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Peripheral Blood Stem Cell Support for Multiple Cycles of Dose Intensive Induction Therapy is Feasible with Little Risk of Tumor Contamination in Advanced Stage Neuroblastoma; A Report from the Childrens Oncology Group

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Abstract

Background—Poor outcome in Stage 4 neuroblastoma may be improved with increased dose intensity of therapy. We investigated the feasibility of sequential collection and infusion of peripheral blood stem cells (PBSC) as hematopoietic support for non-myeloablative dose intensive induction chemotherapy given every 21-28 days.

Methods—Twenty-two children with Stage 4 neuroblastoma (≥ 1 yr of age) received 2 cycles of high dose cyclophosphamide (4 gm/m²), doxorubicin (75mg/m²) and vincristine (2mg/m²) followed by 3 cycles of interpatient dose escalating carboplatin (dose level 0 = 800 mg/m²; dose level 1 = 1000 mg/m²), high dose cyclophosphamide (4 gm/m²) and etoposide (600 mg/m²). PBSC were harvested following cycle 2, 3, and 4 in Cohort 1 and infused after each subsequent cycle. In Cohort 2, PBSC were harvested after cycle 2 and split into 3 aliquots for infusion. Dose limiting toxicity (DLT) and ability to administer cycles within 28 days was assessed.

Results—Sufficient PBSC ($\geq 2 \times 10^6$ CD34 cells/kg per infusion) were collected from 17/21 eligible patients with minimal toxicity and no detectable neuroblastoma cells by immunocytology. Carboplatin at 1000 mg/m² resulted in DLT of delayed platelet recovery > 28 days in 4/8 patients. Despite de-escalation to 800 mg/m², platelet DLT occurred in 4/7 Cohort 1 and 3/7 Cohort 2 patients.

Conclusion—As defined in this protocol, doses of carboplatin were not tolerable with the PBSC dose administered. However, it was feasible to collect sufficient PBSC from small neuroblastoma patients to use as hematopoietic support with minimal risk of tumor contamination and toxicity.

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Keywords

neuroblastoma; peripheral blood stem cell support; dose intensity; carboplatin

INTRODUCTION

Neuroblastoma is the most common extracranial solid tumor in children with more than half presenting with metastatic disease categorized as high risk. Incremental increases in chemotherapeutic dose intensity and consolidation with bone marrow transplant have improved induction response rate and event free survival (EFS), respectively.^{1, 2, 3}

Improving induction response rate increases EFS and survival for patients with high risk neuroblastoma. High-risk patients who achieve complete remission after five cycles of induction therapy have a significantly improved long-term survival.⁴ A retrospective review of 1,592 children with disseminated neuroblastoma demonstrated that dose escalation of the most active agents during induction correlated with significant improvements in induction response and survival.^{5, 6} Further chemotherapy dose intensification through either dose escalation or chemotherapy cycle time compression is primarily limited by hematopoietic toxicity. Reports that autologous peripheral blood stem cell (PBSC) infusions could shorten the period of myelosuppression and allow cycle-compression prompted the development of this trial. In non-myeloablative adult chemotherapy regimens, particularly those incorporating carboplatin and cyclophosphamide, infusion of PBSC shortened the period of myelosuppression and allowed cycle-compression to 14-21 days.^{7, 8}

Prior to this trial, there has been limited pediatric collaborative group experience collecting and infusing PBSC as support for submyeloablative induction regimens. Therefore we designed a 5 drug induction chemotherapy regimen utilizing agents with documented efficacy in neuroblastoma. We chose to escalate only carboplatin because the major dose limiting toxicity of this agent is myelosuppression^{9, 10}. While non-hematopoietic toxicity is tolerable, we recognize that long term side effects occur^{11, 12}. Further dose escalation of cyclophosphamide has potential organ toxicity while both cyclophosphamide and etoposide increase the risk of secondary malignancy^{13, 14, 15}.

In this phase I study, we sought to evaluate the feasibility of repeated PBSC collection and infusion with dose intensive non-myeloablative induction in small children with stage 4 neuroblastoma.

