, X_B, and X_C are binomial random variables with parameters p_A, p_B, p_C, and n_A, n_B, and n_C.

The Bayesian methodology says that p_A, p_B, p_C are not fixed numbers, but rather have a distribution, or range of possible values. So we modeled these distributions as beta with parameters α and β. For our calculations, we will assign the same mean value of 0.20 for all three distributions – but with very little confidence. So for each p (which will be the same for each p_A, p_B, p_C, separately)

$p \approx \text{Beta}(\alpha, \beta)$
where $\alpha/(\alpha+\beta)=0.20$ is the mean of the distribution and $\alpha+\beta=1.0$ is like the "sample size".

We call this our <u>prior distribution</u> of p and p_A, p_B, p_C.

As we observe data (X responders out of n patients treated on each arm separately) then we incorporate these data into our distributions of the true response rate, p. We calculate a new distribution which incorporates both our prior belief (or assumptions) and the data we have observed. So now, after having some data, we have the following distribution for p:

$p|X \approx \text{Beta}(\alpha + X, \beta + (n-X))$
where the mean becomes $(\alpha+X)/((\alpha+X)+(\beta+(n-X))) = c[X/n] + (1-c)[\alpha/(\alpha+\beta)]$
and where $c=n/(\alpha+\beta+n)$ and the effective sample size is $\alpha+\beta+n = n+1$.

We call this our <u>posterior distribution</u> of p.

Since the data in the 3 arms of the study are independent and since we know the posterior distributions for each of the response probabilities, then it is easy to determine the posterior probabilities. For this design, instead of calculating the posterior probabilities, we simulated the distributions with 2,000 realizations for each comparison.

A.2.2 Dropping an Arm / Deciding that the Response Rate is Inferior

After 60 patients (and again after 81 patients, if necessary) we will determine:

(a) $\Pr\{ p_B - p_A > 0.05 \mid X_A, X_B, n_A, n_B\}$ and $\Pr\{ p_C - p_A > 0.05 \mid X_A, X_C, n_A, n_C\}$ and
(b) $\Pr\{ p_C - p_B > 0.05 \mid X_C, X_B, n_C, n_B\}$ and $\Pr\{ p_A - p_B > 0.05 \mid X_A, X_B, n_A, n_B\}$ and
(c) $\Pr\{ p_B - p_C > 0.05 \mid X_C, X_B, n_C, n_B\}$ and $\Pr\{ p_A - p_C > 0.05 \mid X_A, X_C, n_A, n_C\}$.

If the 2 probabilities on any line above are both > 0.85, then we will conclude that Arm A (based on line (a)) or Arm B (based on line (b)) or Arm C (based on line (c)) is inferior and based on this and other considerations, we will close this arm to future randomization. These probabilities were determined based on 2,000 simulations.

A.2.3 Deciding after 90 Patients that we have Sufficiently Strong Data to Stop and Pick the Best Regimen

If an arm is dropped after 60 patients, then we will compare the 2 remaining arms after each has 35 evaluable patients. Without loss of generality, we will assume that Arm A has been dropped. At that time, we will determine the posterior probabilities (where $n_C = n_B = 35$):

$$\Pr\{ p_C - p_B > 0.05 \mid X_C, X_B, n_C, n_B\} \quad \text{and} \quad \Pr\{ p_B - p_C > 0.05 \mid X_C, X_B, n_C, n_B\}$$

If either of these probabilities is > 0.90, then we will close accrual and declare "a winner". If not the study will continue until 104 patients have been randomized and are evaluable. These probabilities were also determined based on 2,000 simulations.

A.3 <u>Evaluating the Characteristics (Statistical Properties) of the Proposed Design</u>

The decision to evaluate data at 60 and 81 patients for the possibility of dropping an inferior arm and at 90 patients to select a superior arm early was based on logistic and practical reasons as well as

preliminary simulations. The choice of the values of 0.05, 0.85 and 0.90 for making the decisions was also based on preliminary simulations.

To evaluate the performance of the proposed design, we considered a range of "true" fixed values of p_A, p_B, p_C. For convenience we always set the value of p_A to be the lowest response rate and the value of p_C to be the highest response rate. For each configuration of (p_A, p_B, p_C), we generated 2,500 trials of 105 patients and evaluated the following design characteristics: probability of correctly selecting the best arm, probability of dropping the inferior arm, probability of dropping the best arm, average number of patients in the inferior arm. The results are summarized in Table A below.

Table A: Probability* of Correctly Selecting the Best Arm and Dropping the Inferior Arm

Response Rate in Inferior & Middle Arms		**Probability of Correctly Selecting Best Arm**			**Probability of Dropping Inferior Arm**			**Probability of Dropping Best Arm**			**Average # of Patients in the Inferior Arm**		
		Difference in Response Rate between Middle & Best Arms			***Difference in Response Rate between Middle & Best Arms***			***Difference in Response Rate between Middle & Best Arms***			***Difference in Response Rate between Middle & Best Arms***		
Inferior	***Middle***	***+0.10***	***+0.15***	***+0.20***	***+0.10***	***+0.15***	***+0.20***	***+0.10***	***+0.15***	***+0.20***	***+0.10***	***+0.15***	***+0.20***
0.10	0.10	87%	94%	98%	21%**	25%	26%	0.4%	0.1%	0.0%	.	.	.
0.15	0.15	81%	92%	97%	25%	26%	30%	0.4%	0.4%	<0.1%	.	.	.
0.20	0.20	78%	90%	96%	25%	29%	32%	1.2%	0.5%	0.3%	.	.	.
0.25	0.25	76%	88%	96%	28%	32%	35%	1.4%	0.6%	0.1%	.	.	.
0.30	0.30	76%	86%	95%	27%	32%	34%	1.5%	0.5%	0.2%	.	.	.
0.35	0.35	73%	88%	94%	28%	30%	34%	1.4%	0.6%	<0.1%	.	.	.
0.05	0.15	88%	96%	99%	50%	51%	52%	0.0%	0.0%	0.0%	28	28	28
0.10	0.20	88%	95%	98%	44%	48%	50%	0.2%	0.1%	0.0%	29	28	28
0.15	0.25	85%	94%	98%	41%	43%	48%	0.3%	0.2%	0.0%	30	29	29
0.20	0.30	85%	92%	97%	41%	45%	46%	0.4%	0.1%	0.0%	30	29	29
0.25	0.35	82%	92%	96%	38%	45%	43%	0.4%	0.0%	<0.1%	30	29	30
0.05	0.20	89%	95%	98%	69%	71%	72%	<0.1%	<0.1%	0.0%	26	25	25
0.10	0.25	86%	92%	98%	60%	65%	68%	0.0%	0.1%	0.0%	27	26	26
0.15	0.30	84%	93%	97%	61%	62%	64%	0.1%	0.0%	0.1%	27	27	26
0.20	0.35	85%	93%	97%	57%	60%	61%	0.2%	0.1%	0.0%	28	27	27
0.25	0.40	83%	93%	97%	56%	60%	62%	0.1%	<0.1%	0.0%	28	27	27

* probabilities estimated based on 2,500 simulations and are reported as percents

** in this box, for the probability of dropping the inferior arm, the percent reflects dropping either Arm A or Arm B (since their response rates are equal for these 6 lines)