PROTOCOL: PROSPECTIVE AND RANDOMIZED STUDY OF FIXED VERSUS FLEXIBLE PROPHYLACTIC ADMINISTRATION OF GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) IN CHILDREN WITH CANCER

Study center: Wayne State University/ Children's Hospital of Michigan

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PROTOCOL SYNOPSIS

Title:	PROSPECTIVE AND RANDOMIZED STUDY OF FIXED VERSUS FLEXIBLE PROPHYLACTIC ADMINISTRATION OF GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) IN CHILDREN WITH CANCER
Study Sites:	Children's Hospital of Michigan, Detroit, MI
Study Duration:	Study duration is anticipated to be approximately 42-48 months
Phase:	Phase III
Objectives:	**Primary objective:** To compare the effect of flexible vs. fixed administration of G-CSF on the parameters of hematological recovery including duration of ANC < 500/μL; time to ANC recovery ≥ 1,000/μL and time to platelet recovery ≥ 75,000/μL in children receiving myelotoxic chemotherapy; **Secondary objectives:** • To compare the effect of flexible vs. fixed administration of G-CSF on the incidence of febrile neutropenia and number of hospital days on antibiotics following myelotoxic chemotherapy ; • To evaluate the number of days of platelet transfusion events after chemotherapy cycles with flexible vs. fixed administration of G-CSF; • To evaluate on the incidence and duration of G-CSF-related side effects including extremities/back pain and headaches after chemotherapy courses followed by flexible vs. fixed administration of G-CSF;
Study Design:	1. Children with solid tumors will receive ICE or equally myelotoxic chemotherapy followed by G-CSF (Neupogen®; 5 μg/kg per day subcutaneously), according to the following schedules: • Fixed schedule: G-CSF starts beginning one day (24 hours) after the last dose of chemotherapy, and continues until ANC recovery > 1,000/μL. • Flexible schedule: G-CSF starts beginning the day when ANC drops < 1,000/μL post-chemotherapy, and continues until ANC recovery > 1,000/μL. 2. The patients will receive fixed or flexible G-CSF schedule in randomized fashion, based on crossover 2 x 2 study design: each patient will receive two identical anticancer chemotherapeutic courses followed by one early and one flexible administration of G-CSF and will serve as his or her own control (see Figure). The time interval between the cycles will be at least 14 days, with the next cycle starting when ANC recovers above 1,000/μL and platelet count recovers above 75,000/μL.

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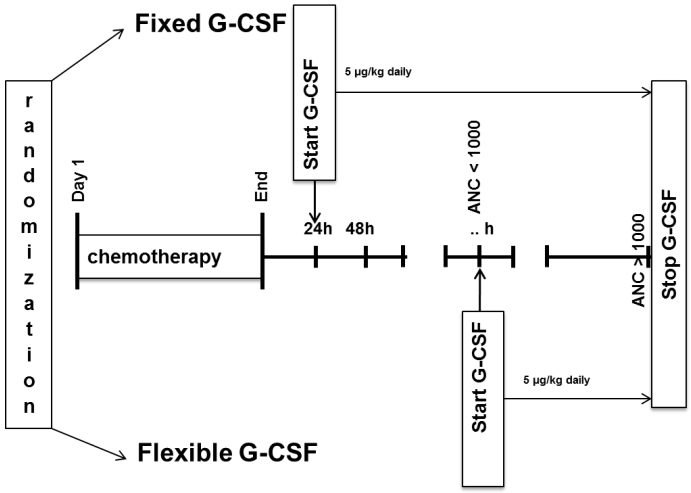
<tr><td></td><td> 
</td></tr>
<tr><td>Number of Patients:</td><td>It is expected that 24 to 30 patients will be enrolled into the trial</td></tr>
<tr><td>Eligibility:</td><td><u>Evidence of disease</u>
Subjects must have or have had at initial diagnosis, histologic proof of their malignancy. Additionally they must have radiographic, nuclear image, or biopsy proof that they have had a recurrence of their disease within four weeks prior to study entry. Subjects must have failed or relapsed from standard first-line chemotherapy for their tumor. Young children with primary embryonal brain tumor treated according to Head Start protocol are eligible as well.
• Subjects with bone marrow involvement are NOT eligible for study.
<u>Age</u>
All subjects must be ≥ 6 months and ≤ 25 years of age at study entry;
<u>Therapy</u>
• Patients will receive repeated cycles of identical chemotherapy that will likely result in grades III-IV hematological toxicity;
• Patients will be treated outside of active Children’s Oncology Group (COG) protocols with specific requirements for schedule of G-CSF administration (patients who are treated “according to” COG protocols are eligible).
The following categories of patients treated at Children’s Hospital of Michigan are eligible for this study:
• Patients with brain tumors treated according to modified Head Start II protocol with vincristine, etoposide, cyclophosphamide, and cisplatin (OPEC) chemotherapy;
• Patients with recurrent Hodgkin lymphoma treated with ICE (Ifosfamide, carboplatin, etoposide) chemotherapy;
• Patients with recurrent solid tumors including sarcomas, Wilms’ tumor, neuroblastomas, or brain tumors treated with high dose ICE or ICT (Ifosfamide, carboplatin, topotecan) or CE (Carboplatin, Etoposide) chemotherapy;
• Patients with Ewing sarcoma and Osteosarcoma treated with IE (Ifosfamide, Etoposide) chemotherapy;
• Patients with soft tissue sarcomas treated with IA (Ifosfamide, Doxorubicin) chemotherapy;
• Patients with osteosarcoma treated with HD Ifo (high dose Ifosfamide) chemotherapy;
<u>Prior therapy</u></td></tr>
</table>

	Subjects must have fully recovered from the toxic effects of any prior therapy. At least 3 weeks should have elapsed since the last dose of chemotherapy (6 weeks in the case of nitrosourea containing therapy). Subjects must have recovered from previous colony-stimulating factor therapy and have been off colony-stimulating factors (G-CSF, GM-CSF, IL-11) for more than 10 days and off erythropoietin for 30 days. Organ Function Requirements ANC and Platelet Count: Subjects must have an ANC >1000/μL and a platelet count >100,000/μL to be eligible for therapy. Renal function: All subjects must have a creatinine clearance or GFR which is greater than or equal to 70 ml/min/1.73 m^2. Hepatic function: All subjects must have a bilirubin less than 1.5 x NL and an SGOT or SGPT less than 2.5 x NL for age. Cardiac function: Subjects should have a normal ejection fraction (per institutional limits), no evidence of cardiac arrhythmias requiring therapy, and a fractional shortening of >28%. Life expectancy: All subjects must have a life expectancy of 12 weeks or more. Diagnostic Categories a) Sarcoma (Soft Tissue and Bone) b) Kidney Tumors c) Brain Tumors d) Other solid tumors (gonadal and germ cell tumors, retinoblastoma, neuroblastoma, and miscellaneous tumors) e) Hodgkin lymphoma Performance Status Must be > 60 from Lansky (age 1 to 16) or Karnofsky (age > 16). Exclusion Criteria Subjects with any of the following will NOT be eligible for study: a) Bone marrow involvement b) Active myelogenous leukemia, or history of myelogenous leukemia Informed Consent and IRB Approval All subjects and/or guardians must sign an institutionally approved informed consent. All institutional, FDA, and NCI requirements for human studies must be met.
Study procedures, frequency	• Staging procedures including imaging, nuclear medicine scans, and bone marrow examination, should be obtained within 4 weeks prior to initiation of chemotherapy; • After enrollment, patients will be randomized to receive either flexible or fixed G-CSF schedule first. • Post chemotherapy, patients are routinely seen in clinic for PE and labs (CBC with differential on every visit; Chem7 – once a week) 2-3 times a week depending on their blood counts and a need for blood transfusion support. • Optional blood samples (3 cc in green top tube) for flow cytometry progenitors assay will be obtained at baseline prior to chemotherapy and upon ANC recovery > 1,000 /μL following fixed and flexible administration of G-CSF (a total of 3 times) • Subjects will be assessed for their disease status after 2 cycles of chemotherapy
Treatments:	1. All patients receive chemotherapy on Days 1-4 (ICE, ICT, OPEC), and G-CSF (5 μg/kg/d) as a daily subcutaneous injection beginning on Day 5 on fixed schedule and on the Day when ANC drops ≤ 1,000/μL according to flexible schedule.