METHODS

Patients

Patients age 1-21 years with newly diagnosed Stage 4 or surgery-only treated low stage disease that progressed to stage 4 at ≥ 1 year of age with weight ≥ 10 kg were diagnosed by tumor histology or presence of tumor in bone marrow with elevated catecholamines¹⁶ at 8 Childrens Cancer Group approved hematopoietic cell transplant centers (listed in appendix I) between May 1996 and October 2001. Patients had normal renal, cardiac, liver function and bone marrow function and no prior systemic chemotherapy or non-emergent radiation therapy. Written informed consent of parent/guardian was obtained according to National Cancer Institute and institutional IRB guidelines.

Treatment

Protocol therapy consisted of three phases: Induction I (cycles 1 and 2 of chemotherapy)¹⁷, Induction II (cycles 3 through 5 of chemotherapy) and Local Control (surgery and radiation

therapy) (Figure 1). Induction II cycles were administered over 3 days. Agents were given as follows: cyclophosphamide 2 gm/m²/day IV over 4 hours with mesna on Day 1 and 2, etoposide 200 mg/m²/day IV over 4 hours on Day 1, 2, and 3, and carboplatin 50% of assigned dose/day IV over 1 hour on Days 1 and 2. Cycles were planned every 21 days once absolute neutrophil count (ANC) >750/ul and platelets > 75,000/ul. Patients completing this induction protocol therapy and those off protocol were allowed to receive consolidation therapy at investigator choice, including myeloablative transplant regimens.

An interpatient carboplatin dose escalation schema was employed after Induction I (Table I). A cohort of 6 patients was accrued per dose level. If ≤1 patient experienced dose limiting toxicity (DLT) during Induction II the dose was considered tolerable and proceeded. If 2 of the 6 experienced DLT, another 6 patients were accrued at same dose level prior to escalation. If ≥3 of the 6 patients experienced DLT this dose was considered too toxic and decreased for the next cohort. The maximum tolerated dose was defined as the level just below the one producing unacceptable DLT.

Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria version 1¹⁸ and DLTs were defined as: recovery of ANC>750/ μl and platelets>75,000/μl after Day 28 of the prior cycle; non-hematologic grade 4 toxicity (excluding fever, infection, elevated transaminases, renal electrolyte wasting, vomiting, diarrhea, or stomatitis which resolved to Grade 1 by Day 28, or grade 3 or 4 pulmonary or cardiac toxicity if due to infection and resolved to grade 1 by day 28); grade 3 toxicity not reversed by day 28 (excluding renal electrolyte wasting, blood pressure changes, or fever/infection if medically controlled to less than grade 1 by day 28); Grade 3 cardiac toxicity even if it resolved by day 28; stomatitis requiring intubation, severe diarrhea resulting in inability to maintain albumin and or serum volume despite medical management; decrease in glomerular filtration rate (GFR)<60 ml/min/1.73m² on day 28 and PBSC collection yield inadequate to support upcoming Induction II cycles.

Patients with hematologic DLT during Induction II who met hematopoietic recovery criteria between days 29-42 received a carboplatin dose reduction of 25% in subsequent Induction II cycles and were removed from protocol therapy if they experienced recurrent DLT or did not meet criteria for further chemotherapy by cycle day 35. Patients not meeting criteria for subsequent therapy by cycle day 42 were removed from protocol therapy unless hematopoietic toxicity was due to marrow infiltration. Patients were also removed from protocol therapy for persistent GFR<60ml/min/1.73m², cardiac ejection fraction <50, shortening fraction <28, or persistent grade 2 dysrhythmia, grade 3 congestive heart failure or grade 4 cardiac toxicity not related to infection or metabolic disturbance, any Grade ≥3 non-hematologic toxicity persisting 6 weeks, severe apheresis procedure toxicity preventing collection of required PBSC cell dose, or insufficient PBSC for re-infusion.

PBSC apheresis and re-infusion

High volume leukopheresis procedures (processing 6 blood volumes) were performed according to institutional guidelines on continuous flow cell separators via double lumen apheresis lines. Priming with irradiated, leukocyte-poor red blood cells was recommended for patients less than 25 kg. PBSC were cryopreserved using 10% dimethyl sulfoxide. Participating institutions could use circulating CD34+ cells to determine timing of PBSC collection once protocol directed collection criteria were attained.

