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Phase I Trial of Dasatinib, Lenalidomide, and Temozolomide in Children with Relapsed or Refractory Central Nervous System Tumors

Nathan J. Robison, MD*, Kee Kiat Yeo, MD*, Adrian P. Berliner, BS, Jemily Malvar, MS, Michael A. Sheard, PhD, Ashley S. Margol, MD MS, Robert C. Seeger, MD, Teresa Rushing, PharmD, Jonathan L. Finlay, Mb ChB, Richard Sposto, PhD, and Girish Dhall, MD

Children's Center for Cancer and Blood Diseases, Division of Hematology, Oncology and Blood & Marrow Transplantation, Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA (NJR, KKY, JM, MAS, AM, RCS, TR, RS, GD), Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA (ABP), Nationwide Children's Hospital and The Ohio State University, Columbus, OH (JLF)

Abstract

Background—Single agent studies targeting the tumor microenvironment in central nervous system (CNS) tumors have largely been disappointing. Combination therapies targeting various pathways and cell types may be a more effective strategy. In this phase I study, we evaluated the combination of dasatinib, lenalidomide, and temozolomide in children with relapsed or refractory primary CNS tumors.

Methods—Patients 1–21 years old with relapsed or refractory CNS tumors were eligible. Starting doses of dasatinib and lenalidomide were 65 mg/m²/dose twice daily and 55 mg/m² once daily, respectively, while temozolomide was constant at 75 mg/m² daily. The study followed a 3+3 phase I design, with a 4-week dose-limiting toxicity (DLT) evaluation period. Serial peripheral blood lymphocyte subsets were evaluated in consenting patients.

Results—Fifteen patients were enrolled and thirteen were DLT-evaluable. DLTs occurred in 5 patients, including somnolence and confusion (1 patient), hypokalemia (1 patient) and thrombocytopenia (3 patients). The maximum tolerated dose for the combination was dasatinib 65 mg/m² twice daily, lenalidomide 40 mg/m² daily, and temozolomide 75 mg/m² daily, for 21 days followed by 7 days rest in repeating 28-day cycles. Transient increases in natural killer effector cells and cytotoxic T-cells were seen after 1 week of treatment. One out of 6 response-evaluable patients showed a partial response.

Corresponding Author: Nathan J. Robison, MD, Children's Hospital Los Angeles, 4650 Sunset Boulevard, MS #54, Los Angeles, CA, 90027. Telephone: 323-361-8147, Fax: 323-361-7128, nrobison@chla.usc.edu.

*Both authors contributed equally to the manuscript

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Conclusions—The combination was feasible and relatively well tolerated in this heavily pre-treated population. The most common toxicities were hematologic. Preliminary evidence of clinical benefit was seen.

Keywords

Child; brain neoplasms; central nervous system neoplasms; tumor microenvironment; antiangiogenic agents; clinical trial, phase I

Introduction

Central nervous system (CNS) tumors are the most common malignancy in children [1]. While significant advances have been made in the understanding of brain tumor biology, prognosis for many pediatric brain tumors remains poor, and the burden of treatment-related morbidity high [2]. The development of new therapies that improve survival and minimize long-term sequelae is essential [3].

Conventional therapeutic concepts in pediatric brain tumors have typically centered on dose-intensive chemotherapy and/or radiation therapy to maximize cell kill. While these strategies have been effective for select diagnoses, they are associated with significant toxicity. Extended treatment-free intervals, required to allow recovery from cytotoxic chemotherapy-related side effects, are implicated in re-growth of tumor and endothelial cells and in development of drug resistance.

The tumor microenvironment is an attractive alternative target [4]. Tumor cells interact with a complex network of epithelial cells, fibroblasts, endothelial cells, pericytes, and tumor-infiltrating immune cells to support proliferation and metastasis [3–8]. Multiple microenvironment-targeting strategies have been developed, including drugs targeting epithelial cell proliferation, monoclonal antibodies or small-molecular inhibitors of pro-angiogenic signaling cascades, and immunomodulatory agents [3, 4, 9]. Single-agent studies in CNS tumors, however, have largely been disappointing [10]. The complexity of tumor-promoting microenvironmental interactions likely limits the potential efficacy of a unimodal approach. A multi-modal strategy targeting multiple components of the microenvironment may therefore be more effective [3, 11–13].