	• Subsequent courses of chemotherapy will begin as soon as ANC recovers to ≥ 1,000/μL and the platelet count to ≥ 75,000/μL between days 14 and 35 • Each patient will sequentially receive G-CSF stimulation according to flexible or fixed administration regimen after 2 consecutive cycles of chemotherapy. The order of the fixed or flexible schedule of G-CSF will be determined randomly for each patient. • After 2 cycles of chemotherapy the study will be completed and patients may receive G-CSF based on their primary oncologist decision. 2. Therapy will continue for maximum of 5 (OPEC) or 6 (ICE, ICT) courses. Patients with brain tumors receiving OPEC will be given high dose consolidation with ABMT support
Criteria for evaluation	The end-points for comparison in this study will be: 1) Duration (days) of ANC < 500/μL; 2) Days to ANC ≥ 1,000/μL from the start of chemotherapy; 3) Days to transfusion unsupported platelet count ≥ 75,000/μL from the start of chemotherapy; 4) Incidence of febrile neutropenia and hospitalization; 5) Number of platelet transfusions per chemotherapy cycle; 6) Days of G-CSF administration; 7) Incidence and duration of G-CSF related pain; 8) Incidence of bacteremia; 9) Percentage of progenitor cells in peripheral blood.
Statistical methods	A crossover 2 x 2 design will be used. Each patient will be sequentially given G-CSF by two different administration regimens after 2 consecutive cycles of chemotherapy. The order of the fixed or the flexible schedule of G-CSF administration will be determined randomly for each patient. The study is completed after 2 cycles of chemotherapy followed by randomly assigned schedules of G-CSF. The primary outcome for analysis will be comparison of time to neutrophil recovery for the two G-CSF administration regimens. Time to hematological recovery will be calculated for each course administered during protocol therapy to the following procedure. Each patient who starts a course of chemotherapy will be evaluated for each of three measures of hematological recovery: 1) duration of ANC < 500/μL; 2) recovery of ANC; 3) recovery of platelet count (PLT). Recovery of ANC is the time from the start of the course until the first date the ANC reaches ≥ 1,000/μL post nadir. Recovery of PLT is the time from the start of the course until the first date the PLT reaches ≥ 75,000/μL without platelet transfusion support. The primary objective of this study is to support the hypothesis (of equivalence) that there is no significant difference in average time to ANC recovery between the two methods of treatment (early, delayed). A bioequivalence test tests that the treatment mean is identical to the current treatment within *a small acceptable equivalence margin*. The analysis will be reported by displaying mean values (for each treatment in each sequence) as well as their differences, and 95% confidence intervals for the mean difference between treatments (adjusting for the period effect). For our primary outcome of time to ANC recovery ≥1,000/μL we hypothesize that the margin of equivalence will equal 2 days, with a standard deviation of 3 days. Setting alpha at 0.05 and type II error at 0.20 the study will have 80% power with a sample size of 7 in each group. Paired Student t-test will be performed to examine differences between groups in outcomes such as duration (number of days) of severe neutropenia with ANC < 1,000/μL,

	number of platelet transfusions, duration of G-CSF administration, and progenitor cell responses. Kaplan-Meier approach will be used for time to event variables such as days to ANC or platelet recovery. Univariately, continuously scaled variables will be presented as means, standard deviations, medians, ranges, and interquartile ranges. Categorically scaled variables will be presented as numbers, ratios, and percentages. The McNemar's test will be used to assess the statistical significance of categorical data like frequency of febrile neutropenias and hospitalizations. Statistically significant results will be considered achieved at a p-value ≤ 0.05. **Sample Size and Study Duration** There will be at least 24 patients enrolled into this trial. We expect to accrue between 5 and 7 patients per year at Children's Hospital of Michigan. Depending upon accrual, the duration of the study will be around 3.5 to 4 years.

1. INTRODUCTION

The use of granulocyte colony-stimulating factor (G-CSF) has been widely accepted into routine clinical practice to reduce the risk of febrile neutropenia (FN) associated with cytotoxic chemotherapy. By accelerating neutrophil recovery, G-CSF shortens neutropenic periods, decreases the need for antibiotics and hospitalizations, and enables intensification of chemotherapy (1). FN is associated with a significant risk of morbidity, mortality, and hospitalization, which ultimately increases the cost of medical care. G-CSF has been demonstrated to reduce the risk of FN in adults when administered immediately after chemotherapy (2). A meta-analysis of G-CSF in pediatric oncology demonstrated that prophylactic G-CSF reduced both the duration of severe neutropenia and the risk of FN, but failed to affect infection related mortality (3). Large-scale clinical use of G-CSF combined with its price significantly contributes to the overall cost of cancer chemotherapy. After more than 20 years of clinical use of G-CSF, its administration schedule remains mostly empirical and is not based on the cellular kinetics of post chemotherapy bone marrow recovery. Optimization of G-CSF use can potentially significantly reduce its side effects and produce significant economic benefits.

2. STUDY OBJECTIVES

G-CSF is customarily used on a fixed schedule when the drug is started 24 hours after completion of chemotherapy and then continued either for a certain number of days or until absolute neutrophil count (ANC) reaches a certain level. This fixed start schedule of G-CSF administration is mostly empirical and is not based on kinetics of post-chemotherapy bone marrow recovery, however, it is not yet known if early (24-48 hours post chemotherapy when ANC is still above 1,000/μL before the nadir) doses of G-CSF significantly contribute towards both shortening of neutropenic periods and decreasing the incidence of FN and hospitalizations. Besides obvious economic reasons to decrease the numbers of G-CSF injections due to its high cost, the prolonged G-CSF administration may result in some negative consequences and side effects. Overstimulation of bone marrow with G-CSF may result in hyperleukocytosis, deeper thrombocytopenia (4), potential exhaustion of bone marrow (5;6), and unpleasant side effects like headaches and back/extremities pains (7). There is evidence from the animal studies (8), that early post-chemotherapy bone marrow lacks dividing myeloid precursors, the target cells

for G-CSF to mediate its effect on accelerated myeloid recovery. These cells reappear later after chemotherapy after recruited stem cells generate them. Thus, the early post-chemotherapy "fixed" doses of G-CSF may not be necessary to achieve the clinical goals of prophylactic G-CSF administration. We therefore hypothesize that a schedule of post-chemotherapy G-CSF administration combining delayed start and timely withdrawal will result in similar parameters of hematological recovery and incidences of FN while providing a significant reduction in number of G-CSF injections and decreasing the side effects of G-CSF compared to fixed early G-CSF administration schedules. To test this hypothesis, we propose a randomized clinical study of "fixed" vs. "flexible" administrations of G-CSF in children with solid tumors receiving myelotoxic chemotherapy with the following specific aims:

Primary Objective

1. To compare the effect of flexible vs. fixed administration of G-CSF on the parameters of hematological recovery including duration of ANC < 500/μL; time to ANC recovery ≥ 1,000/μL and time to platelet recovery ≥ 75,000/μL in children receiving myelotoxic chemotherapy;

Secondary Objectives

1. To compare the effect of flexible vs. fixed administration of G-CSF on the incidence of febrile neutropenia and number of hospital days on antibiotics following myelotoxic chemotherapy ;
2. To evaluate the number of days of platelet transfusion events after chemotherapy cycles with flexible vs. fixed administration of G-CSF;
3. To evaluate on the incidence and duration of G-CSF-related side effects including extremities/back pain and headaches after chemotherapy courses followed by flexible vs. fixed administration of G-CSF;

3. BACKGROUND AND RATIONALE

Neutropenia following chemotherapy and prophylactic use of G-CSF: early versus delayed start

Neutropenia and thrombocytopenia following myelosuppressive chemotherapy are the most important factors limiting dose and intensity of combination chemotherapy. The nadir and duration of neutropenia are the main predictive factors for the occurrence of infection. G-CSF is commonly used after chemotherapy to reduce the incidence of febrile neutropenia (FN) and hospital stays on antibiotics by shortening the duration of severe neutropenia. Recent guidelines from American Society of Clinical Oncology (ASCO) recommend prophylactic administration of G-CSF when the risk of FN is 20% or higher (9), thus significantly increasing the number of chemotherapy regimens for which G-CSF is currently recommended. G-CSF is an expensive medication and combined with its large-scale use, it makes G-CSF one of the significant factors contributing to the overall cost of cancer chemotherapy. ASCO identified G-CSF use as one of the top 5 factors that could be optimized to reduce the cost of cancer care (10). Post-chemotherapy prophylactic G-CSF is customarily started 24 hours after chemotherapy and is given daily until ANC recovery in most of the current Children's Oncology Group (COG) protocols. This early start schedule is mostly empiric and not based on the kinetics of post-chemotherapy bone marrow recovery. At least one prospective randomized clinical study in children with cancer demonstrated that delaying G-CSF until 5 days after completion of chemotherapy did not result in a longer duration of neutropenia or increased the incidence of neutropenia-related problems (11), which indirectly indicates that the doses of G-CSF administered within 24-48 hours after chemotherapy do not significantly contribute to acceleration of granulocytic recovery. One study in adults with ALL (12) also

suggested that post chemotherapy G-CSF administration can be delayed by several days without a negative effect on acceleration of neutrophil recovery.

Stopping of G-CSF: Guidelines for discontinuation of G-CSF are not well documented in the literature. COG protocols typically recommend stopping G-CSF if the following occurs: ANC ≥ 5,000/μL after the nadir is reached; or ANC ≥ 2,000/μL after the nadir is reached; or ANC ≥ 1,500/μL on 2 consecutive days after the nadir. Hyperleukocytosis with WBC up to 30,000's and even 40,000's/μL associated with discomfort related to back and extremity pains and headaches may be seen and is not uncommon when G-CSF stimulation continues beyond ANC recovery >1,000 and the CBC is not checked for several days. Timely G-CSF withdrawal could prevent patients from receiving unnecessary G-CSF doses, experiencing Hyperleukocytosis and prolonged G-CSF related side effects including deeper thrombocytopenia [9].