For Cohort 1 (Dose Level 1 and Dose Level 0, Figure 1.), PBSC collection was conducted prior to each Induction II cycle and infused following the subsequent cycle. Mononuclear cells (MNC) and CD34+ cell yields were measured by flow cytometry. A minimum of 2 × 10⁶ CD 34+ cells/kg were required per reinfusion. For Cohort 2 (Dose Level 0A, Figure 1),

the protocol was amended to employ a single PBSC collection series (minimum of 6×10^6 CD34+/kg) split into 3 aliquots for infusion after each Induction II cycle. PBSC apheresis began when ANC $\geq 1,000$ and platelet count sustained $\geq 20,000$ without transfusion for three consecutive days following count nadir. In Cohort 1, apheresis was performed daily for up to 3 days and for Cohort 2 apheresis was performed up to five consecutive days until the minimum CD34+ cells/kg per cycle was obtained. PBSC infusion was done 48-72 hours after the completion of each Induction II cycle.

Disease status

Disease status was evaluated at the end of each induction phase using the Neuroblastoma International Response Criteria¹⁶. Patients with progressive disease (PD) were removed from protocol therapy. Tumor content in bone marrow, peripheral blood, and PBSC products were analyzed by immunocytochemical analysis as previously described with sensitivity of 1 tumor cell per 100,000 total nucleated cells (1 per 40,000 isolated mononuclear cells) prior to September 2000¹⁹ and 1 tumor cell per 1,000,000 total nucleated cells (1 per 400,000 isolated mononuclear cells) after September 2000²⁰.

Local control

All patients without PD proceeded to delayed surgical resection of residual disease amenable to resection. Hyperfractionated radiation therapy of 2100cGy (1.5 Gy per fraction given BID for 7 days) was administered to the primary site regardless of tumor response or degree of surgical resection. Radiation therapy was given to any residual metastatic disease site identified by positive MIBG scan at end of Induction II.

Statistical Considerations

The primary study aim was to identify an MTD of carboplatin based on the occurrence of DLTs using a sequential dose escalation design with six patients per cohort at a given dose level. Other study objectives were to be assessed in a descriptive fashion; the study was not powered for inferential analyses.

A Wilcoxon rank-sum test was used to test for differences in the number of days of PBSC collection between dose levels. Kaplan-Meier survival rates²¹ with standard errors per Peto²² were calculated and plotted, and quoted in the text as the rate $\pm$ standard error. For EFS, time to event was calculated as the time from study enrollment until the time of first occurrence of relapse, progression, secondary malignancy, or death, or until the time of last contact if no event occurred. For overall survival (OS), time to event was calculated as the time from study enrollment until the time of death, or until the time of last contact if the patient was alive.

RESULTS

Patient Characteristics

From 5/15/96 to 10/1/01, 22 children were enrolled and met the eligibility criteria. Patient characteristics are summarized in Table II. Median age at diagnosis was 33.5 months (range 14-106 months) with a median patient weight of 14.25 kg (range 10 – 25.4 kg).

Treatment Intensification: Carboplatin Dose Escalation

Induction II therapy was initiated in 21 of 22 eligible patients who completed Induction I. Eight patients were assigned to Dose level 1 (Table I) and 7 received Induction II therapy. One patient developed PD and was removed from protocol prior to first apheresis. Five of the 7 patients at Dose Level 1 experienced DLT (4 delayed platelet recovery; 2 with

concomitant inadequate PBSC yield and 1 inadequate PBSC yield alone). No non-hematologic DLT were observed. One patient died of pneumonia during bone marrow transplant after completing protocol therapy. As more than 3 of the patients on Dose Level 1 experienced DLT, dose was de-escalated to Dose Level 0.

Four of the 7 patients assigned to Dose Level 0 in Cohort 1 experienced DLT of delayed platelet recovery of which one had PD and one received less than the required infusion of 2×10^6 CD34+ cells/kg.

Mean time to begin apheresis was Day 20 and lasted average of 1.87 days. Recovery to ANC ≥ 500 and plt $\geq 75,000$ occurred a mean of 6.3 days after apheresis complete. Apheresis may have delayed recovery to platelets $>75,000$ before Day 28 as the patients experienced a decline of platelet number coincident with PBSC apheresis. To minimize this effect, the apheresis schedule was amended to conduct a single high-volume PBSC apheresis series after Induction I, cycle 2 (Figure 1, Cohort 2.).

