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Combination of Bevacizumab, Irinotecan and Temozolomide for Refractory or Relapsed Neuroblastoma: Results of a Phase II Study

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Abstract

Background—The rationale for studying the combination of bevacizumab, irinotecan and temozolomide (BIT) in neuroblastoma is based on: (a) vascular endothelial growth factor (VEGF) expression is associated with an aggressive phenotype, (b) anti-VEGF antibody bevacizumab enhances irinotecan-mediated suppression of neuroblastoma xenografts, (c) bevacizumab safety has been established in pediatric phase I studies and, (d) irinotecan+temozolomide (IT) is a standard salvage chemotherapy.

Procedure—We conducted a phase II study of BIT in patients with measurable/evaluable refractory or relapsed high-risk neuroblastoma (www.clinicaltrials.gov NCT01114555). Each cycle consisted of bevacizumab (15mg/kg intravenously) on days 1 and 15 plus irinotecan (50mg/m²/day intravenously) and temozolomide (150mg/m²/day orally) on days 4–8. Patients could have previously received, but not relapsed on, IT. An early stopping rule mandated continuing therapy only if >5/27 evaluable patients achieved partial (PR) or complete response (CR) after 4 cycles.

Results—33 heavily pretreated patients (9 primary refractory; 24 relapsed) received 1–8 cycles of BIT. Toxicities were expected and transient. Grade 4 toxicities were neutropenia (30%) and thrombocytopenia (24%). Grade 3 toxicities included hepatic transaminitis (15%), proteinuria (9%) and diarrhea (3%). Overall responses were: 3 CR, (all in prior IT-treated patients), 18 no response and 12 progressive disease. Only 1/23 patients assessable for the early stopping rule regarding efficacy achieved PR/CR, so patient accrual was discontinued. Median progression-free and overall survival was 7.7±1.7 and 31.5±5.6 months respectively; all patients continued anti-neuroblastoma therapy post-BIT.

Conclusions—BIT was well tolerated, but addition of bevacizumab did not improve response rates in resistant neuroblastoma compared to historical data for IT.

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Conflict of Interest Statement

The authors declare that they have no competing interests. This study was partially funded by Genentech and the institution receives support from NIH/NCI Cancer Center Support Grant P30 CA008748. Genentech supplied drug and provided partial support for data management.

Keywords

anti-angiogenesis; bevacizumab; irinotecan and temozolomide; neuroblastoma

Introduction

Intensive chemotherapy, aggressive surgery, radiotherapy, and anti-GD2 immunotherapy have improved survival rates for patients with high-risk neuroblastoma (NB), the most common extracranial pediatric solid tumor.[1–3] Nevertheless, many patients develop progressive disease and their long term prognosis is poor.[4] Until recently, the most commonly accepted salvage regimen used for patients with relapsed and refractory disease was the combination of cyclophosphamide and topotecan.[5] However, the Children's Oncology Group (COG), the only cooperative pediatric oncology group in the US, introduced this combination as an upfront treatment for newly diagnosed patients, making it a less attractive option in the setting of relapsed/refractory disease for fear of chemoresistance. Currently, the most widely used second-line chemotherapy regimen for patients with refractory or relapsed NB is the combination of irinotecan and temozolomide (IT).[6,7]

Irinotecan, a camptothecin prodrug activated by carboxylesterases to the active topoisomerase I inhibitor SN-38, has undergone phase I and phase II testing in children with NB using various dosing schedules.[8,9] Toxicities included diarrhea and relatively modest myelosuppression. In general, although prolonged stabilization of disease was often observed, major response rates were <5%.[10,11] Five-day courses of irinotecan alone were shown to be effective palliative therapy for patients with resistant NB.[12] Temozolomide, a methylating agent that generates *O*⁶-methylguanine in DNA, has modest activity against NB with myelosuppression as the main toxicity.[13] Methylation of *O*⁶-guanine by temozolomide may lead to recruitment of topoisomerase I and potentially enhance the probability of inducing camptothecin-mediated damage. This possibility, and the lack of overlapping major toxicities, formed the biochemical rationale for combining temozolomide with irinotecan.[14] A phase I study established the toxicity profile (grade 1–2 diarrhea and myelosuppression) of the combination in heavily prior-treated pediatric patients.[15] Although multiple courses entailed no cumulative toxicity and controlled disease for prolonged periods in many patients, reported objective response rates in patients with relapsed or refractory NB were generally <25%.[6,7]