G-CSF clearance is increased in relation to ANC: An association between the ANC and the elimination of circulating G-CSF was reported and thought to be mediated by internalization and degradation of receptor-bound G-CSF by mature granulocytes. Sturgill et al (13) found a significant negative correlation between ANC and log dose-adjusted AUC and between ANC and Cmax of G-CSF. These observations suggest that the pharmacokinetic profile of G-CSF would be most favorable in neutropenic conditions when most of the dose is available for stimulation of progenitors and not eliminated by mature neutrophils that reside in the bone marrow and circulation during the early days after chemotherapy.

G-CSF related side effects: Pain and irritation at injection sites, extremity/back pain and headaches from medullary bony pressure, as well as thrombocytopenia are the most common side effects of G-CSF reported (7). All of these side effects can be potentially reduced by optimizing G-CSF administration using both delayed start and timely withdrawal.

Chemotherapy: The study will be evaluating G-CSF after myelotoxic chemotherapy regimens with inclusion of alkylating agents and/or carboplatin resulting in predictable severe neutropenia after >80% of the cycles with a nadir during days +7 to +12 and a neutrophil recovery during days +11 to +22 from the first day of chemotherapy. These chemotherapy combinations have proven activity and are commonly used as a standard of care in pediatric solid tumors and lymphomas. They will include Ifosfamide, Carboplatin, Etoposide (ICE, used for the patients with recurrent Hodgkin lymphoma and solid tumors) (14,15), Ifosfamide, Doxorubicin (IA, used for soft tissue sarcomas), Ifosfamide, Etoposide (IE, used for Ewing sarcoma); High-dose Ifosfamide (HDI, patients with osteosarcoma); Vincristine, Cisplatin, Etoposide, Cyclophosphamide (OPEC, brain tumors) (17). All of these chemotherapy regimens are currently used in standard treatment protocols at Children's Hospital of Michigan.

4. PRELIMINARY DATA

Kinetics of post chemotherapy bone marrow cell populations' recovery and G-CSF in mice: an experimental study

We characterized the changes in bone marrow cellular composition in mice after cyclophosphamide (8). We found that early after chemotherapy the bone marrow was predominantly composed of mature residual neutrophils and there were very few progenitors and precursors, on which G-CSF would act to generate granulocytes. We demonstrated, that early after chemotherapy (up to 48 hours), the bone marrow is predominantly composed of mature residual granulocytes and very few progenitors and precursors, and that the point when mature granulocytes significantly decline and immature progenitors reappear occurs in murine bone marrow on days 3 and 4 after a single dose of cyclophosphamide. Since human hematopoiesis is roughly two times slower than murine hematopoiesis, the post-chemotherapy reappearance of immature progenitors may occur even later in a human bone marrow. Although primitive HSCs do not have G-CSF receptors, committed myeloid progenitors do, and are able to respond to direct stimulation by G-CSF (19). **Thus our findings suggest that early after chemotherapy, there may be few if any cells in the bone marrow capable of direct proliferative response to G-CSF.** We also investigated recovery of ANC and WBC after different timing of G-CSF administration in mice and found that fewer G-CSF doses are needed to achieve similar counts if G-CSF is started later after chemotherapy (**Figure**).

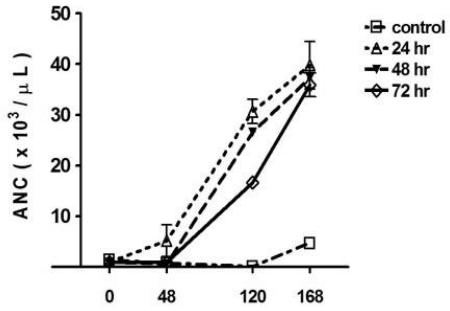


Figure: Recovery of ANC in mice receiving daily G-CSF starting 24, 48, or 72 hours after single dose of cyclophosphamide

Summary: In the studies of this proposal, we hypothesize that optimization of G-CSF administration after chemotherapy with a delayed start of G-CSF injections when ANC drops below 1,000/μL and timely withdrawal upon ANC recovery above 1,000/μL will significantly reduce the duration of G-CSF administration without compromising its positive effects on hematological recovery. The basis of this hypothesis is found by way of published clinical studies and our experimental data indicating that early after chemotherapy, the bone marrow lacks dividing myeloid progenitors and precursors capable of proliferative responses to G-CSF and therefore early post chemotherapy doses of G-CSF may not significantly contribute to acceleration of myeloid recovery. **Optimization of G-CSF administration based on the cellular kinetics of post chemotherapy bone marrow may result in significant economic savings and reduction of G-CSF related side effects.**

5. EXPERIMENTAL DESIGN

Children with solid tumors will receive ICE or equally myelotoxic chemotherapy (ICT or OPEC, see section #) followed by G-CSF (Neupogen®; 5 μg/kg per day subcutaneously), according to the following schedules:

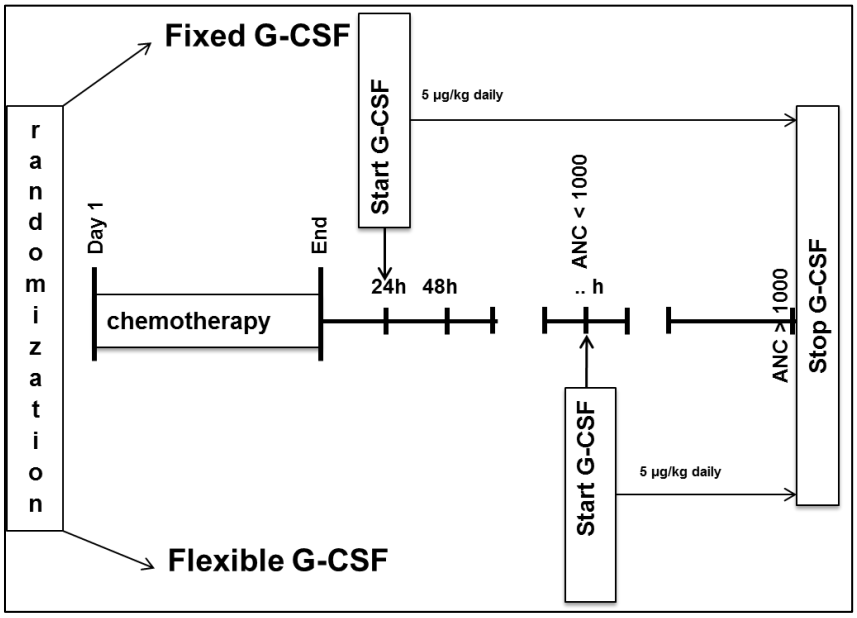
Fixed schedule: G-CSF starts beginning one day (24 hours) after the last dose of chemotherapy, and continues until ANC recovery > 1,000/μL.

Flexible schedule: G-CSF starts beginning the day when ANC drops < 1,000/μL post-chemotherapy, and continues until ANC recovery > 1,000/μL.

The patients will receive fixed or flexible G-CSF schedule in randomized fashion, based on crossover 2 x 2 study design: each patient will receive two identical anticancer chemotherapeutic courses followed by one early and one flexible administration of G-CSF and will serve as his or her own control (see Figure). The time interval between the cycles will be at least 14 days, with the next cycle starting when ANC recovers above 1000/μL and platelet count recovers above 75,000/μL.

6.

Study schema



7. SUBJECT ELIGIBILITY

Evidence of disease
Subjects must have or have had at initial diagnosis, histologic proof of their malignancy. Additionally they must have radiographic, nuclear image, or biopsy proof that they have had a recurrence of their disease within four weeks prior to study entry. Subjects must have failed or relapsed from standard first-line chemotherapy for their tumor. Young children with primary embryonal brain tumor treated according to Head Start protocol are eligible as well.

Subjects with bone marrow involvement are NOT eligible for study.

Age
All subjects must be ≥1 year and ≤ 25 years of age at study entry;

Therapy

- Patients will receive repeated cycles of identical chemotherapy that will likely result in grades III-IV hematological toxicity;
- Patients will be treated outside of active Children's Oncology Group (COG) protocols with specific requirements for schedule of G-CSF administration (patients who are treated "according to" COG protocols are eligible).

The following categories of patients treated at Children's Hospital of Michigan are eligible for this study:

- Patients with brain tumors treated according to modified Head Start II protocol with vincristine, etoposide, cyclophosphamide, and cisplatin (OPEC) chemotherapy;
- Patients with recurrent Hodgkin lymphoma treated with ICE (Ifosfamide, carboplatin, etoposide) chemotherapy;
- Patients with recurrent solid tumors including sarcomas, Wilms' tumor, neuroblastomas, or brain tumors treated with high dose ICE or ICT (Ifosfamide, carboplatin, topotecan) or CE (Carboplatin, Etoposide) chemotherapy;
- Patients with Ewing sarcoma and Osteosarcoma treated with IE (Ifosfamide, Etoposide) chemotherapy;
- Patients with soft tissue sarcomas treated with IA (Ifosfamide, Doxorubicin) chemotherapy;
- Patients with osteosarcoma treated with HD Ifo (high dose Ifosfamide) chemotherapy;

Prior therapy

Subjects must have fully recovered from the toxic effects of any prior therapy. At least 3 weeks should have elapsed since the last dose of chemotherapy (6 weeks in the case of nitrosourea containing therapy). Subjects must have recovered from previous colony-stimulating factor therapy and have been off colony-stimulating factors (G-CSF, GM-CSF, IL-11) for more than 10 days and off erythropoietin for 30 days.