Dasatinib is a small molecule inhibitor that exhibits cell cycle regulation and anti-angiogenic functions through the inhibition of several kinases and growth factor receptors [14–16]. It is known to cross the blood-brain barrier and is postulated to have several anti-tumor immunomodulatory effects [17, 18]. Lenalidomide is another promising agent that acts through the inhibition of angiogenesis by blocking VEGF-induced PI3k-AKT pathway signaling, inhibition of IL-6 and endothelial cell function [6]. Additionally, lenalidomide can induce cell apoptosis and immunomodulate by stimulating T-cell proliferation, decreasing expression of immune-checkpoint inhibitor on both T and NK cells, inhibit TNF-alpha and decrease T-regulatory cells [5, 6, 19–21]. Temozolomide on the other hand is an alkylating chemotherapeutic agent, used in a variety of solids and CSN tumors and has excellent blood-brain barrier penetration. When given metronomically, it has been shown to also have anti-angiogenic activity and selectively deplete T-regulatory cells [7, 8, 22–24]. Several

preclinical studies, including glioma models, have shown enhanced immunomodulatory and cytotoxic activity of dasatinib when combined with temozolomide or lenalidomide [25, 26]. Additionally, these three drugs have been used safely in a variety of different combinations for CNS malignancies in several early phase clinical trials [27–29].

We report the results of a phase I dose-escalation study of dasatinib, lenalidomide, and metronomic temozolomide in children with relapsed, progressive or refractory CNS tumors. The trial's primary objectives were to determine the maximum tolerated dose (MTD) of dasatinib and lenalidomide in combination with a fixed dose of temozolomide, and to describe the combination toxicity profile in children with recurrent or progressive poor-prognosis CNS tumors. We measured peripheral blood lymphocyte-subsets to evaluate the regimen's immunomodulatory effects.

Materials and Methods

The Children's Hospital Los Angeles institutional review board approved the trial. Written informed consent and assent were obtained from all individual participants included in the study and their legal guardians according to institutional guidelines. The study was open to accrual from January 29, 2010 to January 15, 2013. An independent Data Safety Monitoring Committee (DSMC) monitored the trial conduct.

Eligibility

Children aged 1 – 21 years with refractory or progressive primary CNS tumors for which there were no standard treatment options were eligible. Histological confirmation was required, except for patients with brainstem or optic pathway glioma. Inclusion criteria included performance status > 50 (Karnofsky or Lansky for patients > 16 years, respectively), life expectancy > 8 weeks, and adequate bone marrow function (absolute neutrophil count $> 1000/\mu\text{L}$, platelet count $> 100\,000/\mu\text{L}$, hemoglobin $> 8.0\text{ g/dL}$), renal function (age-adjusted normal serum creatinine or glomerular filtration rate $> 70\text{ mL/min/1.73 m}^2$), and liver function (total bilirubin $< 2\times$ and aspartate and alanine aminotransferase $< 3\times$ institutional upper limit of normal for age). Patient must not have received marrow suppressive chemotherapy within 4 weeks (6 weeks for nitrosureas), biologic agents within 2 weeks, focal irradiation within 1 month, and craniospinal irradiation or autologous hematopoietic cell transplantation within 3 months prior to enrollment. Exclusion criteria included concurrent use of enzyme-inducing anticonvulsants or anticoagulant therapy, evidence of intratumoral hemorrhage, gastrointestinal bleeding, pregnancy or lactation, and a history of coronary artery disease.

Study Treatment and Dose Escalation

This study followed a modified standard 3+3 dose escalation plan (Supplement 1, Table 1) [30]. In brief: only dasatinib and lenalidomide were subject to dose escalation. The starting doses were dasatinib $65\text{ mg/m}^2/\text{dose}$ twice daily and lenalidomide 55 mg/m^2 daily, both 80% of the single-agent MTD established in Children's Oncology Group phase I studies [14, 20]. Temozolomide dose was 75 mg/m^2 daily. All 3 drugs were given for 21 days, followed by 7 days' rest. Each cycle was 28 days. For patients who were unable to swallow pills,

lenalidomide capsule contents were sprinkled onto applesauce prior to administration, and dasatinib and temozolomide were administered as extemporaneously prepared oral solution. Dasatinib was dissolved (not crushed) in liquid and temozolomide solution was prepared as described by Trissel et al [31].

