HHS Public Access

Author manuscript

Bone Marrow Transplant. Author manuscript; available in PMC 2016 June 06.

Published in final edited form as:

Bone Marrow Transplant. 2012 November ; 47(11): 1448–1454. doi:10.1038/bmt.2012.51.

A Phase I/II Study of Cyclophosphamide and Topotecan in Patients with High-Risk Malignancies Undergoing Autologous Hematopoietic Cell Transplantation: The St. Jude Long-term Follow-up

Kimberly A. Kasow, DO[1], **Clinton F. Stewart, PharmD**[2], **Raymond C. Barfield, MD, PhD**[3], **Nancy L. Wright, MBA**[4], **Chenghong Li, MS**[5], **Deo Kumar Srivastava, PhD**[5], **Wing Leung, MD, PhD**[6], **Edwin M. Horwitz, MD, PhD**[7], **Laura C. Bowman, MD**[4], **Rupert Handgretinger, MD, PhD**[8], and **Gregory A. Hale, MD**[9]

[1]Division of Pediatric Hematology/Oncology, University of North Carolina at Chapel Hill, Chapel Hill, NC

[2]Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN

[3]Department of Hematology/Oncology, Duke University, Durham, NC

[4]Transplant and Gene Therapy Program, Cancer Center, St. Jude Children's Research Hospital, Memphis, TN

[5]Department of Biostatistics, St. Jude Children's Research Hospital, Memphis, TN

[6]Division of Bone Marrow Transplantation and Cellular Therapy, St. Jude Children's Research Hospital, Memphis, TN

[7]Department of Pediatric Oncology, Children's Hospital of Philadelphia, Philadelphia, PA

[8]Department of Hematology/Oncology and General Pediatrics, University Hospital of Tuebingen, Tuebingen, Germany

[9]Division of Pediatric Hematology, Oncology, Blood and Marrow Transplantation, All Children's Hospital, St. Petersburg, FL

Abstract

Fifty-eight consecutive children with high-risk malignancies were treated with cyclophosphamide and targeted topotecan followed by autologous hematopoietic cell transplantation (AHCT) in a phase I/II IRB-approved study. Twelve participants enrolled in phase I; 5 received dose level 1 of topotecan 3mg/m^2/day with subsequent doses targeted to total systemic exposure of 100 ± 20 ng °hr/ml and cyclophosphamide 750mg/m^2/day. Seven participants received dose level 2. Cyclophosphamide dose escalation to 1g/m^2/day was considered excessively toxic; 1 died from

Correspondence: Kimberly A. Kasow, DO, Division of Pediatric Hematology/Oncology, University of North Carolina at Chapel Hill, 1108 Physicians Office Building170 Manning Drive, CB #7236, Chapel Hill, NC 27599-7236, Phone 919-843-2072, Fax: 919-966-7629, ; Email: kkasow@med.unc.edu

CONFLICT OF INTEREST

The authors have no financial conflicts to disclose.

irreversible veno-occlusive disease and 2 experienced reversible hepatotoxicity. These adverse events halted further dose escalation. Forty-six participants were enrolled in phase II; results are on the 51 participants who received therapy at dose level 1, the maximum tolerated dose. Diagnoses included neuroblastoma (26), sarcoma (9), lymphoma (8), brain tumors (5), Wilms (2) and retinoblastoma (1). Twenty participants (39.3%) were in ≥ CR1 at enrollment; median age was 5.1 years. Most common non-hematologic grade 3/4 toxicity was gastrointestinal (n=37). Neutrophil and platelet engraftment occurred at a median of 15 and 24 days, respectively. Twenty-six (51%) remain alive at a median of 6.4 years after AHCT. Cyclophosphamide 3.75g/m^2 and targeted topotecan followed by AHCT is feasible and produces acceptable toxicity in children with high-risk malignancies.

Keywords

cyclophosphamide; topotecan; autologous BMT; neuroblastoma; sarcoma; lymphoma

INTRODUCTION

Autologous hematopoietic cell transplantation (AHCT) is used as consolidation therapy to treat patients with high-risk malignancies; however, only a fraction of these patients are cured with the majority developing recurrent disease.[1, 2] Clinical and laboratory studies support dose intensification as a strategy for improving survival for adults and children with malignancies at high risk of treatment failure. In our preclinical xenograft model system and in phase I and II trials, topotecan has shown promising anti-tumor activity in several pediatric malignancies such as neuroblastoma, rhabdomyosarcoma, CNS tumors, and Wilms tumor.[3-7] Results of a Pediatric Oncology Group phase I trial identified the maximum tolerated dose (MTD) for topotecan (0.75 mg/m^2/d) and cyclophosphamide (250 mg/m^2/d) when each was given once daily for five consecutive days without stem cell rescue but followed by filgrastim.[8] No significant toxicity other than myelosuppression was identified, and responses were observed in osteosarcoma, neuroblastoma, Wilms tumor, and rhabdomyosarcoma. The broad anti-tumor activities of this combination in lower doses made it ideal to consider escalation to higher doses with AHCT.

Doses up to 7 g/m^2 of cyclophosphamide have been given in combination with maximum doses of etoposide (a topoisomerase II inhibitor) followed by stem cell rescue, with acceptable toxicity. Therefore, cyclophosphamide 3.75 g/m^2 combined with topotecan was considered safe for study in the setting of autologous stem cell rescue. In children with recurrent solid tumors, researchers at St. Jude Children's Research Hospital in Memphis, Tennessee completed a phase I trial of topotecan, 10 doses delivered with dose adjustments to a targeted systemic exposure (TSE) of 100 ng°hr/ml. In another St. Jude trial for metastatic neuroblastoma, researchers successfully targeted the topotecan dose in 22 of 27 days (81%), but more importantly attained the targeted topotecan AUC in each patient studied.[9] Based on this experience, a phase I/II dose escalation study using ten doses of topotecan and five doses of cyclophosphamide followed by AHCT in children with high-risk malignancies was proposed.

MATERIALS AND METHODS

Patients with solid tumor and lymphomas at high-risk of treatment failure were eligible. Additional eligibility criteria included age < 25 years at diagnosis, ECOG performance score 0-2, ANC > 750/mm^3, echocardiographic shortening fraction > 25%, BUN < 40 mg/dl, creatinine < 2.0 mg/dl, bilirubin < 3.5 mg/dl and SGOT < 500 U/dl. All patients had documented chemotherapy-responsive disease and were greater than 2 weeks from previous therapy and had recovered from toxicity. The St. Jude Institutional Review Board approved this clinical trial, and participants and/or legal guardians provided informed consent prior to the initiation of the preparative regimen.

Toxicity Grading

Toxicities were collected prospectively by a clinical research nurse for the first 100 days after transplantation and followed for resolution; a transplant physician concurrently reviewed all toxicities and assigned level of severity and attribution to the treatment regimen. All cases of non-hematologic organ dysfunction were considered regimen-related toxicities (RRT). Toxicities were scored according to the National Cancer Institute (NCI) Common Toxicity Criteria Version 2.0.[10] An external Data Safety Monitoring Board was not used for this trial; rather the study's investigators discussed and monitored the toxicities internally.