Organ Function Requirements

ANC and Platelet Count

Subjects must have an ANC >1000/μL and a platelet count >100,000/μL to be eligible for therapy.

Renal function

All subjects must have a creatinine clearance or GFR which is greater than or equal to 70 ml/min/1.73 m^2.

Hepatic function

All subjects must have a bilirubin less than 1.5 x NL and an SGOT or SGPT less than 2.5 x NL for age.

Cardiac function

Subjects should have a normal ejection fraction (per institutional limits), no evidence of cardiac arrhythmias requiring therapy, and a fractional shortening of >28%.

Life expectancy

All subjects must have a life expectancy of 12 weeks or more.

Diagnostic Categories

a) Sarcoma (Soft Tissue and Bone)
b) Kidney Tumors
c) Brain Tumors

d) Other solid tumors (gonadal and germ cell tumors, retinoblastoma, neuroblastoma, and miscellaneous tumors)
e) Hodgkin lymphoma

Performance Status
Must be > 60 from Lansky (age 1 to 16) or Karnofsky (age > 16).

Exclusion Criteria
Subjects with any of the following will NOT be eligible for study:
a) Bone marrow involvement
b) Active myelogenous leukemia, or history of myelogenous leukemia

Informed Consent and IRB Approval
All subjects and/or guardians must sign an institutionally approved informed consent.
All institutional, FDA, and NCI requirements for human studies must be met.

8. TREATMENT PLAN

All patients receive chemotherapy on Days 1-4 (ICE, ICT, OPEC) or Days 1-5 (IE, IA) and G-CSF (5 μg/kg/d) as a daily subcutaneous injection beginning on Day 5-6 (24 hours after completion of chemotherapy) on fixed schedule and on the Day when ANC drops ≤ 1,000/μL according to flexible schedule. Subsequent courses of chemotherapy will begin as soon as ANC recovers to ≥ 1,000/μL and the platelet count to ≥ 75,000/μL between days 21 and 35, provided there is at least 48 hours interval between the last dose of G-CSF and the next cycle of chemotherapy. Each patient will sequentially receive G-CSF stimulation according to flexible or fixed administration regimen after first 2 consecutive cycles of chemotherapy. The order of the fixed or flexible schedule of G-CSF will be determined randomly for each patient so that some patients will get flexible schedule first; others will get fixed schedule first. After first 2 cycles of chemotherapy the study will be completed and patients may receive G-CSF based on their primary oncologist decision.
Therapy will continue for maximum of 5 (OPEC) or 6 (ICE, ICT) courses. Patients with brain tumors receiving OPEC will be given high dose consolidation with ABMT support

CHEMOTHERAPY

ICE (Recurrent solid tumors, high dose ICE): Etoposide 150 mg/m^2/day in NS (final concentration 0.4 mg/mL) IV over 1 hour at hours 1-2 on Days 1-3; Ifosfamide 3,000 mg/m^2/day in 600 mL/m2 NS IV over 3 hours at hours 1 – 4 on Days 1 – 3; Carboplatin 600 mg/m^2/day in 250 mL/m^2 NS IV over 2 hours on Day 4; Mesna 750 mg/m^2 over 15 min prior to Ifosfamide infusion on Days 1 - 3; Mesna 2250 mg/m^2 in D5½NS IV over 23 hours after Ifosfamide on days 1 – 3.
Recurrent Hodgkin Lymphomas: Same schedule as for high dose ICE, but Ifosfamide and Etoposide will be given for 2 days (days 1-2), Carboplatin will be given on day 3.

ICT: Same as ICE, except Topotecan 1.2 mg/m^2/day in 30 mL NS IV over 30 min prior to Ifosfamide on days 1 – 3 is substituted for Etoposide.

IE (Ewing Sarcoma, Osteosarcoma): Etoposide 100 mg/m^2/day in NS (final concentration 0.4 mg/mL) IV over 1 hour at hours 1-2 on Days 1-5; Ifosfamide 1,800 mg/m^2/day in 600 mL/m2 NS IV over 3 hours at hours 1 – 4 on Days 1 – 5.

IA (Soft Tissue Sarcomas): Doxorubicin 37.5 mg/m^2/day in NS IV over 1 hour at hours 1-2 on Days 1 and 2; Ifosfamide 3,000 mg/m^2/day in 600 mL/m2 NS IV over 3 hours at hours 1 – 4 on Days 1 – 3.

OPEC: If patients receive high-dose methotrexate (HDMTX 270 mg/kg IV over 4 hours with IV hydration and leucovorin rescue), OPEC starts after they clear methotrexate (serum level < 0.1 mcmol/L); Vincristine 0.05 mg/kg/day on Days 1, 8, 15; Etoposide 2.5 mg/kg/day IV over 1 hour on Days 1 - 3, Cyclophosphamide 55 mg/kg/day IV over 1 hour on Days 1 – 2 followed by Mesna 13 mg/kg x 3 doses after each Cyclophosphamide dose; Cisplatin 3.5 mg/kg IV over 6 hours on Day 4.

ADMINISTRATION OF G-CSF

Fixed administration
G-CSF (Neupogen®) 5 µg/kg once daily started at 24 hours after completion of chemotherapy and stopped when ANC reaches at least 1,000/µL post nadir.

Flexible administration
G-CSF (Neupogen®) 5 µg/kg once daily started on the first day after chemotherapy when ANC falls below 1,000/µL and stopped when ANC reaches at least 1,000/µL post nadir.

Dose Modification of Study Drug
There will be no dose or schedule modifications of G-CSF in this study.
G-CSF beyond Day 21 of Course
If the subject fails to achieve the desired ANC (≥ 1,000/µL) in any course, G-CSF may be continued until the ANC is sufficiently elevated to permit the next course of chemotherapy

Definition of Dose-Limiting Toxicity (DLT)
DLT is defined as any Grade IV non-hematological toxicity that is definitely, probably or possibly related to G-CSF.

9. REQUIRED OBSERVATIONS

Pretreatment

History and physical examination: A complete history and physical examination including accurate assessment and documentation of measurable disease, adenopathy, hepatomegaly, splenomegaly, blood pressure, body surface area, etc.

Hematology (must be within one week prior to starting therapy): CBC, manual differential, hematocrit, reticulocyte count, and platelet count; Bone marrow aspirate and biopsy (rhabdomyosarcoma, Ewing sarcoma, retinoblastoma, and medulloblastoma/PNET only).

Chemistries (must be within one week prior to therapy): Serum creatinine, creatinine clearance or GFR, total bilirubin, SGPT/SGOT, albumin, alkaline phosphatase, electrolytes, calcium, phosphorus, magnesium, uric acid.

Cardiac evaluation: EKG and echocardiogram are required prior to entrance on study.

Performance status;

Tumor evaluations: Studies will be performed as appropriate to access the size and extent of tumor involvement. These studies may include roentgenogram, CT or MRI scans, ultrasound, nuclear medicine scans or tumor markers as appropriate.

During study

Routine examinations: Both interval history and physical examination including vital signs are to be done once a week during each chemotherapy treatment course.

Hematology: CBC, manual differential, reticulocyte count, and platelet count will be done on Day 1, and then 3 times a week until recovery of ANC > 1,000/μL and platelets > 75,000/μL.

To decrease the number of clinic visits and blood draws during the study, the following practical rules will be used for making a decision to start or stop G-CSF: 1) if the ANC is within > 1,000/μL and < 1,200/μL range in a patient with falling counts who has not reached the nadir and was assigned to the flexible schedule, the next day's ANC will be considered to be < 1,000/μL and the G-CSF will be initiated; 2) if the ANC is within > 800/μL and < 1,000/μL range in a flexible G-CSF group patient with rising counts post nadir, the next day's ANC will be considered to be > 1,000/μL and the G-CSF will be stopped;
In cases when ANC > 200/μL from the protocol specified landmarks of 1,000/μL and 2,000/μL, the next day CBC will be obtained.

Chemistries: Electrolytes, creatinine, calcium, phosphorus, magnesium, total bilirubin, SGOT and SGPT and alkaline phosphatase will be done on Days 1, 3, 5 and 11 of each treatment course.

Tumor measurement: Studies used for pretreatment tumor evaluation should be repeated at the end of second course.