In the initial design, the DLT-evaluation period was 12 weeks (3 cycles). Participants received dasatinib with temozolomide for the first cycle, lenalidomide with temozolomide for the second cycle, and all three drugs for the third cycle. After four patients had been enrolled and completed the DLT evaluation period, the protocol was amended per DSMC recommendation (Amendment 1): the first 2 cycles were omitted, such that subsequent participants began all 3 drugs simultaneously in cycle 1, and the DLT evaluation period was decreased to 4 weeks (1 cycle).

Toxicities were graded based on the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. DLT was defined as any of the following occurring during the DLT-evaluation period and at least possibly related to study treatment: grade 4 neutropenia or thrombocytopenia any time during the first 21 days; grade 4 neutropenia or thrombocytopenia lasting > 7 days; grade 3 thrombocytopenia requiring a platelet transfusion on more than 2 occasions during a cycle; myelosuppression causing a 7 days delay between treatment cycles; any grade 4 non-hematologic toxicity; any grade 3 non-hematologic toxicity (with the following exceptions: grade 3 nausea/vomiting/diarrhea/stomatitis < 3 days duration, grade 3 fever or infection, grade 3 transaminase elevation resolving prior to next cycle, and grade 3 hypophosphatemia, hypokalemia, hypocalcemia or hypomagnesemia that responded to oral supplementation within 7 days); or any grade 2 non-hematologic toxicity lasting >7 days and considered intolerable by the patient. Patients were considered DLT-evaluable if they experienced a DLT, if they completed at least 85% of prescribed dose during the DLT-evaluation period and were for observed for at least seven additional days without a DLT, or if treatment was halted for unacceptable toxicity or death that was at least possibly related to treatment.

Dose Modification Criteria

If grade 4 neutropenia or thrombocytopenia occurred during the 21 days of drug administration, treatment was suspended for at least 7 days. Patients meeting criteria for hematologic DLT during any subsequent cycle were allowed to continue on study but received subsequent doses at the next lower dose level. Patients who experienced dose-limiting hematological toxicity after a single dose level reduction were removed from protocol therapy.

Patients experiencing a non-hematological DLT that returned to baseline within 7 days after planned start the next treatment course were allowed to continue on study but received subsequent doses at the next lower dose level. If non-hematological DLT recurred at the reduced dose, the patient was removed from study. Patients who had any grade 3 or 4 non-hematologic toxicity resulting in 7 days delay in the start of a cycle were removed from protocol therapy.

Response Definitions

Patients were considered response-evaluable if they began study treatment and had at least one measurable lesion at least 2 cm in maximal diameter at enrollment. Response evaluations were conducted at baseline and after every other course. Complete response (CR) was defined as complete disappearance of target lesions on MRI with stable or decreasing corticosteroid dose and stable neurologic examination; partial response (PR):

30% reduction in bidimensional tumor measurements; progressive disease (PD): worsening neurologic status or >20% increase in bidimensional measurements, or appearance of one or more new lesions; stable disease (SD): criteria for CR, PR and PD not met.

Immune Cell Subtypes

For patients who consented to correlative studies, peripheral blood was drawn on cycle 1 days 0, 8, 22 and 28 and analyzed within 24 hours. Peripheral blood mononuclear cells (PBMCs) were prepared using Histopaque-1077 (Sigma-Aldrich, St. Louis, MO) for density centrifugation separation and washed with PBS (without Ca⁺⁺ and Mg⁺⁺) containing 0.2% bovine serum albumin and 0.1% NaN₃. Fc-receptors were neutralized using FcR blocking agent (Miltenyi, Cambridge, MA) for 15 minutes. PBMCs were stained with anti-CD56-PerCP-Cy5.5, anti-CD16-Alexa Fluor 700, anti-CD3-Pacific Orange, and anti-CD8-APC-H7 (BD Biosciences, San Diego, CA) mABs in the dark for 45 minutes, and then washed twice. Data were acquired using a four-laser LSRII flow cytometer (BD Biosciences) with ultraviolet laser excitation to exclude DAPI⁺ dead cells. Data were analyzed using FCS Express software (De Novo Software, Glendale, CA). Debris was excluded by gating on forward and side scatter parameters. Compensation for optical spillover of fluoresced light into unintended channels was performed using antibody capture beads (anti-mouse kappa chain; BD Biosciences) incubated with the same antibodies as used in the experimental tubes, and the AutoComp software routine was employed to calculate appropriate compensation (DIVA software version 6.0, BD Biosciences). Minimum 300,000 events were acquired for each sample.