Study Design and Analysis

The trial was originally designed to study 4 dosage levels in the phase I portion of the study:

Level 1: cyclophosphamide dosage 3.75 grams/m^2; targeted topotecan to 100 ± 20 ng °hr/ml;

Level 2: cyclophosphamide dosage 5 grams/m^2; targeted topotecan to 100 ± 20 ng °hr/ml;

Level 3: cyclophosphamide dosage 5 grams/m^2; targeted topotecan to 140 ± 20 ng °hr/ml;

Level 4: cyclophosphamide dosage 5 grams/m^2; targeted topotecan to 160 ± 20 ng °hr/ml.

The objectives of this trial in defining the maximum tolerated dose (MTD) were assessed according to a traditional phase I study. MTD was defined as the dose level below the unacceptable dose limiting toxicity (DLT) level. A DLT was defined as an irreversible toxicity, present at the time of death, or reversible non-hematologic grade IV toxicity that persisted for greater than 10 days. Any participant who had not engrafted by day 45 post-transplantation was also considered a failure.

Once the MTD was determined, the phase II portion of the study began with the aim to better understand the toxicity profile due to this regimen. This portion of the study was set up using a Simon's two-stage design to try and insure that not many patients were on a toxic chemotherapy preparative regimen.[11] Those in the MTD cohort in phase I were included in the phase II part of the study. Although the study was originally written to enroll up to an additional 19 patients at the MTD, because stopping rules had not been met and there was no

subsequent protocol for these patients, IRB approval was obtained to continue enrollment on study. Hence, 51 participants were enrolled and treated on dose level 1.

Survival was defined as the time interval from date of transplant to death from any cause or to last follow-up. For event-free survival (EFS), an event was defined as a relapse, disease progression, or death (due to any cause). The date of the first event was used in calculating EFS. For this survival analysis, time was censored at the last follow-up date if no failure was observed. Kaplan-Meier survival estimates were obtained and the comparisons between survival distributions were made using the log-rank test.[12,13]

The cumulative incidence of relapse was estimated by the method of Kalbfleisch and Prentice (1980) which accounts for competing risks.[14] The length of time at risk for relapse was computed from the date of AHCT to the date of relapse, death, or last contact, whichever came first. Deaths from non-relapse causes were considered as competing events. The cumulative incidences of relapse for different diagnosis group were compared by Gray's test[15]. The criteria for significance for all analyses were a p-value significant at a level of α=0.05. All statistical analyses used SAS Release 9.2.

Disease status, such as complete remission, very good partial response, partial response, stable disease, and progressive disease, was not defined by this study. Participant's disease response was categorized as determined by the participant's induction chemotherapy.

Stem Cell Collection and Cryopreservation

All patients had marrow morphologically free of tumor at the time of peripheral blood stem cell collection or marrow harvest. PBSC collection was performed after routine chemotherapy administration followed by G-CSF 10 mcg/kg/day until apheresis was completed. PBSC collection goal was at least 2×10^6 $CD34^+$ cells/kg. A marrow harvest was performed in patients unable to mobilize stem cells; targeted marrow cell dose was greater than 1.0×10^8 total nucleated cells/kg. Two patients had stem cell products purged on another institutional protocol using $CD34^+$ positive selection and FACS analysis. A minimum of 1×10^6 $CD34^+$ cells/kg from an apheresis product or 1×10^8 total nuclear cells/kg from a marrow product was required to proceed to AHCT. All stem cell products were stored at −150° C in the vapor phase of liquid nitrogen using 10% dimethylsulfoxide (DMSO) and a controlled rate freeze program.

Conditioning Regimen

For the Phase I portion of the study, topotecan was administered intravenously once daily as a 30-minute infusion for 10 days (Days −11 to −2). At levels 1 and 2, patients initially received topotecan 3 mg/m^2/day with subsequent dosages adjusted to achieve a targeted topotecan lactone AUC (area under the concentration-time curve) value of 100 ± 20 ng °hr/ml.[16] Dosages of chemotherapeutic agents were calculated using actual body weight. All patients but one received full-prescribed doses of each agent.

On days −6 through −2, cyclophosphamide was infused intravenously over 1 hour according to the dose escalation schedule. At level 1, patients also received cyclophosphamide 750 mg/m^2/day for 5 days (total 3.75$grams/m^2$). Mesna at 25% of the cyclophosphamide dose

was administered intravenously prior to each dose of cyclophosphamide and again at 3, 6, and 9 hours after each dose of cyclophosphamide, equaling 100% of the cyclophosphamide dose. At level 2, cyclophosphamide was administered at a dose of 1000 mg/m^2/day for 5 days (total 5 grams/m^2). Patients began pre-hydration with D5W + 0.45% Sodium Chloride at 125 ml/m^2/hr at the time of admission to the inpatient transplant unit on day −7, the evening prior to the first dose of cyclophosphamide. This intravenous fluid rate continued until day 0, the day of the stem cell infusion. Day −1 was a rest day. Prior to stem cell infusion on day 0, the graft was rapidly thawed in a 37° C water bath at the bedside and then infused through a central venous catheter. On day +1, filgrastim (G-CSF) was given at 5 μg/kg/day subcutaneously or intravenously until ANC > 3000/mm^3 on 2 consecutive days. No prophylaxis for veno-occlusive disease of the liver was administered.

Pharmacokinetics

For each patient, blood samples were obtained prior to the topotecan infusion, and at 0.25, 1, and 6 hours after the end of the topotecan infusion on days −6 and −11. Each sample was processed immediately, and topotecan lactone concentrations were measured by high performance liquid chromatography.[16] Topotecan lactone systemic clearance and single day AUC were calculated for each patient. If the single day topotecan lactone AUC was within the targeted AUC range on day-11 or day-6, then no dosage adjustment was required on the subsequent day and the patient remained at the same topotecan dosage. If the single day topotecan lactone AUC was not within the targeted topotecan AUC range on day-11 or day-6, the subsequent topotecan dosage was adjusted based on that patient's topotecan lactone clearance, to attain a single day topotecan lactone AUC in the targeted range.

If the topotecan target AUC range was not achieved with the dosage adjustment on day-10 or −5, additional dosage adjustments were made until the target was achieved. If topotecan blood samples or pharmacokinetic studies were not evaluable, then blood samples were obtained on the next available day. Dose adjustment followed the same procedure as outlined above.

RESULTS

Patient characteristics

Fifty-eight patients with high-risk solid malignancies were enrolled on this Institutional Review Board- approved study. Twelve patients received therapy on the phase I portion.. Five received dose level 1 (topotecan target AUC of 100 ± 20 ng°hr/ml and cyclophosphamide 750 mg/m^2/day × 5 days). One of the 5 participants experienced a dose-limiting toxicity, stomatitis. Seven patients were treated at dose level 2 (topotecan target of 100 ± 20 ng°hr/ml and cyclophosphamide 1000 mg/m^2/day × 5 days). One dose level 2 patient experienced an irreversible grade IV non-hematologic regimen- related-toxicity and eventually died of veno-occlusive disease. Two other participants experienced grade 2 hyperbilirubinemia and were clinically diagnosed with mild veno-occlusive disease. Although these 2 events did not meet stopping rule guidelines, for the safety of the patients, the principal investigator in collaboration with other study team members, including the study's biostatistician and co-investigators, decided to close the phase I safety portion of the

study. Dose level 1 was deemed the MTD. Characteristics dose level 2 participants are described in Table 1. Three participants died from primary disease. The next 46 consecutive patients were treated on the trial's phase II portion at dose level 1. Results will be described based on the 51 patients who received therapy at dose level 1 (5 from phase I and 46 from phase II). Patient demographics for these participants, including diagnoses, disease status at the time of AHCT are outlined in Table 2.