Angiogenesis is a significant regulator of NB growth and tumor vascularity correlates with high-risk disease.[16,17] The pro-angiogenic vascular endothelial growth factor (VEGF) and its receptor (VEGFR) are considered to play a critical role in the initiation of tumor angiogenesis.[18] Expression of VEGF and VEGFR correlate with a higher stage of NB.[19,20] Furthermore, VEGF may act as a growth factor for NB cells independent of its pro-angiogenic action.[21] Bevacizumab, a humanized monoclonal antibody[22] binding all five isoforms of human VEGF, has been extensively studied in adults with cancer and has FDA approval for the therapy of several cancers usually in combination with chemotherapy.[23] Like other anti-angiogenic agents, its anti-tumor effect is attributed to normalization of

tumor vasculature. The safety of bevacizumab was established in children with resistant solid tumors in a phase I trial conducted by the COG. Dose-limiting toxicity was not reached up to a dose of 15mg/kg administered intravenously every 14 days, and the toxicity profile was similar to that seen in adults.[24] In anecdotal reports in pediatric patients with relapsed malignancies, significant adverse events appeared to be more frequent especially in children with brain tumors.[25,26] Pharmacokinetic parameters in children were similar to those measured in adults[27] as were objective responses when used as a single agent.

In preclinical studies of NB xenografts, bevacizumab transiently normalized tumor vasculature[28] but only partially suppressed tumor growth.[29] Diminished VEGF gene and protein expression were associated with response of NB xenografts to irinotecan.[30] In experiments performed in our laboratory we observed insignificant tumor suppression with bevacizumab alone but noted significantly enhanced tumor suppression and improved survival of LAN-1, NMB7, SKNBE(2)C and SKNLP NB xenograft-bearing mice treated with bevacizumab plus irinotecan when compared to irinotecan alone (Modak, Chung and Cheung unpublished data). We therefore added bevacizumab to the salvage regimen of IT and tested the combination (BIT) in a phase II single-arm clinical trial for patients with relapsed or refractory NB (Clinicaltrials.gov NCT identifier NCT01114555). The trial was approved by the institutional review board (IRB) of Memorial Sloan Kettering Cancer Center (MSKCC).

METHODS

Patient Selection

High-risk NB patients with a history of tumor progression or recurrence, or failure to achieve complete remission after induction chemotherapy were eligible. They were required to have measurable or evaluable disease and a minimum life expectancy of 8 weeks. Patients could have previously received irinotecan and/or temozolomide provided their disease had not progressed while on therapy. At least 2 weeks should have elapsed since prior biologic therapy. Three weeks should have elapsed since last dose of chemotherapy. Salient exclusion criteria included: absolute neutrophil count <500/ μ l, platelet count <35,000/ μ l and hemoglobin <8g/dl, chronic non-healing wound, ulcer, or bone fracture, major surgery within 28 days or minor surgery within 7 days before enrollment, known bleeding diathesis, history of thrombosis <3 months before study entry, cerebrovascular accident, abdominal fistula, gastrointestinal perforation, or intra-abdominal abscess <6 months of study entry, known brain metastasis, proteinuria with urine protein: creatinine ratio $\geq$ 1.0, or uncontrolled hypertension. Informed consent was obtained according to MSKCC IRB procedures.