Required Studies			
Studies to be obtained	Baseline	Course # 1	Course # 2
History	X	X	X
Physical exam with vital signs	X	X	X
Tanner stage	X		
Height and weight	X	X	X
Performance status	X	X	X
CBC, differential, platelets	X	3 times a week	3 times a week
Urinalysis	X	X	X
Electrolytes (Na, K, Ca, PO4, Mg)	X	2 times a week	2 times a week
Serum glucose	X	Weekly	Weekly
Creatinine, SGPT, bilirubin; LDH; uric acid	X	X	X
Total protein/albumin	X	Weekly	Weekly
Triglycerides, cholesterol	X	X	X
Disease evaluation	X		After the second course
Pregnancy test	X		
Echocardiogram, EKG	X		12 months
Hematopoietic progenitor cells responses (optional)	X	X	X

Assessment of pain: G-CSF related bone pain will be assessed by nurses and physicians on the days of clinic visits and documented in the charts and CRFs. The Numeric Rating Scale (NRS) with grading from 0 to 10 is commonly used in Children's Hospital including patients with sickle cell anemia and

vasoocclusive pain crises. The NRS is a self-reporting tool for patients 10 years and older. Pain will be graded as no pain – 0; mild pain (1 - 3); moderate pain (4 - 6), and severe pain (7 - 10 levels). Analogous Faces Pain Scale 1 – 10 will be used for children from 5 to 11 years old and Revised Face, Legs, Activity, Cry, Consolability (r-FLACC) scale will be used for younger nonverbal children.

Hematopoietic progenitor cells responses: This is an optional exploratory aim where peripheral progenitor studies will be obtained prior to chemotherapy and upon ANC recovery that follows both the fixed and the flexible administration of G-CSF (a total of 3 time points for a patient). An expression of a panel of markers including CD34, CD33, CD41, CD61, CD 10, CD 19, CD117, and CD11b will be analyzed on peripheral blood mononuclear cells using flow cytometry. We plan to assess the percentages of total CD34+ cells as well as committed myeloid, granulocytic, megakaryocytic, and lymphoid progenitors using co-expression of CD34/CD33; CD34/CD41; CD34/CD61; CD34/CD117; CD34/CD11b; CD34/CD10, 19 in peripheral blood. The percentage of the population and the change from baseline to the time of ANC recovery will be assessed.

No additional tests/ blood draws other that routinely done for patients receiving cancer chemotherapy at CHM will be required for this study, except for progenitor responses studies.

10. SUPPORTIVE CARE

Surgery
Surgery after the second or subsequent course may be performed to obtain a partial or complete response during this protocol.

Radiation Therapy
Radiation for palliation and/or to obtain a partial or complete response of local lesions is acceptable during the third or subsequent courses.

Blood Products Support

The number and days of random and/or phereses transfusions, as well as red blood cell transfusions, will need to be quantified and entered on the data capture forms. It is recommended that all blood products be irradiated with at least 1500 cGy prior to administration to prevent graft vs. host disease.

Subjects should receive prophylactic platelet concentrate transfusions for platelet count < 20,000/μL. Additional platelet transfusions may be administered to subjects with active bleeding or for whom the investigator feels it is clinically indicated. Record of transfusion and reasons for the transfusions should be made on the appropriate form.

Subjects should receive packed red blood cell transfusions in order to maintain the hemoglobin level > 8.0 g/dL (Hct > 25%). If clinically indicated, additional red cell transfusions may be administered at the discretion of the investigator.

Presumed Bacterial Infection
Bacterial infection will be presumed in subjects who develop a fever > 38^{o}C at a time when their ANC is < 500/μL.

Subjects with neutropenic fever as defined above will have appropriate cultures, will be hospitalized, and will be placed empirically on at least a regimen of a third-generation cephalosporin with or without an aminoglycoside.

Subjects with clinically or microbiologically documented infections who have responded to initial therapy will be treated for 10 to 14 days, depending on the nature and site of infection, even if the granulocyte count recovers first. In subjects with documented infections who have defervescence but who remain leukopenic, antibiotics will be cautiously discontinued after 14 days of antibiotic therapy, and they will be re-instituted if fever recurs.

If the initial fever is of undetermined origin, and the subject has become afebrile on empiric therapy, then the antibiotics should be discontinued with the return of the ANC to >200/µl on two occasions, 24 hours apart. For subjects with undefined fever who have defervesced but who remain leukopenic, all antibiotics should be cautiously discontinued at 14 days after the initiation of empiric therapy; antibiotic treatment will be re-instituted if the fever recurs.

Subjects who are persistently or recurrently febrile and leukopenic despite 5-7 days of antibiotic therapy should be started on a course of empiric amphotericin B therapy to be continued at least until the ANC is >500 µ1. If clinically indicated, amphotericin B may be started at an earlier date at the discretion of the investigator.

11. OFF PROTOCOL THERAPY GUIDELINES

Discontinuation of Chemotherapy and G-CSF
a) Recurrent or progressive disease during any course of therapy.
b) At the discretion of the responsible physician if stable disease status without CR or PR at the end of two courses of chemotherapy.
c) Grade IV renal toxicity secondary to ifosfamide (less than 25% creatinine clearance).
d) A maximum of 6 courses of chemotherapy.
e) Failure to achieve ANC ≥ 1000/µL by Day 35 of any course.
Off Study Criteria
Subjects will be considered off study only if the following criteria are met:
a) Death
b) Entry onto another COG therapeutic study.
c) Lost to follow-up. A subject may be declared lost to follow-up if there has been no patient contact, after repeated attempts, for a 24 month period.

12. RESPONSE CRITERIA

All subjects who are registered will be accounted for in the report of the results. Subjects who complete at least 1 course of therapy will be evaluable for response. All subjects who receive the first course of treatment will be included in the analysis of survival. All tumor measurements are recorded in centimeters; the largest diameter and its perpendicular will be recorded for each measurable lesion.
Disease Status
Measurable disease: Bidimensionally measurable lesions with clearly defined margins by 1) plain x-ray, with at least one diameter, .5 cm or greater (bone lesions not included) or 2) CT, MRI, or other imaging scan, with both diameters greater than the distance between cuts of the imaging study.
Objective status:
(To be recorded at each evaluation). If an organ has too many measurable lesions at each evaluation, choose three to be followed before the patient is entered on study. The remaining measurable lesions in that organ will be considered evaluable for the purpose of objective status determination. Unless progression is observed, objective status can only be determined when all measurable and evaluable sites and lesions are assessed.
Evaluable disease:

Unidimensionally measurable lesions, masses with margins not clearly defined, lesions with both diameters less than 0.5 cm, lesions on scan with either diameter smaller than the distance between cuts, palpable lesions with either diameter less than 2 cm, bone disease.

Complete response (CR):

Complete disappearance of all measurable and evaluable disease. No new lesions. No disease related symptoms. No evidence of nonevaluable disease, including normalization of markers and other abnormal lab values. All measurable, evaluable, and nonevaluable lesions and sites must be assessed using the same technique as baseline. Refers to clinical CR.

Partial response (PR):

Applies only to subjects with at least one measurable lesion. Greater than or equal to 50% decrease under baseline in the sum of products of perpendicular diameters of all measurable lesions. No progression of evaluable disease. No new lesions. All measurable and evaluable lesions and sites must be assessed using the same techniques as baseline.

Stable/No response:

Does not qualify for CR, PR, or progression. All measurable and evaluable sites must be assessed using the same techniques as baseline.

Progression:

25% increase OR an increase of 10 cm2 (whichever is smaller) in the sum of the products of the Perpendicular diameters of all measurable lesions over smallest sum observed (over baseline if nodecrease) using the same techniques as baseline, OR clear worsening of any evaluable disease, OR reappearance of any lesion that had disappeared, OR appearance of any new lesion/site, OR failure to return for evaluation due to death OR deteriorating condition (unless clearly unrelated to this cancer). For "scan-only" bone disease, increased uptake does not constitute clear worsening. Worsening of existing nonevaluable disease does not constitute progression. Exception: Lesions that appear to increase in size due to presence of necrotic tissue will not be considered to have progressed.

Unknown:

Progression has not been documented and one or more measurable or evaluable sites have not been assessed.

Nonevaluable disease does not affect objective status except in determination of CR (all disease must be absent - a patient who otherwise has a CR, but who has nonevaluable disease present or not assessed, will be classified as having a PR) and in determination of progression (if NEW sites of nonevaluable disease develop). Patients with only nonevaluable disease cannot be assessed for response.

13. DATA MANAGEMENT PLAN

Research records for this study can be divided into following three categories:

1. General information, including patient demographic, diagnostic and staging data.
2. Treatment phase - including all other data such as the biological and therapeutic data pertinent to this study

Above data will be collected by a CRA, Research Nurse, and the Principal Investigator oversees data collection.

Registration - All patients must be registered with the Pediatric Hematology Oncology Research Office (313 966-7918 or 313-966-8541), located on the second floor of the Carl's Building. Data is retained by the Research Office for at least 5 years after study completion. The Principal Investigator oversees record management

14. SAFETY CONSIDERATIONS

All chemotherapy combinations including ICE, ICT, and OPEC are based on FDA approved drugs /regimens and have been routinely used at Children's Hospital of Michigan. Data concerning efficacy and toxicity of these regimens have been extensively published and Grade III-IV hematological toxicity is expected to occur after 100% of courses with around 50% of courses associated with febrile neutropenia and hospitalizations.
G-CSF is routinely administered at Children's Hospital of Michigan after all of these chemotherapy combinations and the usual start time of G-CSF is 1 to 4 days after completion of chemotherapy. G-CSF has been commonly used according to the flexible start schedule after OPEC chemotherapy in young children with brain tumors and all 5 patients tolerated treatment well without significant delays. We do not expect any severe DLTs from G-CSF administration.