Regimen-Related Toxicities

Fifty of the 51 participants were able to receive full doses of the preparative regimen. One patient with NHL was only able to received 9 of 10 doses of topotecan and 3 of 5 doses of cyclophosphamide. This participant developed premature ventricular contractions with short runs of ventricular tachycardia and further chemotherapy was omitted. The patient, who had no previous history of cardiac disease and had a normal echocardiogram and electrocardiography prior to the initiation of this therapy, remained hemodynamically stable, had no electrolyte abnormalities, and did not require medical intervention. Although there was also a high incidence of other Grade III-IV toxicity in this heavily pretreated population (Table 3), three patients had no Grade III-IV non-hematologic toxicity. Toxicities were reviewed in real time at transplant research meetings and determined to be acceptable and expected in the setting of high dose chemotherapy followed by stem cell rescue, particularly gastrointestinal toxicities (Table 4). Due to these expected toxicities, de-escalation below level 1 was not considered. Three patients (5.9%) died of non-relapse toxicity, including multi-system organ failure and respiratory failure.

Transplant outcome

All patients achieved neutrophil engraftment, defined as the first day of 3 consecutive days with an ANC > 0.5×10^9/l, at a median of day +15 (range, 8-33 days) and platelet engraftment, defined as platelets > 20,000/μl without transfusion for seven consecutive days, at a median of day +24 (range, 8-109 days). The median time to neutrophil engraftment for patients receiving marrow grafts was day +18 (range, 10-33 days) and for those receiving PBSC grafts day +10 (range, 8-11 days) ($p < 0.0001$). The median time to platelet engraftment for marrow recipients was day +27 (range, 16-109 days) while those receiving PBSC grafts achieved platelet engraftment at a median of day +17.5 (range, 8-88 days) ($p = 0.001$). The 3-year and 5-year overall survival (OS) for the 51 participants was 66.7 ± 6.5% and 58.7 ± 7.7% respectively (Figure 1). The 3-year and 5-year event-free survival (EFS) were 43.1 ± 6.8% and 41.2 ± 8.2% respectively (Figure 1). The cumulative incidence of relapse for all participants was 54.9 ± 7.1 at 3 years and 56.9 ± 7.1 at 5 years (Figure 2A). Although not statistically significant (for all diagnoses, p-value = 0.83) (Figure 2B), neuroblastoma (the most common tumor in this study) showed a tendency of being more likely to relapse at both 3 and 5 years (57.7 ± 10.0% and 61.5 ± 9.9%, respectively) compared to lymphomas (50.0 ± 19.4% and 50.0 ± 19.4%, respectively) or sarcomas (44.4 ± 17.9% and 44.4 ± 17.9%, respectively). Upon univariate analysis of OS and EFS in respects to gender, product infused, diagnosis, and disease status prior to AHCT, none of these variables were statistically significant (data not shown). Twenty-two patients (43.2%) died of recurrent, persistent, or progressive disease; 2 (3.9%) died from multi-organ failure

and 1 (2%) died from respiratory failure. Twenty-six participants (51%) are surviving at a median follow-up time of 6.4 years (range, 4.0-9.8 years).

Pre- and Post-transplant Therapy

Four patients received radiation therapy before AHCT, four received radiation after AHCT and five received radiation therapy both before and after AHCT. Three patients with neuroblastoma received post-transplant therapy with cis-retinoic acid beginning at a median of 32 days post-AHCT (range, 32-53 days) and continuing for 6 months as directed by their primary treatment plan. No patient received anti-tumor antibody therapy post-HSCT.

Topotecan Pharmacokinetics

The median (range) topotecan lactone clearance was 32.1 L/hr/m^2 (range 15.3 to 44.2). A total of 138 pharmacokinetic studies were performed in 55 patients during cycle 1. All patients received a fixed 3 mg/m^2 fixed topotecan dosage for the first dose of cycle 1, and of the 55 studies 39 (71%) were within the desired target range. Of the remaining 83 pharmacokinetic studies, 67 were within the target range for a pharmacokinetic targeting success of 81%. The median (range) topotecan dosage for the patients that were within the target range was 3.1 mg/m^2 (1.1 to 4.6). Those studies outside the target range were evenly distributed among participants and after a second study, all patients were within the target range. No difference in topotecan pharmacokinetics or targeting results was noted between those patients receiving cyclophosphamide 750 or 1000 mg/m^2.

DISCUSSION

In the past 25 years, the prognosis for pediatric solid tumors has improved largely because of a multi-modal approach including multi-agent chemotherapy regimens, aggressive surgery, and conformal radiation therapies. However patients with metastatic or recurrent disease often have a dismal prognosis and are candidates for aggressive therapy including AHCT. Although neuroblastoma is the only pediatric malignancy in which randomized studies have demonstrated an increase in survival rate with the addition of AHCT to the treatment schema compared to standard chemotherapy alone, this treatment is frequently used to treat other high-risk pediatric solid tumors such as Ewing sarcoma, rhabdomyosarcoma, Wilms tumor, and lymphomas.[17–21] Topotecan has shown promising anti-tumor activity in pediatric malignancies, such as neuroblastoma, rhabdomyosarcoma, Wilms tumor, retinoblastoma, and Ewing sarcoma.[22–24] In a Children's Oncology Group trial in which 119 patients with relapsed or refractory neuroblastoma were randomized to receive either topotecan alone (2 mg/m^2/day for 5 days) or in combination with cyclophosphamide (topotecan 0.75 mg/m^2/day with cyclophosphamide 250 mg/m^2/day for 5 days), the two-drug combination demonstrated superior outcomes in terms of progression-free survival, but no difference in overall survival.[3]

Combining alkylating agents with topoisomerase inhibitors is attractive because of the broad activity in pediatric malignancies and the non-overlapping extramedullary toxicities. Gastrointestinal, fever/neutropenia, metabolic disturbances, and skin abnormalities were the most common Grade III-IV toxicities observed. Skin toxicities were common in areas that

had received prior radiation therapy as well as in non-radiated regions.[25] Topotecan is known to be a radiosensitizing agent, suggesting these skin toxicities were a form of radiation recall.[26,27] One patient each who had received thoracic radiation therapy developed cardiac arrhythmias or pleuro-pericardial effusions.