Treatment and Monitoring

Each cycle of BIT comprised: bevacizumab at 15mg/kg/dose infused intravenously (IV) over 30–90 minutes on days 1 and 15, and irinotecan at 50mg/m²/day IV over 1 hour, followed by temozolomide at 150mg/m²/day orally, on days 4–8. Prophylactic use of oral antibiotics (cefixime or equivalent) was permitted. Bevacizumab was provided as a study drug by Genentech. Cycles could be repeated on day 28 in the absence of wound dehiscence, gastrointestinal perforation, brain or pulmonary hemorrhage, tracheoesophageal fistula,

reversible posterior encephalopathy, grade 4 non-hematological toxicities attributable to protocol therapy, or grade 3 hypertension, allergic reaction, venous thromboembolism, bowel obstruction or congestive cardiac failure. Dose reductions were not permitted. If grade 3 proteinuria occurred, bevacizumab could be held until proteinuria resolved to grade 2. All toxicities should have resolved to <grade 2 by day 42 of each cycle in order to continue therapy. Extent-of-disease evaluations were performed after every two cycles with computed tomography or magnetic resonance imaging, ¹²³I-metaiodobenzylguanidine (MIBG) scan and bilateral bone marrow (BM) aspirations and biopsies. Response was assessed using modified International Neuroblastoma Response Criteria (INRC) [31]: Complete remission (CR): no evidence of disease; very good partial remission: >90% decrease in volume of primary tumor and no other evidence of active disease; partial remission (PR): 50–90% decrease in measurable disease, no new lesions, 1 positive BM site allowed; no response (NR): <25% increase in any existing lesion; progressive disease (PD): any new lesion, increase of any measurable lesion by >25%. In addition, responses on MIBG scan were noted using modified Curie score.[32] An objective response was defined as any improvement in modified Curie score. Toxicity was scored by Common Terminology Criteria for Adverse Events version 4.0.

Biostatistical Considerations

Since best responses in NB patients treated with IT can occur beyond two cycles, the primary endpoint chosen was efficacy of BIT as assessed by response after four cycles.[6,7] If patients had PD before completing four cycles, treatment was considered to have failed, and such patients were not replaced on study. Patients withdrawing from study prior to completing four cycles due to reasons other than PD e.g. BIT toxicity or parent/physician preferences were replaced. Based on reported historical responses to IT, we proposed declaring treatment effective if probability of response was 0.35. A Simon two-stage design that differentiated between response rates of 0.2 (undesirable rate) and 0.35 (desirable rate) was used with plans to treat 27 evaluable NB patients with BIT in the first phase and to add an additional 34 NB patients only if >5 responses (CR+PR) were observed in the first cohort. BIT would be considered effective if 17/63 patients showed response. Besides reporting response based on the efficacy endpoint described above, we assessed overall response in all treated patients. Toxicity and survival evaluations were secondary endpoints. Since BIT had not been previously used in pediatric trials, we adopted early stopping rules to avoid undue toxicity: grade 4 diarrhea or severe myelosuppression or grade 3 other toxicities in 4/12 patients. Survival was assessed using Kaplan Meier analysis (SPSS, IBM).

RESULTS

Patient Characteristics

33 patients with a median age of 6.4 (range 2.2–25.8) years were enrolled, including 25 (76%) previously treated with irinotecan and/or temozolomide (Table 1). At study entry all patients had evaluable MIBG-avid skeletal disease and/or measurable soft tissue NB. Ten patients had primary refractory disease and 23 had progressive or relapsed NB. They

received 1–8 (median 4) cycles. Median time interval between cycles was 28 ± 3.4 (range 28–42) days. All patients were eligible for toxicity, response and survival assessment.