Changes over time in lymphocyte subsets were analyzed using repeated measures ANOVA. The analysis was performed on the rank of the cell populations, to minimize the effects of the outlying observations. Given the large number of observations, a false discovery rate (FDR) adjustment was performed to keep the type I error at 5%.

Results

Fifteen eligible patients were enrolled (four before and eleven after Amendment 1). Two patients were not DLT-evaluable: one came off study due to disease progression with neurologic deterioration before receiving any study drug; the second came off study due to clinical and radiographic disease progression during the DLT-evaluation period, without experiencing a DLT. The remaining 13 patients were considered DLT-evaluable. Patient characteristics and treatment regimens received prior to enrollment are shown in Tables 2 and 3.

Of three patients enrolled at dose level 1, two had a DLT: grade 3 somnolence, confusion, and dizziness in one patient and prolonged grade 4 thrombocytopenia in the second. In addition, one patient enrolled at dose level 1 prior to Amendment 1 experienced grade 4 neutropenia in cycle 3; this event was reviewed by the DSMC, and was not considered a DLT (see study design, above). Of four patients enrolled at dose level 0A, two had a DLT, including grade 4 hypokalemia in one patient, and prolonged thrombocytopenia in the second. Six patients were enrolled at dose level 0B. One patient suffered a DLT of grade 4 thrombocytopenia. The remaining five patients tolerated the first cycle well without grade 3 or 4 toxicities during the DLT-evaluation period. Dose level 0B was determined to be the MTD.

Among all 13 evaluable patients, the most common adverse events were hematologic, followed by rash, headache and electrolyte abnormalities (Table 4). Six patients came off therapy due to progressive disease, five due to myelosuppression (four for neutropenia and one for thrombocytopenia) and two by parental request. Among the six patients treated at dose level 0B, three required dose reduction to dose level -1 at the end of the second cycle, two of whom eventually came off therapy due to delayed neutrophil recovery. The median number of cycles completed was three (range zero to six). All therapy-related toxicities resolved on follow-up.

Response and Clinical Outcome

Six patients had measurable tumor and were evaluable for response. Of these, the best response was PR in one patient with pilocytic astrocytoma previously refractory to temozolomide, prolonged stable disease in two patients with diffuse intrinsic pontine glioma (DIPG) and ependymoma (6.6 and 15.9 months, respectively), and progressive disease in three patients.

Immunologic Findings

The majority of analyzed patients (seven of 10) exhibited an approximate doubling of the frequency of effector/cytotoxic NK cells ($CD56^{\text{low}}CD16^+CD3^-$) in peripheral blood at day 7; these frequencies had generally returned to their pre-treatment values by day 21 (Figure 1A; $P < 0.001$; FDR adjusted $P=0.015$). There was a similar change in total NK cells ($P < 0.001$, FDR adjusted $P=0.01$), but there was no consistent change in the frequency of $CD56^{\text{high}}CD16^-CD3^-$ NK cells at any time point. There was a trend towards a transient increase in frequency of effector T-lymphocytes ($CD3^+CD8^+$) on day 7; this was not statistically significant (Figure 1B; $P=0.16$). The frequency of B-lymphocytes was decreased at day 7 in nine of nine patients and at day 21 in seven of eight analyzed patients, returning to pre-therapy values at day 28 ($P < 0.001$, FDR adjusted $P=0.01$). The frequency of regulatory T-lymphocytes ($CD3^+CD4^+CD25^+CD45RO^+$) did not change in any consistent manner. Taken together, these findings suggest a transient increase in the frequencies of cytotoxic NK and T cells relative to non-cytotoxic (B) cells with this drug combination.

Discussion

In this phase I study, we hypothesized that the combination of dasatinib, lenalidomide and metronomic temozolomide would be feasible and well tolerated, with anti-angiogenic and immunomodulatory synergy, resulting in efficient anti-tumor activity and avoiding the limitations of monotherapy.