Three patients died of non-relapse causes, including multi-system organ failure and single organ failure. This toxicity rate is lower compared to those reported in other series of AHCT in pediatrics.[28] The reminder of those who died in this trial experienced progressive or recurrent disease. This is consistent with the results of prior studies in which disease recurrence has remained the main cause of treatment failure after AHCT.[29] VOD and liver toxicity have not been observed frequently with topotecan, but was the most common toxicity leading to the determination of the MTD.[30,31] Cyclophosphamide may have also contributed to the observed hepatotoxicity as this side effect has been well described [32, 33] and the participants were heavily pre-treated prior to AHCT. Topotecan, on the other hand, has not been studied extensively as a component of transplant regimens. The majority of published experience is in the setting of high-risk or recurrent ovarian cancer or multiple myeloma, used in combination with other agents.[34–37] For patients with CNS tumors, topotecan (2 mg/m^2/day for 5 days) was given with thiotepa (300 mg/m^2/day for 3 days) and carboplatin (approximating 500 mg/m^2/dose for 3 days, with Calvert formula under the curve 7) to 10 patients.[38] There were two toxic deaths and 4 were surviving disease free at a median of 6 years after treatment. Other trials using topotecan-containing regimens with and without stem cell support demonstrate feasibility and therapeutic efficacy in patients with leukemias, lymphomas, and other solid tumors in children and adults.[39–44] These studies have demonstrated acceptable response rates to the treatment regimen. Toxicities in these studies were similar to ours but cutaneous toxicity was not commonly observed. It is important to note that this study was the only one to target topotecan doses and the only one to include pediatric patients. Other published studies primarily included adult patients with non-pediatric malignancies, a markedly different population than this study.

Five-year event-free-survival (EFS) for all patients on this study was 41.2% which compares favorably with the literature as these were high-risk diseases. Mesenchymal tumors, such as sarcomas had promising results with the 5-year EFS being 44.4%, suggesting this regimen merits further study in this cohort. In addition, patients with lymphoma demonstrated responses (5-year EFS 50%), but patient numbers were quite low. Additional studies are needed in this group to better categorize efficacy. For patients with neuroblastoma, it is important to note that none of these patients received post-transplant antibody therapy. Overall survival rates at 5-years were favorable allowing patients to proceed to other salvage therapies. This observation suggests that this regimen is well tolerated and does not lead to long-term organ dysfunction and prohibiting further relapse therapies.

In this study, the MTD dose was determined to be 3 g/m^2 cyclophosphamide and ten doses of topotecan with a TSE of 100 ± 20 ng°hr/ml when administered with hematopoietic stem cell rescue. Topotecan administration was easily administered on an outpatient basis, although early admissions were necessary due to fever in some patients. Our study demonstrates the feasibility and relative safety of this combination of cyclophosphamide and topotecan. Future efforts may be directed at increasing the dose of topotecan or

incorporating an additional agent into the regimen. As has been demonstrated in other malignancies, most notably acute lymphoblastic leukemia, individualized drug treatment may enhance treatment efficacy while reducing toxicity.[45, 46]

Acknowledgments

We would like to thank research nurses Margaret Armond, Cynthia Walker and Patricia Johnson for data collection; Frances Curran for data entry and Sara Williams for manuscript preparation. The authors also wish to thank Paula Condy, Margaret Edwards, Lisa Walters, Teri Kuehner, and Sheri Ring for assistance in obtaining the topotecan pharmacokinetic samples. Thank you to the pharmacokinetic and clinical pharmacists who assisted in the care of these patients.

This work was supported in part by NIH Cancer Center Support grant P 30CA 21765 and American Lebanese Syrian Associated Charities (ALSAC).

REFERENCES

1. Shih CS, Hale GA, Gronewold L, Tong X, Laningham FH, Gilger EA, et al. High-dose chemotherapy with autologous stem cell rescue for children with recurrent malignant brain tumors. Cancer. 2008; 112:1345–1353. [PubMed: 18224664]
2. Hale GA. Autologous hematopoietic stem cell transplantation for pediatric solid tumors. Expert Rev Anticancer Ther. 2005; 5:835–846. [PubMed: 16221053]
3. London WB, Frantz CN, Campbell LA, Seeger RC, Brumback BA, Cohn SL, et al. Phase II randomized comparison of topotecan plus cyclophosphamide versus topotecan alone in children with recurrent or refractory neuroblastoma: a Children's Oncology Group study. J Clin Oncol. 2010; 28:3808–3815. [PubMed: 20660830]
4. Zage PE, Kletzel M, Marcus R, Castleberry R, Zhang Y, London WB, et al. Outcomes of the POG 9340/9341/9342 trials for children with high-risk neuroblastoma: a report from the Children's Oncology Group. Pediatr Blood Cancer. 2008; 51:747–753. [PubMed: 18704922]
5. Carol H, Houghton PJ, Morton CL, Kolb EA, Gorlick R, Reynolds CP, et al. Initial testing of topotecan by the pediatric preclinical testing program. Pediatr Blood Cancer. 2010; 54:707–715. [PubMed: 20017204]
6. Metzger ML, Stewart CF, Freeman BB 3rd, Billups CA, Hoffer FA, Wu J, et al. Topotecan is active against Wilms' tumor cells: results of a multi-institutional phase II study. J Clin Oncol. 2007; 25:3130–3136. [PubMed: 17634492]
7. Daw NC, Santana VM, Iacono LC, Furman WL, Hawkins DR, Houghton PJ, et al. Phase I and pharmacokinetic study of topotecan administered orally once daily for 5 days for 2 consecutive weeks to pediatric patients with refractory solid tumors. J Clin Oncol. 2004; 22:829–837. [PubMed: 14990638]
8. Saylors RL 3rd, Stewart CF, Zamboni WC, Wall DA, Bell B, Stine KC, et al. Phase I study of topotecan in combination with cyclophosphamide in pediatric patients with malignant solid tumors: a Pediatric Oncology Group study. J Clin Oncol. 1998; 16:945–952. [PubMed: 9508177]
9. Santana VM, Furman WL, Billups CA, Hoffer F, Davidoff AM, Houghton PJ, et al. Improved response in high-risk neuroblastoma with protracted topotecan administration. J Clin Oncol. 2005; 23:4039–4037. [PubMed: 15961757]
10. National Cancer Institute Cancer Therapy Evaluation Program. Common Toxicity Criteria, Version 2.0. DCTD, NCI, NIH, DHHS; Mar. 1998
11. Simon R. Optimal two-stage designs for phase II clinical trials. Controlled Clinical Trials. 1989; 10:1–10. [PubMed: 2702835]
12. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Amer Statist Assn. 1958; 53:457–481.
13. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep. 1966; 50:163–170. [PubMed: 5910392]