Adverse Event reporting - Serious Adverse Events (SAE) - The investigator shall notify the IRB and Data Safety Committee of any unexpected fatal or life threatening experience associated with the use of the drug as soon as possible, but not later than (7) seven calendar days after the occurrence. The investigator is responsible for notifying their IRB within their timelines required. CTCAE version 4.0 will be used to code and grade this study.

Non-serious but Significant Adverse Events- As with serious adverse events, pre-existing conditions that would otherwise meet the definition of an adverse event do NOT need to be reported. A CRF will be provided to capture these events. A tabulation of the adverse events for a given safety assessment time period (see Data Safety Monitoring Committee section) will be made available to the Data Safety Review Committee for their periodic assessments and will be used for tabulation and analysis of adverse events in the final study report.

Post-Study Adverse Events - Adverse events which are identified through 4 weeks after study completion will be captured and reported as noted in the procedures described above. Subjects with unresolved previously reported adverse events or new adverse events should be followed by the investigator until the events are resolved, stabilized, the child is lost to follow-up or an explanation is evident.
Resolution means that child has returned to baseline state of health or that the investigator does not expect any further improvement or worsening of the adverse event.

15. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

The end-points for comparison in this study will be:
1) Duration (days) of ANC $< 500/\mu L$;
2) Days to ANC $\geq 1{,}000/\mu L$ from the start of chemotherapy;
3) Days to transfusion unsupported platelet count $\geq 75{,}000/\mu L$ from the start of chemotherapy;
4) Incidence of febrile neutropenia and hospitalization;
5) Number of platelet transfusions per chemotherapy cycle;
6) Days of G-CSF administration;
7) Incidence and duration of G-CSF related pain;
8) Incidence of bacteremia;
9) Percentage of progenitor cells in peripheral blood.

A crossover 2 x 2 design will be used for this study. Each patient will be sequentially given G-CSF by two different administration regimens after 2 consecutive cycles of chemotherapy. The order of the fixed or the flexible schedule of G-CSF administration will be determined randomly for each patient. The randomization scheme will be generated by using the Web site Randomization.com (http://www.randomization.com). Some patients will get the flexible schedule first; others will get the fixed schedule first. The study is completed after 2 cycles of chemotherapy followed by randomly assigned schedules of G-CSF.

The primary outcome for analysis will be comparison of time to neutrophil recovery for the two G-CSF administration regimens. Time to hematological recovery will be calculated for each course administered during protocol therapy to the following procedure. Each patient who starts a course of chemotherapy will be evaluated for each of two measures of hematological recovery: 1) duration of ANC < 500/μL; 2) recovery of ANC; 3) recovery of platelet count (PLT). Recovery of ANC is the time from the start of the course until the first date the ANC reaches ≥ 1,000/μL post nadir. Recovery of PLT is the time from the start of the course until the first date the PLT reaches ≥ 75,000/μL without platelet transfusion support.

The primary objective of this study is to support the hypothesis (of equivalence) that there is no significant difference in average time to ANC recovery between the two methods of treatment (early, delay). Sample size is calculated based upon the methodology provided by Chow, Shao and Wang (20). The procedure computes sample size for bioequivalence in balanced 2×2 cross-over designs in which the outcome is a continuous random variable, using the parametric t-test approach. A bioequivalence test tests that the treatment mean is identical to the current treatment within *a small acceptable equivalence margin*. The analysis will be reported by displaying mean values (for each treatment in each sequence) as well as their differences, and 95% confidence intervals for the mean difference between treatments (adjusting for the period effect).

For our primary outcome of time to ANC recovery ≥1,000/μL we hypothesize that the margin of equivalence will equal 2 days, with a standard deviation of 3 days. Setting alpha at 0.05 and type II error at 0.20 the study will have 80% power with a sample size of 12 in each group.

General Linear Model (GLM) procedure will be performed to examine differences between groups in outcomes such as duration (number of days) of severe neutropenia with ANC < 1,000/μL, number of platelet transfusions, duration of G-CSF administration, and progenitor cell responses. For our conventional two-treatment, two-period, two-sequence (2 x 2) randomized crossover design, the statistical model now will include factors accounting for the following sources of variation: sequence, period, and treatment (and also subjects nested in sequences). Estimates will be obtained for the adjusted differences between treatment means in time to ANC recovery and the standard error associated with these differences.

Kaplan-Meier approach will be used for time to event variables such as days to ANC or platelet recovery. Univariately, continuously scaled variables will be presented as means, standard deviations, medians, ranges, and interquartile ranges. Categorically scaled variables will be presented as numbers, ratios, and percentages. The McNemar's test will be used to assess the statistical significance of categorical data like frequency of febrile neutropenias and hospitalizations. Statistically significant results will be considered achieved at a p-value ≤0.05.

Sample Size and Study Duration

There will be at least 24 patients enrolled into this trial.

We expect to accrue between 5 and 7 patients per year at Children's Hospital of Michigan. Depending

upon accrual, the duration of the study will be around 3.5 to 4 years.

16. DRUG INFORMATION

GRANULOCYTE COLONY-STIMULATING FACTOR, (r-metHuG-CSF, G-CSF, Filgrastim, Neupogen®)

Source and Pharmacology: r-metHuG-CSF (produced in E. coli by recombinant DNA technology) stimulates the production of neutrophils in the bone marrow and selected end-cell activation. The 175 amino acid protein (M.W. of 18,800 daltons) differs from the natural protein in that the N-terminal amino acid is a methionine and it is not o-glycosylated. 3.45 ug to 11.5 ug of G-CSF administered subcutaneously resulted in a maximum serum concentration of 4 ng/ml to 49 ng/ml within 2 to 8 hours. The elimination half-life is similar for SQ and IV, approximately 3.5 hours.

Toxicity:

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug		Local irritation at injection site	Allergic reaction, low grade fever
Prompt: Within 2-3 weeks, prior to the next course	Medullary bone pain, leukocytosis	Increased alkaline phosphatase, increased lactate dehydrogenase, increased uric acid, thrombocytopenia	Splenomegaly, worsening of pre-existing skin rashes, alopecia
Delayed: Anytime later during therapy, excluding the above conditions			Cutaneous vasculitis
Late: Anytime after the completion of treatment			

Formulation and Stability: Supplied as a clear solution in 300 µg/ml (1 ± 0.6 x 108 U/mg) (1 ml or 1.6 ml) 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 µg/ml; G-CSF is incompatible with normal saline. At dilutions from 5 µg/ml to 14 µg/ml, add human serum albumin to a final albumin concentration of 2 mg/ml to protect against absorption of the G-CSF 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 µg/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. The suggested starting dose is 5 µg/kg.

17. DATA COLLECTION AND REPORTING

Investigators are required to notify the FDA and IRB of all serious and unexpected drug related AE's. All reactions in a 'reportable' category must be reported unless it is documented in the medical record chart that treatment is definitely not responsible for the toxicity. Serious and unexpected AE's will be reported to the following sources within the stated time frame. The reporting of adverse reactions is in addition to and does not supplant the reporting of toxicities as part of the data reporting for this study.

18. DATA SAFETY MONITORING:

Scheduled meetings will be held every 6 months or more frequently depending on the activity of the protocol. These meetings will include the protocol investigators and research staff involved with the conduct of the protocol

A Data Safety Monitoring Committee will be composed of members of the Department of Pediatrics at WSU not actively involved in this trial (Dr. Ronald Thomas, biostatistician, Dr. Deepak Kamat, Professor of Pediatrics). They will examine reports of adverse events and may ask for an analysis of the adverse events. They will examine any potential excess adverse events at 25% and 50% enrollment. Adverse events and serious adverse events will be recorded in accordance with FDA GCP guidelines. All adverse effects will be documented in the source documents and case report forms. For each occurrence causality and relationship to drug will be documented and the information reported to the IRB as per standard operating procedures.