Overall, while the combination of these three agents was relatively well tolerated, myelosuppression, including both neutropenia and thrombocytopenia, was common. This should not be surprising given that many patients in this cohort had previously been treated with multiple courses of myelosuppressive chemotherapy. Further dose reduction may be appropriate for heavily pretreated patients. Although response was not a primary endpoint of this study, evidence of clinical benefit was seen in several patients, including partial response in one child with low-grade glioma, and stable disease in patients with ependymoma and DIPG. The latter patient had prolonged stable disease while receiving six cycles of therapy, with an unusually prolonged overall survival of 10 months after initial progression [32].

The MTD for both dasatinib and lenalidomide in this combination was lower than the previously established respective single-agent pediatric MTD [14, 20]. Documented single agent pharmacodynamic and clinical activity of both agents at even lower doses (10–15 mg per day of lenalidomide in adults with multiple myeloma and 40–50 mg per dose twice daily of dasatinib in patients with chronic myeloid leukemia) supports the potential efficacy of the individual agents at the combination MTD [14, 19, 20, 33]. Temozolomide was kept at a fixed dose 75 mg/m² daily due to demonstrated efficacy and tolerability at this low dose in patients with glioblastoma and low-grade glioma [34]. However, given reports of efficacy at even lower metronomic doses temozolomide in adults with CNS tumors, further temozolomide dose reduction may be appropriate in future studies of this regimen [35, 36].

We explored the immunomodulatory effect of this regimen by measuring lymphocyte subsets and NK cells. A transient increase in NK effector cells and CD8⁺ effector T-lymphocyte was observed at day 7; this did not persist at day 21. We hypothesize that the increase may reflect an immunostimulatory effect of the regimen, while the subsequent return to baseline may represent clonal exhaustion, migration of the cells into tissue, or temozolomide-mediated interval immunosuppression [37]. Previous studies have shown a decrease in T-regulatory cells associated with metronomic temozolomide, although findings have been inconsistent [38, 39]. We saw no change in frequency of T regulatory cells, but did not assess absolute cell numbers. We posit that the increased ratio of effector NK and T cells to regulatory cells seen in our study could result in a net positive anti-tumor immunomodulatory effect.

To evaluate further the immunomodulatory activity and disease-specific efficacy of this regimen, a stratified phase II study, including patients with recurrent/progressive low and high-grade glioma and ependymoma, is proposed. The study would involve a one-month lead-in without temozolomide to evaluate drug-specific immunomodulation. Association of baseline and on-treatment immunologic findings with diagnosis and response would be assessed. Given the multiple potential mechanisms of action of the three drugs, analysis of

both tumor and microenvironmental cells for response-predictive biomarkers would be a key exploratory endpoint in the phase II setting.

In conclusion, the combination of dasatinib, lenalidomide and temozolomide at low metronomic doses was safe and feasible in this heavily pre-treated population of children with recurrent/progressive brain tumors, although hematologic toxicity was common. The MTD in pediatric patients with recurrent CNS tumors is dasatinib 65 mg/m² twice daily, lenalidomide 40 mg/m² daily, and temozolomide 75 mg/m² daily, in 28-day cycles consisting of 21 days of treatment followed by 7 days of rest. A transient immunomodulatory effect was observed in most patients. The clinical importance of this immunomodulation merits further evaluation in a disease-specific phase II setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Transient increase in frequency of CD56^{low}CD16⁺CD3⁻ effector/cytotoxic natural kill cells (a) and a trend toward increase in effector T lymphocytes (b) was seen at day 7. Y-axis: peripheral blood cell subset percentage; X-axis: cycle 1 study day. DL: Patient dose level (1, OA, or OB)

Table 1

Dose Levels

All drugs given by mouth for 21 days followed by 7 days rest, in repeating 28-day cycles	
Dose Level –1	Dasatinib 55 mg/m ² twice daily Lenalidomide 40 mg/m ² daily Temozolomide 75 mg/m ² daily
Dose Level 0A	Dasatinib 55 mg/m ² twice daily Lenalidomide 55 mg/m ² daily Temozolomide 75 mg/m ² daily
Dose Level 0B	Dasatinib 65 mg/m ² twice daily Lenalidomide 40 mg/m ² daily Temozolomide 75 mg/m ² daily
Dose Level 1	Dasatinib 65 mg/m ² twice daily Lenalidomide 55 mg/m ² daily Temozolomide 75 mg/m ² daily
Dose Level 2A	Dasatinib 65 mg/m ² twice daily Lenalidomide 70 mg/m ² daily Temozolomide 75 mg/m ² daily
Dose Level 2B	Dasatinib 85 mg/m ² twice daily Lenalidomide 55 mg/m ² daily Temozolomide 75 mg/m ² daily
Dose Level 3	Dasatinib 85 mg/m ² twice daily Lenalidomide 70 mg/m ² daily Temozolomide 75 mg/m ² daily