14. Kalbfleisch, JD.; Prentice, RL. Relative Risk (Cox) Regression Models. In: Kalbfleisch, JD.; Prentice, RL., editors. The statistical analysis of failure time data. John Wiley & Sons; New York: 1980. p. 163-188.
15. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat. 1988; 16:1141–1154.
16. Hubbard KE, Schaiquevich P, Bai F, Fraga CH, Miller L, Panetta JC, et al. Application of a highly specific and sensitive fluorescent HPLC method for topotecan lactone in whole blood. Biomed Chromatogr. 2009; 23:707–713. [PubMed: 19277971]
17. Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. N Engl J Med. 1999; 341:1165–1173. [PubMed: 10519894]
18. Ladenstein R, Potschger U, Le Deley MC, Whelan J, Paulussen M, Oberlin O, et al. Primary disseminated multifocal Ewing sarcoma: results of the Euro-EWING-99 trial. J Clin Oncol. 2010; 28:3284–3291. [PubMed: 20547982]
19. Presson A, Moore TB, Kempert P. Efficacy of high-dose chemotherapy and autologous stem cell transplant for recurrent Wilms' tumor: a metanalysis. J Pediatr Hematol Oncol. 2010; 32:454–461. [PubMed: 20505538]
20. Stiff PJ, Agovi MA, Antman KH, Blaise D, Camitta BM, Cairo MS, et al. High-dose chemotherapy with blood or bone marrow transplants for rhabdomyosarcoma. Biol Blood Marrow Transplant. 2010; 16:525–532. [PubMed: 19961947]
21. Bradley MB, Cairo MS. Stem cell transplantation for pediatric lymphoma: past, present, and future. Bone Marrow Transplant. 2008; 41:149–158. [PubMed: 18084337]
22. Dunkel IJ, Khakoo Y, Kernan NA, Gershon T, Gilheeney S, Lyden DC, et al. Intensive multimodality therapy for patients with stage 4a metastatic retinoblastoma. Pediatr Blood Cancer. 2010; 55:55–59. [PubMed: 20486171]
23. Hunold A, Weddeling N, Paulussen M, Ranft A, Liebscher C, Jürgens H. Topotecan and cyclophosphamide in patients with refractory or relapsed Ewing tumors. Pediatr Blood Cancer. 2006; 47:795–800. [PubMed: 16411206]
24. Panetta JC, Schaiquevich P, Santana VM, Stewart CF. Using pharmacokinetic and pharmacodynamic modeling and simulation to evaluate importance of schedule in topotecan therapy for pediatric neuroblastoma. Clin Cancer Res. 2008; 14:318–325. [PubMed: 18172284]
25. Penson RT, Seiden MV, Goodman A, Fuller AF Jr, Berkowitz RS, Matulonis UA, et al. Phase 1 trial of escalating doses of topotecan in combination with a fixed dose of pegylated liposomal doxorubicin in women with müllerian malignancies. Gynecol Oncol. 2004; 93:702–707. [PubMed: 15196868]
26. Chen AY, Choy H, Rothenberg ML. DNA topoisomerase 1-targeting drugs as radiation sensitizers. Oncology (Williston Park). 1999; 13:39–46. [PubMed: 10550825]
27. Cho LC, Choy H. Topoisomerase 1 inhibitors in the combined-modality therapy of lung cancer. Oncology (Williston Park). 2004; 18:29–39. [PubMed: 15255165]
28. Horn B, Reiss U, Matthay K, McMillan A, Cowan M. Veno-occlusive disease of the liver in children with solid tumors undergoing autologous hematopoietic progenitor cell transplantation: a high incidence in patients with neuroblastoma. Bone Marrow Transplant. 2002; 29:409–415. [PubMed: 11919731]
29. Fraser CJ, Weigel BJ, Perentesis JP, Dusenbery KE, DeFor TE, Baker KS, et al. Autologous stem cell transplantation for high-risk Ewing's sarcoma and other pediatric solid tumors. Bone Marrow Transplant. 2006; 37:175–181. [PubMed: 16273111]
30. Cheuk DK, Wang P, Lee TL, Chiang AK, Ha SY, Lau YL, et al. Risk factors and mortality predictors of hepatic veno-occlusive disease after pediatric hematopoietic stem cell transplantation. Bone Marrow Transplant. 2007; 40:935–944. [PubMed: 17768390]
31. Kushner BH, Cheung NK, Kramer K, Dunkel IJ, Calleja E, Boulad F. Topotecan combined with myeloablative doses of thiotepa and carboplatin for neuroblastoma, brain tumors and other poor-risk solid tumors in children and young adults. Bone Marrow Transplant. 2001; 28:551–556. [PubMed: 11607767]

32. de Jonge ME, Huitema AD, Beijnen JH, Rodenhuis S. High exposures to bioactivated cyclophosphamide are related to the occurrence of veno-occlusive disease of the liver following high-dose chemotherapy. Br J Cancer. 2006; 94:1226–1230. [PubMed: 16622453]
33. McDonald GB, Slattery JT, Bouvier ME, Ren S, Batchelder AL, Kalhorn TF, et al. Cyclophosphamide metabolism, liver toxicity, and mortality following hematopoietic stem cell transplantation. Blood. 2003; 101:2043–2048. [PubMed: 12406916]
34. Donato ML, Aleman A, Champlin RE, Weber D, Alexanian R, Ippoliti CM, et al. High-dose topotecan, melphalan, and cyclophosphamide (TMC) with stem cell support: a new regimen for the treatment of multiple myeloma. Leuk Lymphoma. 2004; 45:755–759. [PubMed: 15160952]
35. Kazmi SM, Saliba RM, Donato M, Wang M, Hosing C, Qureshi S, et al. Phase II trial of high-dose topotecan, melphalan, and CY with autologous stem cell support for multiple myeloma. Bone Marrow Transplant. 2011; 46:510–515. [PubMed: 20581887]
36. Litzow MR, Peethambaram PP, Safgren SL, Keeney GL, Ansell SM, Dispenzieri A, et al. Phase I trial of autologous hematopoietic SCT with escalating doses of topotecan combined with CY and carboplatin in patients with relapsed or persistent ovarian or primary peritoneal carcinoma. Bone Marrow Transplant. 2010; 45:490–497. [PubMed: 19648970]
37. Donato ML, Aleman A, Champlin RE, Saliba RM, Wharton JT, Burke TW, et al. Analysis of 96 patients with advanced ovarian carcinoma treated with high-dose chemotherapy and autologous stem cell transplantation. Bone Marrow Transplant. 2004; 33:1219–1224. [PubMed: 15122311]
38. Gilheeney SW, Khakoo Y, Souweidane M, Wolden S, Boulad F, Dunkel IJ. Thiotepa/topotecan/carboplatin with autologous stem cell rescue in recurrent/refractory/poor prognosis pediatric malignancies of the central nervous system. Pediatr Blood Cancer. 2010; 54:591–595. [PubMed: 19998470]
39. Bernbeck B, Bahci S, Meisel R, Troeger A, Schönberger S, Laws HJ, et al. Serial intense chemotherapy combining topotecan, etoposide, carboplatin and cyclophosphamide (RTECC) followed by autologous hematopoietic stem cell support in patients with high risk soft tissue sarcoma (STS). Klin Padiatr. 2007; 219:318–322. [PubMed: 18050041]
40. Pérez-Martinez A, Lassaletta A, González-Vicent M, Sevilla J, Díaz MA, Madero L. High-dose chemotherapy with autologous stem cell rescue for children with high risk and recurrent medulloblastoma and supratentorial primitive neuroectodermal tumors. J Neurooncol. 2005; 71:33–38. [PubMed: 15719272]
41. Pérez-Martinez A, Quintero V, Vicent MG, Sevilla J, Díaz MA, Madero L. High-dose chemotherapy with autologous stem cell rescue as first line of treatment in young children with medulloblastoma and supratentorial primitive neuroectodermal tumors. J Neurooncol. 2004; 67:101–106. [PubMed: 15072454]
42. Crump M, Couban S, Meyer R, Rudinskas L, Zanke B, Gluck S, et al. Phase II study of sequential topotecan and etoposide in patients with intermediate grade non-Hodgkin's lymphoma: a National Cancer Institute of Canada Clinical Trials Group study. Leuk Lymphoma. 2002; 43:1581–1587. [PubMed: 12400600]
43. Steinherz PG, Shukla N, Kobos R, Steinherz L. Remission re-induction chemotherapy with clofarabine, topotecan, thiotepa, and vinorelbine for patients with relapsed or refractory leukemia. Pediatr Blood Cancer. 2010; 54:687–693. [PubMed: 20205253]
44. Tiersten A, Selleck M, Smith DH, Wertheim I, Kaufman E, Hershman D, et al. Phase I/II study of tandem cycles of high-dose chemotherapy followed by autologous hematopoietic stem cell support in women with advanced ovarian cancer. Int J Gynecol Cancer. 2006; 16:57–64. [PubMed: 16445611]
45. McLeod HL, Krynetski EY, Relling MV, Evans WE. Genetic polymorphisms of thiopurinemethyltransferase and its clinical relevance for childhood acute lymphoblastic leukemia. Leukemia. 2000; 14:567–572. [PubMed: 10764140]
46. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science. 1999; 286:487–491. [PubMed: 10521338]