Seven patients were enrolled into Cohort 2 and received carboplatin 800mg/m² (Table I, Dose Level 0a). All had adequate PBSC yield for three infusions. Three experienced DLT of delayed platelet recovery. No other DLTs were noted. As the protocol defined a dose level to be considered too toxic if 3 or more patients experienced DLT, the proposed treatment plan was deemed infeasible and the trial was concluded. An MTD was not determined using the specified interval.

PBSC Cell Yields and Engraftment

CD34/kg yields were adequate in the 21 patients collected after Induction I, cycle 2 (Table III). Among the 26 sequential collections performed in 14 patients (Cohort 1, Dose Levels 0 and 1), 4 patients had inadequate PBSC yield. Single high volume collections (Cohort 2, Dose level 0A) were all successful and required fewer days of collection to meet the required PBSC yield than did those patients who underwent sequential collections (Cohort 1) following Dose Level 0. The median CD34/kg $\times 10^6$ cell dose infused and median days to hematopoietic recovery following Cycle 3 (first cycle of Induction II) are shown in Table IV. For all Induction II cycles, median time to neutrophil recovery $> 750/\mu\text{l}$ and platelets $> 75,000/\mu\text{l}$ were 15 days (12 - 21) and 24 days (14 - 41) respectively for Cohort 1 and 16 days (13 - 20) and 28 days (20 - 42) for Cohort 2. None of the 46 PBSC products tested were positive for tumor contamination by immunocytologic testing despite persistent morphologic marrow disease after Induction I therapy in 9 of 21 patients.

Non-Dose Limiting Toxicity

All patients experienced Grade 3-4 hematologic toxicity. Non-hematologic toxicity during the 44 cycles of Induction I and 56 cycles of Induction II is depicted in Table V. Among the six patients with pulmonary dysfunction in Induction I, two were intubated, one each from tumor compression and CMV pneumonitis. Toxicity from PBSC infusion was tolerable with nausea and vomiting most common (16/57 infusions). Other PBSC toxicity included facial flushing (4/57), cough (5/57), shaking or chills (2/57), hypertension (1/57) and headache (2/57).

Response and Survival

Sixty-four percent of patients (14/22) achieved at least a PR by the end of Induction I and one patient progressed. Eleven of 16 patients (69%) who completed Induction II were CR, VGPR, or PR prior to local control, of whom 4/16 (25%) achieved CR /VGPR with chemotherapy. Two patients were not evaluated at the end of Induction II chemotherapy, however both were CR when evaluated after completing surgery for local control.

The overall 3-year EFS and OS rates (n=22) are $20\% \pm 10\%$ and $26\% \pm 11\%$, respectively (Figure 2). Of note, 12 of 15 patients who completed protocol therapy without PD underwent myeloablative therapy with stem cell transplant and nine of these 12 reported receiving 13-cis-retinoic acid post-transplant.

DISCUSSION

Severe myelosuppression has been dose-limiting for many intensified treatment regimens and led to the investigation of PBSC support as a means of mitigating hematologic toxicity²³⁻²⁷. We sought to assess the feasibility of utilizing sequential reinfusions of autologous PBSC to allow administration of dose intensive non-myeloablative induction cycles every 28 days. The primary aim was to determine an MTD of carboplatin, with fixed dose cyclophosphamide and etoposide in the treatment of children with advanced neuroblastoma. Within the context of this study, PBSC re-infusion at protocol specified dose was not sufficient to permit administration of induction cycles every 28 days due to prolonged thrombocytopenia.

This study provides important data regarding the technique, timing, and applicability of PBSC harvest and reinfusion in the treatment of young patients with advanced neuroblastoma. Prior to beginning this study, apheresis and reinfusion had been utilized effectively in patients as small as 20 kg, but very limited data existed regarding the use in smaller children²⁸. In this study, 21/22 patients weighed less than 17.5 kg. Apheresis was accomplished with acceptable toxicity. Another key finding of this study was that the PBSC apheresis schedule could be amended from three sequential collections to a single large-volume apheresis. It was possible to perform PBSC collection after 2 induction cycles to obtain the required CD34/kg yield. This could be done without immunocytochemical evidence of PBSC tumor contamination, regardless of BM disease status at the time of apheresis. Theoretically, future studies utilizing a single large volume apheresis series early in induction would reduce the number of apheresis procedures, minimize the impact of platelet depletion known to occur with apheresis²⁹, and potentially provide an extra aliquot of PBSC for use with novel therapy in the event of relapse³⁰.