19. REFERENCES

Reference List

1. Aapro M, Crawford J, Kamioner D. Prophylaxis of chemotherapy-induced febrile neutropenia with granulocyte colony-stimulating factors: where are we now? Support.Care Cancer 2010 May;18(5):529-41. PMCID:PMC2846279

2. Crawford J, Ozer H, Stoller R, Johnson D, Lyman G, Tabbara I, Kris M, Grous J, Picozzi V, Rausch G, et al. Reduction by granulocyte colony-stimulating factor of fever and neutropenia

induced by chemotherapy in patients with small-cell lung cancer. N.Engl.J.Med. 1991 Jul 18;325(3):164-70

3. Sung L, Nathan PC, Lange B, Beyene J, Buchanan GR. Prophylactic granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor decrease febrile neutropenia after chemotherapy in children with cancer: a meta-analysis of randomized controlled trials. J.Clin.Oncol. 2004 Aug 15;22(16):3350-6

4. Timmer-Bonte JN, de Mulder PH, Peer PG, Beex LV, Tjan-Heijnen VC. Timely withdrawal of G-CSF reduces the occurrence of thrombocytopenia during dose-dense chemotherapy. Breast Cancer Res.Treat. 2005 Sep;93(2):117-23

5. Hornung RL, Longo DL. Hematopoietic stem cell depletion by restorative growth factor regimens during repeated high-dose cyclophosphamide therapy. Blood 1992 Jul 1;80(1):77-83

6. van OR, Robinson S, Sheridan T, Mislow JM, Dawes D, Mauch PM. Granulocyte colony-stimulating factor enhances bone marrow stem cell damage caused by repeated administration of cytotoxic agents. Blood 1998 Sep 15;92(6):1950-6

7. Murata M, Harada M, Kato S, Takahashi S, Ogawa H, Okamoto S, Tsuchiya S, Sakamaki H, Akiyama Y, Kodera Y. Peripheral blood stem cell mobilization and apheresis: analysis of adverse events in 94 normal donors. Bone Marrow Transplant. 1999 Nov;24(10):1065-71

8. Yankelevich M, Goodell MA, Kaplan J. Efficacy of delayed administration of post-chemotherapy granulocyte colony-stimulating factor: evidence from murine studies of bone marrow cell kinetics. Exp.Hematol. 2008 Jan;36(1):9-16. PMCID:PMC2223182

9. Smith TJ, Khatcheressian J, Lyman GH, Ozer H, Armitage JO, Balducci L, Bennett CL, Cantor SB, Crawford J, Cross SJ, et al. 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. J.Clin.Oncol. 2006 Jul 1;24(19):3187-205

10. Schnipper LE, Smith TJ, Raghavan D, Blayney DW, Ganz PA, Mulvey TM, Wollins DS. American Society of Clinical Oncology identifies five key opportunities to improve care and reduce costs: the top five list for oncology. J.Clin.Oncol. 2012 May 10;30(14):1715-24

11. Rahiala J, Perkkio M, Riikonen P. Prospective and randomized comparison of early versus delayed prophylactic administration of granulocyte colony-stimulating factor (filgrastim) in children with cancer. Med.Pediatr.Oncol. 1999 May;32(5):326-30

12. Hofmann WK, Seipelt G, Langenhan S, Reutzel R, Schott D, Schoeffski O, Illiger HJ, Hartmann F, Balleisen L, Franke A, et al. Prospective randomized trial to evaluate two delayed granulocyte colony stimulating factor administration schedules after high-dose cytarabine therapy in adult patients with acute lymphoblastic leukemia. Ann.Hematol. 2002 Oct;81(10):570-4

13. Sturgill MG, Huhn RD, Drachtman RA, Ettinger AG, Ettinger LJ. Pharmacokinetics of intravenous recombinant human granulocyte colony-stimulating factor (rhG-CSF) in children receiving myelosuppressive cancer chemotherapy: clearance increases in relation to absolute neutrophil count with repeated dosing. Am.J.Hematol. 1997 Feb;54(2):124-30

14. Cairo MS, Shen V, Krailo MD, Bauer M, Miser JS, Sato JK, Blatt J, Blazar BR, Frierdich S, Liu-Mares W, et al. Prospective randomized trial between two doses of granulocyte colony-stimulating factor after ifosfamide, carboplatin, and etoposide in children with recurrent or refractory solid tumors: a children's cancer group report. J.Pediatr.Hematol.Oncol. 2001 Jan;23(1):30-8

15. Yankelevich M, Dolgopolov I, Ravshanova R, Kamaeva O, Lebedev V, Izshogin D, Socolova I, Siegel SE, Mentkevich G. Efficacy and toxicity of Ifosfamide/ cyclophosphamide, carboplatin, and etoposide (ICE/CCE) chemotherapy with or without GM-CSF in relapsed or refractory Wilms' tumor. A single institution study. Int.J.Pediatr.Hematol.Oncol. 6[5], 331-338. 2000.

Ref Type: Generic

16. Amini E, Roffidal T, Lee A, Fuller GN, Mahajan A, Ketonen L, Kobrinsky N, Cairo MS, Wells RJ, Wolff JE. Central neurocytoma responsive to topotecan, ifosfamide, carboplatin. Pediatr.Blood Cancer 2008 Jul;51(1):137-40

17. Chi SN, Gardner SL, Levy AS, Knopp EA, Miller DC, Wisoff JH, Weiner HL, Finlay JL. Feasibility and response to induction chemotherapy intensified with high-dose methotrexate for young children with newly diagnosed high-risk disseminated medulloblastoma. J.Clin.Oncol. 2004 Dec 15;22(24):4881-7

18. Dolgopolov I, Andreeva L, Yankelevich M, Mscheidze D, Siegel S, Mentkevich G. Delayed introduction of G-CSF after chemotherapy does not affect peripheral blood stem cell yield and engraftment kinetics in children with high-risk malignancies: retrospective study of 45 cases. Am.J.Hematol. 2003 Aug;73(4):225-9

19. Ebihara Y, Xu MJ, Manabe A, Kikuchi A, Tanaka R, Kato S, Nakahata T, Tsuji K. Exclusive expression of G-CSF receptor on myeloid progenitors in bone marrow CD34+ cells. Br.J.Haematol. 2000 Apr;109(1):153-61

20. Chow S-C, Shao J, Wang H. Sample size calculations in clinical research. 63-68. 2003. New York, Marcel Dekker.

Ref Type: Generic

20. SAMPLE INFORMED CONSENT

Medical Research Informed Consent

Title of Study: PROSPECTIVE AND RANDOMIZED STUDY OF FIXED VERSUS FLEXIBLE PROPHYLACTIC ADMINISTRATION OF GRANULOCYTE COLONY-STIMULATING (G-CSF) FACTOR IN CHILDREN WITH CANCER

Principal Investigator (PI): Maxim Yankelevich MD
Children's Hospital of Michigan/Wayne State University
3901 Beaubien Blvd, Detroit, MI 48201
313-745-5515

Funding Source: Departmental

If you are the parent or legal guardian of a child who may take part in this study, permission from you is required. When we say "you" in this consent form, we mean you or your child; "we" means the doctors and other staff.

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

You are being asked to take part in this research study because you have been undergoing treatment for cancer. This study is to see if there is a difference between a "fixed schedule" or a "flexible schedule" of a supportive care medicine called Granulocyte Colony-Stimulating Factor (G-CSF). G-CSF is often used to reduce the risk of neutropenia as a side effect from chemotherapies. Neutropenia occurs when the level of certain white blood cells in the blood drops below normal, which increases the risk of infections.

A "fixed schedule" often means G-CSF is started 24 hours after chemotherapy and is stopped after a set number of days or once counts recover. A "flexible schedule" means only giving G-CSF when the blood test called ANC drops below a certain level, and discontinuing it once that count has recovered.

This study is called a clinical trial. A clinical trial is a research study involving treatment of a disease in human patients. It is common to enroll children and adolescents with cancer in a clinical trial that seeks to improve cancer treatment over time. Clinical trials include only people who choose to take part.

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

WHAT IS THE CURRENT STANDARD FOR G-CSF USE?

The standard schedule for the use of G-CSF is usually a fixed schedule with the drug started 24 hours after chemotherapy ends. It is continued for a certain number of days or until white blood cell counts recover.

WHY IS THIS STUDY BEING DONE?

This study is to see if there is a difference between a "fixed schedule" or a "flexible schedule" of a supportive care medicine called Granulocyte Colony-Stimulating Factor (G-CSF). If the "flexible schedule" works, there could be a reduction in the G-CSF side effects and could save a lot of money in the cost of cancer treatments.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

It is expected that a maximum of 15 patients will be treated on this study, which is taking place at Wayne State University/Children's Hospital of Michigan.

WHAT IS INVOLVED IN THIS STUDY?

This study will happen during standard chemotherapy treatment. 3 treatment designs could be followed:

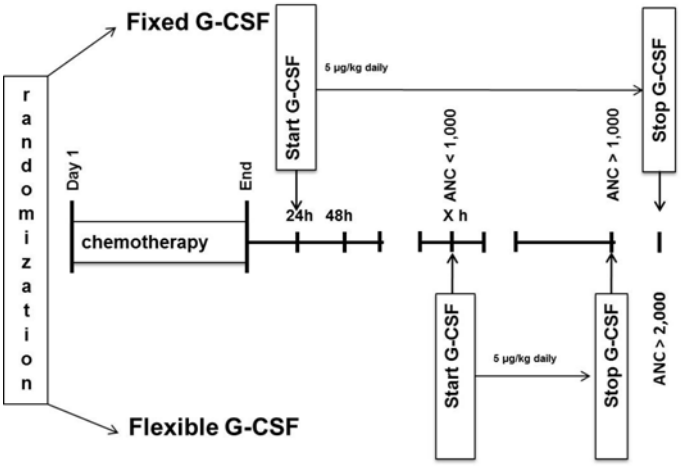
ICE (Ifosfamide, Carboplatin, Etoposide, Mesna) chemotherapy

ICT (Ifosfamide, Carboplatin, Topotecan, Mesna) chemotherapy

OPEC (Vincristine, Cisplatin, Etoposide, Cyclophosphamide, Mesna) chemotherapy

These chemotherapy treatment plans are not the experimental part of this study; you could be treated on these without participating.