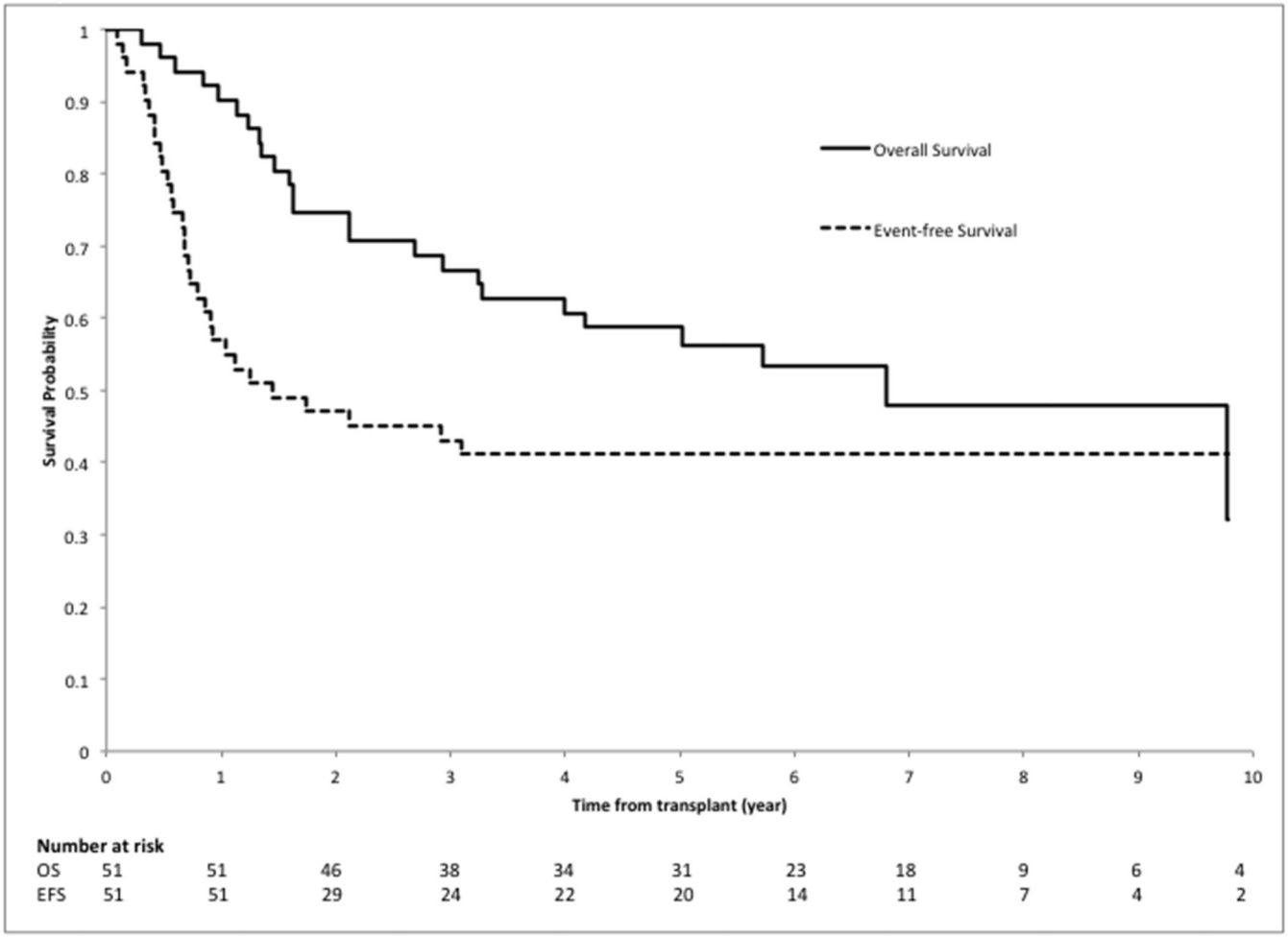

Figure 1.

Overall survival and event-free survival of the 51 participants and the number at risk treated on dose level 1.