As this was a phase I dose escalation study of induction chemotherapy, followed by transplant on other protocols after study completion, it was not designed to evaluate survival. Sixty-nine percent (11/16) of patients completing Induction II had either a CR/VGPR (4) or PR (7) following chemotherapy alone. Unlike most induction regimens, response was assessed prior to delayed surgical resection and radiation to the primary tumor. Given that response rates on prior CCG or POG studies have measured outcomes based on the combination of chemotherapy and local control, it is difficult to compare the response rates on this protocol to historically reported data. It is also difficult to compare EFS/OS to historical data as this protocol only included patients with Stage 4 disease > 365 days of age and patients did not receive uniform therapy following completion of the A3951 regimen.

There are two major implications of this study. First, sequential PBSC collection and reinfusion is possible in young patients weighing less than 20 kg. A single large-volume apheresis after 2 induction chemotherapy cycles provides an adequate tumor-free yield to support multiple reinfusions. Second, within the parameters defined by this study the carboplatin doses in combination with cyclophosphamide and etoposide were not tolerable using the administered PBSC doses. Perhaps an agent associated with less thrombocytopenia, such as topotecan, may be a more feasible candidate for dose escalation. In the immediate future, high dose chemotherapy remains a key element in the treatment of neuroblastoma, however this research did not address the urgent need for new approaches to treat high risk disease.

The results of this trial were integral in developing a standardized collection and re-infusion procedure for peripheral blood stem cells in small pediatric cancer patients. These results proved instrumental in the design and implementation of a large, multi-center, randomized Phase III trial in the Children's Oncology Group, COG A3973, in which PBSC apheresis was performed following the second cycle of induction chemotherapy.

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Figure 1.
CCG 3951 therapy schema.

Figure 2. Kaplan-Meier event-free survival (EFS) and overall survival (S) curves for Stage 4 neuroblastoma patients from diagnosis treated with CCG 3951 intensified induction and peripheral blood stem cell support (N=22).

Table I

Carboplatin Dose Levels

Cohort	Dose Level (N = # per level)	Total Dose per Cycle	
		mg/m ² for patients > 12 kg:	mg/kg for patients < 12 kg:
1	0 (N=7)	800	26.6
	1 (N=7)	1000	33.4
2	0a (N=7)	800	26.6

Table II

Patient Characteristics

Level	Age (months), Gender	Wt (kg)	Disease Sites	Histology ³¹ /MYCN	Adequate PBSC Yield (Yes/No)	DLT (cycle/harvest # occurred)	BM Status Cycle 2	PBSC Immuno (# pos /tested)	Response End Induction I	Response End Induction II	EFS
1	20, M	11	A, BM, B	UH/ND	Yes	PLT (1, 2)	Pos	0/3	PR	Off	DOD
1	31, M	13.7	A, BM, B	UH/amp	Yes	-	Neg	0/3	PR	CR*	DOD
1	60, F	17.2	A, BM, B	ND	No	PLT (1) PBSC (1)	Neg	0/5	PR	off	CCR
1	25, M	10.4	A, B, LN	UH/amp	Yes	-	Neg	0/2	PR	PR	DOT ⁺
1	54, M	14.8	A, BM, B, LN	ND	Yes	PLT (2, 3)	Pos	0/3	PR	PD	DOD
1	14, M	10.2	A, BM, B	ND	N/A	N/A	Pos	N/A	PD	-	DOD
1	15, M	13	C, BM, B	FH/amp	No	PLT (1) PBSC (2)	Neg	0/5	PR	off	DOD
1	93, F	21.7	RP, BM, B, LN	ND/non	No	PBSC (2)	Neg	0/4	PR	off	CCR
0	32, M	17.3	A, BM, B, LN, L	ND	Yes	PLT (1)	Pos	0/1	MR	PD after Cycle 5	DOD
0	35, F	16	A, BM, B, L, LN	ND	Yes	PLT (3)	Pos	0/4	PR	PR	DOD
0	31, M	11.2	C, BM, B	UH/amp	Yes	-	Neg	0/2	MR	PR	DOD
0	106, M	25.4	A, BM, B, LN	ND	Yes	-	Neg	ND	MR	VGPR	DOD
0	20, M	17	A, RP, BM, B, L	UH/amp	No	PLT (3) PBSC (3)	Neg	0/2	SD	MR	DOD
0	39, M	16	A, BM, B	UH/non	Yes	-	Pos	0/2	MR	MR	DOD
0	38, M	15.2	A, BM, B, LN	UH/non	Yes	PLT (3)	Neg	0/6	PR	CR*	DOD