Responses

Best overall responses by INRC in all 33 patients were 3 (9%) CR, 18 (55%) NR and 12 (36%) PD (Table 3). Sixteen patients were withdrawn before receiving 4 cycles: six had PD and were not replaced, 2 had unacceptable toxicities (one with prolonged myelosuppression and one with persistent proteinuria) and were replaced, and a further eight patients were withdrawn before 4 cycles due to parental request. Among the 23 patients evaluable for the defined efficacy endpoint (i.e. response to BIT after 4 cycles), responses were 1 (4%) CR, 13 (57%) NR and 9 (27%) PD. Thirty-two patients had MIBG-avid skeletal disease at enrollment, with a median modified Curie score of 7 (range 1–20); 9 had objective responses with modest reductions in MIBG score (median –1; range –1 to –4). BM and soft tissue responses were documented in 5 (27%) and 1 (10%) patients, respectively. Responses did not correlate with disease status at study entry: objective responses were noted in 5/10 patients with primary chemorefractory disease versus 7/23 patients who had progressed or relapsed ($p=0.28$ by Chi-squared test) prior to BIT. All 3 patients who had CR had low burden disease: one had a single MIBG-avid rib-based soft tissue mass that resolved after 4 cycles, one had MIBG-avid clival and upper cervical bony lesions (the former having been irradiated six months before BIT) which resolved after 6 cycles, and one patient had a single MIBG-avid acetabular site irradiated two months before BIT which resolved after 4 cycles. Of the 25 patients who had previously received IT, 8 had objective responses (including all 3 patients who achieved CR) compared to 2/8 patients who did not receive prior IT ($p=0.78$ by Chi-squared test).

Toxicities

In general, BIT was well tolerated (Table 2). Treatment was outpatient and all toxicities were expected based on prior reports of separately administered IT and bevacizumab. Toxicities were transient, resolving without any intervention apart from supportive care. The commonest toxicity was grade 3 or 4 myelosuppression, in 22/33 (67%) patients, though only one patient required admission for (uncomplicated) febrile neutropenia. There were no non-hematological grade 4 toxicities. Grade 3 proteinuria developed in 2 patients (after 2 cycles in each) and resolved spontaneously after 48 and 56 days (from cycle 2, day 1 of bevacizumab) respectively, necessitating removal of the latter patient from study. One patient developed transient elevations of hepatic aspartate aminotransferase and alanine aminotransferase. Grade 1–2 diarrhea and vomiting were common (82%) as expected with IT. Epistaxis was another common adverse event (39%) as expected with bevacizumab, but did not require any intervention other than nasal pressure and/or platelet transfusion for associated thrombocytopenia. One patient developed transient grade 2 hypertension. All patients were followed for late toxicities: no unexpected toxicities were observed, however one patient who had received extensive prior treatment developed secondary acute myeloid leukemia 2.3 years after completing BIT.

Post-protocol therapy and survival

All patients went on to receive additional therapy after coming off study. Subsequent therapies included immunotherapy with the anti-GD2 monoclonal antibody 3F8 (n=14). The contribution of BIT, if any, to prolonging survival could not be evaluated. Median progression-free survival (PFS) and overall survival (OS) were 7.7 ± 1.7 months and 31.5 ± 5.6 months, respectively (Figure 1). Three-year PFS and OS probabilities were $10 \pm 5\%$ and $39 \pm 9\%$, respectively. Seven patients are currently long-term (>3 years from BIT) survivors: four with residual disease, two in CR despite post-BIT relapses, and one in continuous CR. Survival did not correlate with response to BIT.