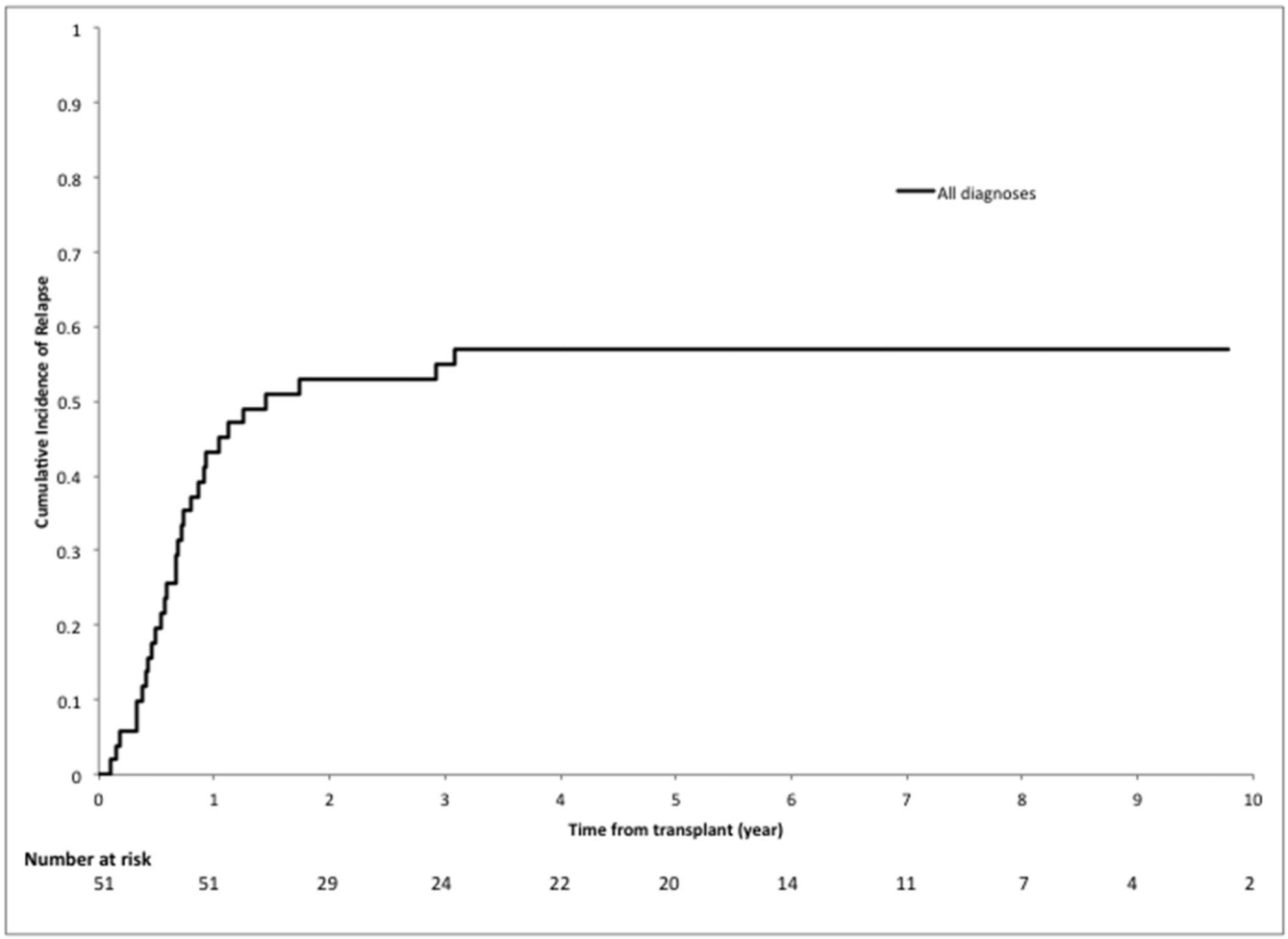

Figure 0002

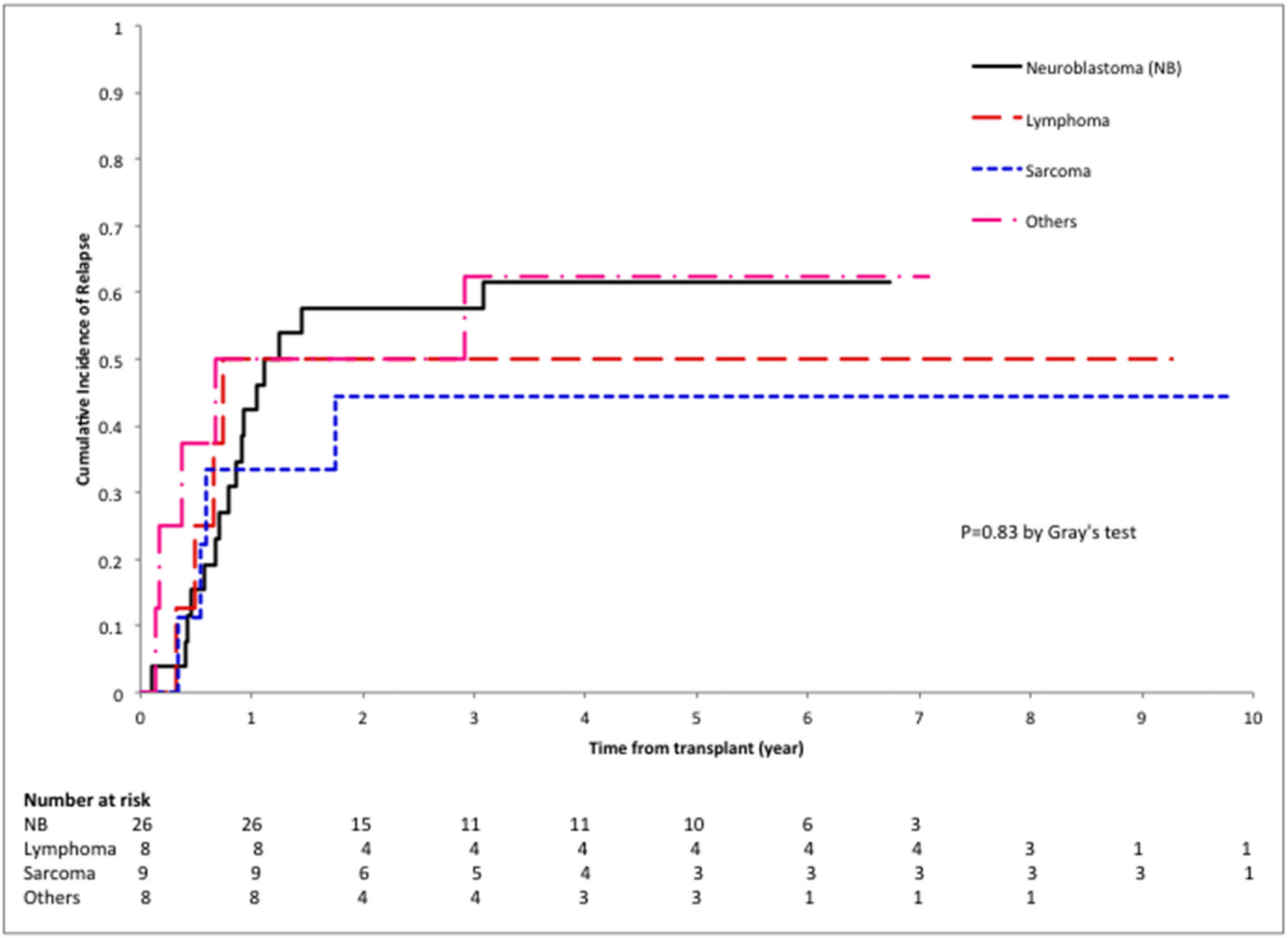

Figure 0003

Figure 2.

A. Cumulative incidence of relapse among the 51 participants treated on dose level 1. B. Cumulative incidence of relapse by disease type and the number at risk. No statistical significance among disease type (p-value = 0.83). Neuroblastoma (NB), lymphoma, and sarcoma were the 3 most common diagnoses treated in this study. The others category included brain tumor, Wilms tumor, and retinoblastoma.

Table 1

Characteristics of the 7 participants who were treated on dose level 2.

Patient	Age at HCT (years)	Gender	Diagnosis	Disease Status at HCT	Stem Cell Graft	DLT	If death, cause
1	14.9	Male	LYMPHOMA	PD	PBSC		
2	20	Male	SARCOMA	CR2	PBSC	Hyperbilirubinemia *	Recurrence or persistence of primary disease
3	4.7	Male	WILMS	CR2	PBSC		Recurrence or persistence of primary disease; Hepatic failure; Respiratory failure
4	14.7	Male	SARCOMA	CR1	PBSC	VOD	Veno-occlusive disease of the liver; MSOF
5	1.8	Male	NB	VGPR	BM		
6	14.4	Male	SARCOMA	PR	PBSC	Hyperbilirubinemia *	
7	1.2	Male	BRAIN TUMOR	PD	BM		Progressive disease

Abbreviations: HCT, hematopoietic cell transplantation; NB, neuroblastoma; PD, progressive disease; CR, complete remission; VGPR, very good partial response; PR, partial response; PBSC, peripheral blood stem cell; BM, bone marrow; DLT, dose limiting toxicity; VOD, veno-occlusive disease; MSOF, multi-system organ failure.