Table 2

Patient characteristics

Characteristic	Number
Patients enrolled	15
DLT evaluable	13
Sex	
Male	11
Female	4
Age, year	
Median	8.7
Range	5.1 – 17.7
Diagnosis	
Atypical Teratoid/Rhabdoid Tumor	1
Medulloblastoma	3
Ependymoma	2 *
CNS Embryonal Tumor NOS	1 *
DIPG	4
Leptomeningeal Glioneural Tumor	1
Mixed Malignant Astro-oligodendroglioma	1
Diffuse High-Grade Astrocytoma	1
Pilocytic Astrocytoma	1

CNS central nervous system, *DIPG* diffuse intrinsic pontine glioma, *DLT* dose-limiting toxicity.

* Includes two non-DLT evaluable patients

Table 3

Treatment received prior to enrollment

Pt no.	Diagnosis	Treatment received prior to enrollment
1	Recurrent mixed malignant astro-oligodendroglioma	i. Headstart III ii. Irinotecan and bevacizumab iii. Focal irradiation
2	Recurrent posterior fossa anaplastic ependymoma	i. ACNS0121 ii. Focal irradiation with carboplatin
3	Recurrent medulloblastoma	i. Headstart II ii. Craniospinal irradiation
4	Recurrent medulloblastoma	i. Headstart III ii. Craniospinal irradiation
5	Recurrent diffuse high-grade astrocytoma	i. Temozolomide ii. Focal irradiation with temozolomide
6	Refractory pilocytic astrocytoma	i. Carboplatin and vincristine ii. Temozolomide and irinotecan iii. Vinblastine
7	DIPG	i. Focal irradiation with temozolomide
8	Progressive leptomeningeal glioneural tumor	i. Temozolomide ii. Carboplatin and vincristine iii. Vinblastine iv. Temozolomide and irinotecan v. Focal irradiation
9	DIPG	i. Focal irradiation with carboplatin and PO etoposide
10	Recurrent medulloblastoma	i. Headstart III ii. Craniospinal irradiation
11	DIPG	i. Focal irradiation with oral etoposide
12	Recurrent ATRT	i. Headstart III ii. Craniospinal irradiation
13	DIPG	i. Focal irradiation

Headstart: induction & myeloablative consolidation chemotherapy followed by autologous stem cell rescue

Table 4

Summary of Grade 2 and higher adverse events based on assigned dose level

Adverse event	Grade 2	Grade 3	Grade 4
	Number of events (number of patients affected)		
Dose Level 1			
Confusion/Somnolence		1 (1) [*]	
Neutropenia		1 (1)	3 (3)
Leukopenia		4 (3)	
Thrombocytopenia	2 (2)	1 (1)	1 (1) [*]
Rash	2 (2)		
Pain	1 (1)		
Hypotension	1 (1)		
Hypokalemia		1 (1)	
Dose Level 0A			
Neutropenia	1 (1)	1 (1)	3 (2)
Leukopenia	1 (1)	2 (2)	
Thrombocytopenia		3 (3) [*]	2 (2)
Anemia	3 (3)	1 (1)	
Rash	2 (2)		
Hypokalemia			1 (1) [*]
Infection	1 (1)		
Fever	1 (1)		
Dose Level 0B			
Somnolence	1 (1)		
Lethargy	2 (1)		
Headache	1 (1)	2 (1)	
Neutropenia	2 (2)	6 (4)	8 (5)
Leukopenia	4 (3)	2 (2)	3 (2)
Thrombocytopenia	1 (1)	2 (2)	3 (1) [*]
Anemia	5 (2)		
Headache	1 (1)		
Hypokalemia		1 (1)	
Nausea	1 (1)		

* Includes dose limiting toxicities during the dose-finding period