You will be randomized to a "fixed schedule" or a "flexible schedule" group. Randomization is used in studies that involve two or more groups. Each group gets a different treatment, and patients have to be placed in one of the groups. Randomization means that the patients are placed in a group by chance. You will be randomized for cycle 1, and then move to the other group for cycle 2. For example – some patients will get flexible schedule first, others will get fixed schedule first. If you get flexible schedule cycle 1, you will get fixed schedule for cycle 2. A "fixed schedule" often means G-CSF is started 24 hours after chemotherapy and is stopped after a set number of days or once counts recover. A "flexible schedule" means only giving G-CSF when the blood test called ANC drops below a certain level, and discontinuing it once that count has recovered.



Methods for Giving Drugs

G-CSF is given by a shot underneath the skin.

Standard Medical Tests

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

- Physical exam
- Blood tests
- Bone marrow tests
- Various scans
- Tests of kidney function
- Tests of lung and heart function
- Urine tests
- Imaging studies

HOW LONG WILL I BE ON THIS STUDY?

The treatment portion of the study will last about 6 months (2 cycles). After first 2 cycles of chemotherapy the study will be completed and you may receive G-CSF based on your doctors' decision. Therapy will continue for maximum of 5 (OPEC) or 6 (ICE, ICT) courses.

The researchers may decide to remove you from the study if your cancer gets worse, or you experience effects from the treatment that are considered too severe. You can remove yourself from the study at any time. However, if you consider removing yourself from the study, we encourage you to talk to your regular physician and to the research physician before making a final decision.

WHAT ARE THE RISKS OF THE STUDY?

While on the study, you are at risk for the side effects listed below. There may also be other side effects that cannot be predicted. You should discuss these potential risks with the researcher and/or your regular doctor. Other drugs will be given to make side effects less serious and less uncomfortable. Patients are watched carefully and treatment is stopped if serious side effects develop.

Risks and side effects related to Filgrastim (G-CSF) include those which are:

Likely	Less Likely	Rare but serious
• Aching or pain in the bones	• Local irritation at the site of the injection • Headache Higher than normal levels of liver enzymes in the blood which may indicate liver irritation or damage and uric acid in the blood • Increase of uric acid in the blood	• Allergic reactions which can be life threatening with shortness of breath, low blood pressure, rapid heart rate, hives and facial swelling. This reaction is very rare and has been associated mainly with intravenous administration. • If you are known to have sickle cell

	• A low number of platelets in the blood which may cause you to bruise and bleed more easily • Low fever Enlargement of the spleen (an organ in the abdomen/belly which stores blood cells) which may cause pain in the abdomen or left shoulder Rash or worsening of skin rashes Inflammation of a blood vessels in the skin leading to a raised purple rash and bruising that has been seen mainly in patient who are treated for a long time Higher than normal white blood count • Skin condition marked by fever and painful skin lesions that appear mainly on the face, neck, back and arms	disease, filgrastim may cause a sickle cell crises. • Severe damage to the spleen (an organ in the abdomen/belly which stores blood cells) which could lead to pain and loss of blood into the abdomen (belly) and maybe life threatening • Difficulty breathing and lung damage that may be due to the white blood cells that are stimulated by filgrastim traveling to the lungs when they are inflamed or infected. • *A blood disorder or leukemia that has only been seen in patients with certain immune disorders who are treated for a very long time.*

No additional tests/ blood draws other that routinely done for patients receiving cancer chemotherapy at Children's Hospital of Michigan will be required for this study, except for peripheral blood progenitor assays

Optional study (Peripheral Blood Progenitor Assay Study): Drawing samples of blood is often a part of the routine evaluation of patients who have cancer. When blood is drawn for routine tests, patients will be asked to give an extra teaspoonful of blood for special research studies.

There will be no cost to you for any blood collected and studied in the laboratory for research purposes. There are no plans for you to be paid for allowing your blood to be used in research. Should any laboratory discoveries be made that have commercial value (able to be sold), there are no plans for you to have any rights or ownership in such commercial products, and you will not be paid anything for providing your blood for research.

The choice to allow extra laboratory research of your blood sample is up to you. No matter what you decide to do, it will not affect your care. If you decide now that your blood can be kept for research, you can change your mind at any time.

WILL I BENEFIT FROM THIS STUDY?

There may not be any direct medical benefit to you if you participate in the study. It is hoped that the information learned from this study may help future patients with cancer.

A combination of Ifosfamide, Carboplatin, and Etoposide/Topotecan or Vincristine, Cyclophosphamide, Etoposide, Cisplatin is being recommended with the addition of bone marrow growth-promoting factor G-CSF. The potential benefit of this treatment is suppression of the growth and spread of your (child's)

malignant disease as well as a possible reduction in the blood cell toxicity that may result from the combination chemotherapy.

If the new flexible schedule of G-CSF is effective you (your child) will receive fewer shots and may experience less side effects of G-CSF while getting the same reduction of bone marrow inhibition and low white blood cell counts.

ARE THERE OTHER TREATMENT OPTIONS?

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

- Treatment with routine clinical use of G-CSF
- Treatment that is considered in your best interest according to your treating physician.

Please talk to your doctor about these and other options.

WILL MY MEDICAL INFORMATION BE KEPT PRIVATE?

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

Research Related Injuries

In the event that this research related activity results in an injury, treatment will be made available including first aid, emergency treatment, and follow-up care as needed. Cost for such care will be billed in the ordinary manner to you or your insurance company. No reimbursement, compensation, or free medical care is offered by Wayne State University, the Detroit Medical Center, University Physician Group, sponsor, and any other facility involved with this study. If you think that you have suffered a research related injury, contact the PI right away at 313-745-5515.

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. You will be identified in the research records by a code name or number. Information that identifies you personally will not be released without your written permission. However, the study sponsor, the Institutional Review Board (IRB) at Wayne State University, The Detroit Medical Center or federal agencies with appropriate regulatory oversight [e.g., Food and Drug Administration (FDA), Office for Human Research Protections (OHRP), Office of Civil Rights (OCR), etc.) may review your records.

When the results of this research are published or discussed in conferences, no information will be included that would reveal your identity

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

WILL I HAVE TO PAY FOR THIS TREATMENT?

Taking part in this study may lead to added costs to you or your insurance company. Please ask about any expected added costs or insurance problems. Staff will be able to assist you with this.

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

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

WHAT ARE MY RIGHTS AS A STUDY PARTICIPANT?

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

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

Whether you participate or not, you will continue to get the best medical care this hospital can provide.

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you decide to take part in the study you can later change your mind and withdraw from the study. You are free to only answer questions that you want to answer. You are free to withdraw from participation in this study at any time. Your decisions will not change any present or future relationship with Wayne State University, Detroit Medical center or its affiliates, or other services you are entitled to receive.

While taking part in this study you will be told of any important new findings that may change your willingness to continue to take part in the research.

You can decide to stop being in the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. Your doctor will still take care of you.

Whether you participate or not, you will continue to get the best medical care this hospital can provide.

Questions

If you have any questions about this study now or in the future, you may contact Dr. Yanekelevich or one of her research team members at the following phone number 313-745-5515. If you have questions or concerns about your rights as a research participant, the Chair of the Institutional Review Board can be contacted at (313) 577-1628. If you are unable to contact the research staff, or if you want to talk to someone other than the research staff, you may also call (313) 577-1628 to ask questions or voice concerns or complaints.

Optional study (Peripheral Blood Progenitor Assay Study):

Making Your Choice

This study aims to evaluate immature (progenitor) white cells into circulation in response to different schedules of G-CSF. The evaluation will be performed during the first and the second courses of

treatment only, and will consist of blood samples drawn from a vein or a catheter. Small (1 teaspoon) samples of blood will be drawn on Day 1 before the first dose of chemotherapy and after white blood cell count recovery. Blood will be drawn at the same time with other labs so no additional draws will be required. This is an optional study that you may choose to participate:

Please indicate whether you choose to participate in extra tests on your blood:

Instruction: Circle YES to this instruction if you agree to let researchers use a sample of your blood to be used for research related to progenitor cell counts in response to G-CSF.

My blood may be kept for use in research to learn about, prevent, or treat cancer.

YES _____ NO _____ _____

Initial and date

Consent to Participate in a Research Study

To voluntarily agree to take part in this study, you must sign on the line below. If you choose to take part in this study you may withdraw at any time. You are not giving up any of your legal rights by signing this form. Your signature below indicates that you have read, or had read to you, this entire consent form, including the risks and benefits, and have had all of your questions answered. I have already read the above information. I have asked all my questions and I have gotten answers. I agree to enroll in this study. I have been given a copy of all 10 pages of this form.

_____ _____

Name of Participant Date of Birth

_____ _____

Signature of Parent/ Legally Authorized Guardian Date

_____ _____

Printed Name of Parent Authorized Guardian Time

_____ _____

*Signature of Parent/ Legally Authorized Guardian Date

_____ _____

*Printed Name of Parent Authorized Guardian Time

_____ _____

**Signature of Witness (When applicable) Date

_____	_____
Printed Name of Witness	Time
_____	_____
Signature of Person Obtaining Consent	Date
_____	_____
Printed Name of Person Obtaining Consent	Time

_____