* Hyperbilirubinemia was determined to be a dose limiting toxicity by the study's principal investigator in collaboration with study co-investigators, biostatisticians, and other team members.

Table 2

Patient characteristics of the 51 participants who received the preparative regimen at dose Level 1.

	N	%
Gender		
Female	24	47.1
Male	27	52.9
Age at Transplant	Mean±Std Dev	Median (min-max)
	7.6 ± 6.0	5.1 (1.5 – 21.3)
Diagnosis at Transplant		
NEUROBLASTOMA	26	51.0
LYMPHOMA	8	15.7
SARCOMA	9	17.7
BRAIN TUMOR	5	9.8
WILMS TUMOR	2	3.9
RETINOBLASTOMA	1	2.0
Disease Status at Transplant		
CR1	11	21.6
CR2	7	13.7
CR3	1	2.0
CR4	1	2.0
PD	2	3.9
PR	8	15.7
Rel 1	2	3.9
SD	10	19.6
VGPR	9	17.6
Product Type		
BM	33	64.7
PBSC	18	35.3

Abbreviations: BM, bone marrow; PBSC, peripheral blood stem cell; CR, complete remission; PD, progressive disease; PR, partial response; Rel, relapse; SD, stable disease; VGPR, very good partial response; Std Dev, standard deviation.

Table 3

Maximum grades 1-4 adverse events divided by category.[+] The relationship of the adverse event to the study was defined as definite, possible, probable, or remote. The numbers in the cells represent the number of participants who experienced this maximum grade adverse event and the total number of participants who experienced an adverse event in the specific category.

CATEGORY	MAXIMUM GRADE				TOTAL
	1	2	3	4	
ALLERGY/IMMUNOLOGY	1	5	2	0	8
AUDITORY/HEARING	2	0	1	0	3
APPENDIX VI BMT EVENTS	0	0	2	0	2
BLOOD/BONE MARROW	0	4	10	2	16
CARDIOVASCULAR (ARRHYTHMIA)	1	2	0	0	3
CARDIOVASCULAR (GENERAL)	2	7	5	1	15
CONSTITUTIONAL SYMPTOMS	13	10	3	0	26
DERMATOLOGY/SKIN	4	10	14	0	28
ENDOCRINE	0	2	1	0	3
GASTROINTESTINAL	3	6	9	28	46
HEMORRHAGE	6	0	8	1	15
HEPATIC	2	4	9	1	16
INFECTION/FEBRILE NEUTROPENIA	1	5	32	0	38
METABOLIC/LABORATORY	0	4	16	2	22
NEUROLOGY	3	1	2	0	6
PAIN	4	7	12	0	23
PULMONARY	2	2	9	2	15
RENAL/GENITOURINARY	5	9	3	0	17

[+]AEs were coded according to the Common Toxicity Criteria, version 2.0.

Table 4

Grades 3 and 4 adverse events. The numbers represent the number of patients who experienced this particular adverse event of the indicated grade. For example, among the 51 patients in the analysis sample, 1 experienced grade 3 “Allergic Reaction, Blood Products”, no one experienced grade 4 of this event.

CATEGORY Adverse event	Grade 3	Grade 4
ALLERGY/IMMUNOLOGY		
Allergic Reaction, Blood Products	1	0
Allergic Reaction, Bone Marrow Infusion	1	0
AUDITORY/HEARING		
Otitis media	1	0
Appendix VI BMT Events		
Veno-occlusive disease of the liver	2	0
Blood/Bone Marrow		
Anemia	2	1
Leukopenia	1	1
Neutropenia	3	0
Thrombocytopenia	2	0
Transfusion, packed red blood cells	4	0
Transfusion, platelets	6	0
CARDIOVASCULAR (GENERAL)		
Hypertension	1	0
Hypotension	4	1
CONSTITUTIONAL SYMPTOMS		
Fever without Neutropenia	3	0
DERMATOLOGY/SKIN		
Cellulitis	1	0
Desquamation	1	0
Pruritus	1	0
Rash	11	0
Rash, Generalized	1	0
Skin Excoriation, Hickman Line Site	1	0
Urticaria	1	0
ENDOCRINE		
Syndrome of Inappropriate Anti-Diuretic Hormone	1	0
GASTROINTESTINAL		
Colitis	3	0
Dehydration	1	0
Diarrhea	12	0
Esophagitis	1	0
Loss of Appetite	1	27
Mucositis	2	0

CATEGORY Adverse event	Grade 3	Grade 4
Nausea	2	0
Pancreatitis	1	0
Stomatitis	1	1
Stomatitis and Pharyngitis	0	1
Typhlitis	7	0
Vomiting	3	0
HEMORRHAGE		
Epistaxis	4	0
Hematuria	3	0
Hemorrhagic Cystitis	3	1
HEPATIC		
Alanine Aminotransferase, Abnormal Level	1	1
Aspartate Aminotransferase, Abnormal Level	2	1
GGT, Abnormal Level	1	0
Hepatomegaly	4	0
Hyperbilirubinemia	2	0
LDH, Abnormal Level	1	0
SGPT Measurement, Abnormal Level	1	0
INFECTION/FEBRILE NEUTROPENIA		
Febrile Neutropenia	26	0
Infection, Adenovirus, Stool	4	0
Infection, Candida, Rectum	2	0
Infection, Candida, urine	1	0
Infection, Clostridium Difficile, Stool	1	0
Infection, Coagulase Neg Staph, Nares	1	0
Infection, E. Coli, Blood	1	0
Infection, Enterobacter, Blood/Hickman Line	1	0
Infection, Fungal, Disseminated	1	0
Infection, Gamma-hemolytic Strep, Blood	1	0
Infection, Herpes zoster dermatitis	1	0
Infection, Human Herpes Simplex Virus, Oral	2	0
Infection, Pseudomonas, Stool	2	0
Infection, Staphylococcus Epidermidis, Blood	1	0
Infection, Staphylococcus, Hickman Catheter	1	0
METABOLIC/LABORATORY		
Amylase Measurement, Abnormal Level	1	0
Hyperglycemia	4	0
Hyperuricemia	1	0
Hypocalcemia	1	0
Hypokalemia	16	3
Hypophosphatemia	6	0

CATEGORY Adverse event	Grade 3	Grade 4
Lipase Measurement, Abnormal Level	1	0
NEUROLOGY		
Hallucinations	2	0
PAIN		
Abdominal Pain	6	0
Headache	1	0
Pain, Back	1	0
Pain, Generalized, Multiple Sites	3	0
Pain, Skin	1	0
Pain, radiation related	1	0
PULMONARY		
Dyspnea	1	0
Hypoxia	4	1
Infiltrates, Pulmonary	0	1
Pleural effusion	3	0
Pneumonia	3	1
Pneumonitis	1	0
Pulmonary Nodules	1	0
Pulmonary edema	1	0
RENAL/GENITOURINARY		
BUN, elevated	1	0
Renal failure	1	0
Spasm, Bladder	1	0