Level	Age (months), Gender	Wt (kg)	Disease Sites	Histology ³¹ /MYCN	Adequate PBSC Yield (Yes/No)	DLT (cycle/harvest # occurred)	BM Status Cycle 2	PBSC Immuno (# pos /tested)	Response End Induction I	Response End Induction II	EFS
0A	50, M	15.9	A, BM, B, L	UH/ non	Yes	-	Pos	ND	MR	PR	DOD
0A	20, F	10	A, BM, B, L	UH/ non	Yes	PLT (2, 3)	Neg	ND	SD	PR	DOD
0A	29, F	12.9	A, BM, B	FH/ non	Yes	-	Neg	ND	PR	CR	CCR
0A	35, M	13.5	A, BM, B	UH/ amp	Yes	PLT (3)	Neg	0/3	PR	PR	CCR
0A	25, M	14	RP, LN	UH/ ND	Yes	PLT (2, 3)	Neg	ND	PR	VGPR	CCR
0A	36, M	14.5	A, BM, B	UH/ND	Yes	-	Pos	ND	PR	VGPR	DOD
0A	46, F	13.7	A, BM, B, LN	UH/ ND	Yes	-	Pos	0/1	PR	PR	CCR

A= adrenal primary, C= celiac primary, RP=retroperitoneal primary, BM= bone marrow, B= bone, LN= lymph node, FH= favorable Shimada histology, UH= unfavorable Shimada histology, amp = amplified MYCN, non= non-amplified MYCN, PLT = dose limiting toxicity of platelet recovery, PBSC= dose limiting toxicity of inadequate PBSC collection, DOD = dead of disease, DOT= dead of toxicity, N/A= not applicable, ND= not done.

* patients not evaluated for response until after surgery for local control

+ patient died of toxicity (pneumonia) during non protocol BMT one month after completing protocol therapy.

Table IIIMedian CD34/kg $\times 10^6$ cell yields from PBSC apheresis

Cohort	Dose Level	Collection 1 (Range)	Collection 2 (Range)	Collection 3 (Range)
1	1 (N=7)	25.8 (5.7 – 960)	8.8 (0.97 - 28.2)	6.0 (2.9 - 7.7) *
	0 (N=7)	30.6 (4.3 - 163)	7.1 (2.6 - 22.4)	4.5 (1.3 – 17.8)
2	0A (N=7)	15.7 (7.5 - 24)	NA	NA

* Four patients collected at this time point

Table IV

Median Hematopoietic Recovery and Median $CD34 \times 10^6$ Cell Dose Infused by Dose Level Following Cycle 3

Cohort	1		2
Dose Level	1 N=7	0 N=7	0A N=7
CD 34/kg infused (range)	9.2 (3.4 - 20)	10.0 (4.4 - 86)	4.9 (1.8 - 8.1)
Days to ANC>750 (range)	16 (14 - 20)	14 (12 - 21)	16 (13 - 20)
Days to PLTS>75K (range)	22 (18-39)	24 (14 - 28)	25 (16 - 28)
Days to Next cycle (range)	29 (26-40)	26 (24 - 32)	34 (26 - 40)

Table V

Non-Hematologic Toxicity

Episodes Induction I Toxicity (N=44 cycles)	
Grade 3 or 4 Non-Hematologic Toxicity	Number
Stomatitis	9
Pulmonary	6
Nausea/vomiting	3
Abdominal pain	2
Constipation	2
Diarrhea	2
Hematuria	1

Episodes Induction II Toxicity (N = 56 cycles)	
Grade 3 or 4 Non-Hematologic Toxicity	Number
Infection	6
Nausea/ vomiting	6
Transaminitis	5
Diarrhea	4
Abdominal pain	3
Pulmonary	3
Decreased creatinine clearance	2
Hematuria	2
Cardiac Dysrhythmia	1