DISCUSSION

The combination of irinotecan and temozolomide has been widely used as salvage therapy for chemorefractory or relapsed NB, especially after 2005 when topotecan was added to upfront therapy in COG protocols.[33] We previously reported our experience with a five-day regimen of the combination for resistant high-risk NB and demonstrated that IT could be delivered as outpatient therapy with modest toxicity. Mild myelosuppressive effects allowed for use when BM reserve was limited. However, major responses (CR+PR by International Neuroblastoma Response Criteria (INRC), as used in this current study) were uncommon (<10%). We and others have attempted to enhance the anti-NB activity of irinotecan or IT by combining standard chemotherapeutic agents[34] newer agents such as temsirolimus[35], gefitinib[36], or alisertib[37] or targeted radiotherapy with ^{131}I -MIBG.[38] However, response rates were not significantly improved, ranging from 0-25%. We hypothesized that adding an agent that provides inhibition of angiogenesis, an important oncogenic pathway that was not previously targeted in high-risk NB, would improve the CR +PR rates to a benchmark of 35%. Unfortunately, in our study the response rate to BIT, as assessed by INRC, was only 9%. Even if response criteria are modified to allow 50% reduction in Curie scores on MIBG scans to be deemed PR, the response rate was only 18% (6/33 patients). This sub-optimal response rate resulted in termination of the study after the first phase as mandated by the Simon two-stage design. All three patients who achieved CR and 7/9 patients with objective responses by MIBG scan had previously received, though had not progressed on, IT suggesting that prior IT therapy did not account for the low response rate. Based on our data and experience with the above-mentioned combination studies, it is unlikely that adding anti-VEGF agents to the “backbone” of IT will improve response rates significantly beyond 25%.

In children, the combination of bevacizumab and irinotecan has primarily been studied in patients with brain tumors. Bevacizumab confers PFS benefit in adults with glioblastoma multiforme and received FDA approval for this indication.[39] However, in the pediatric population, the combination of bevacizumab and irinotecan was ineffective in recurrent malignant glioma and ependymoma.[40] For other pediatric tumors, there are only anecdotal reports on the use of BIT. Responses have been observed in patients with Ewing sarcoma[41] and Wilms tumor.[42]

BIT toxicities encountered in our study were similar to those reported in adults with hypertension, fatigue, epistaxis and proteinuria being the commonest. Compared to our

study, a higher number of children receiving BIT for brain tumors (6% versus 24%) stopped therapy due to toxicity.[43] Although there were no long-term adverse events in NB patients treated with BIT, the addition of bevacizumab did add several toxicities that interfered with patient quality of life. The most common of these (39%) was epistaxis, which even though not severe, required nasal pressure and infrequently, nasal packing. Severe side-effects of bevacizumab such as severe hypertension, gastrointestinal perforation, etc, albeit rarer than in adults, have been described in children necessitating vigilance while using bevacizumab in this population.[43]

Ours is the first pediatric phase II study of bevacizumab either as a single agent or in combination with chemotherapy, for any non-CNS tumor. As observed in brain tumor patients, <10% of our high-risk NB study patients had major responses. We did not evaluate possible mechanisms for this lack of efficacy. However, several mechanisms for treatment failure could be considered including dependence on VEGF-independent angiogenesis pathways, upregulation of pro-angiogenic and other tumor stromal factors. Despite overall manageable toxicity, the results from this phase II study do not support the addition of bevacizumab to standard chemotherapy regimens for high-risk NB.

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Table of Abbreviations

BIT	bevacizumab with irinotecan and temozolomide
VEGF	vascular endothelial growth factor
IT	irinotecan+temozolomide
PR	partial response
CR	complete response
NB	neuroblastoma
COG	Children's Oncology Group
VEGFR	vascular endothelial growth factor receptor
IRB	institutional review board
MSKCC	Memorial Sloan Kettering Cancer Center
MIBG	¹²³ I-metaiodobenzylguanidine
NR	No response
PD	Progressive disease

PFS	Progression free survival
OS	Overall survival

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Figure 1. Survival in neuroblastoma patients treated with bevacizumab, irinotecan and temozolomide (BIT)

Table 1

Patient Demographics at study entry

Characteristics (n=33 patients)	Distribution
Age at therapy	Median ($\pm$ Stdev.): 6.4 ($\pm$ 5.7) years; Range: 2.2–25.8 years
Gender	Male: 25; Female: 8
INSS stage at diagnosis	Stage 4: 32; Stage 3: 1
Tumor Biology	
<i>MYCN</i> status	Amplified: 5 ; Non-amplified: 28
<i>ALK</i> status	Mutated: 1; Wild-type: 23; Unknown: 9.
Relapse status prior to study entry	
Primary Refractory	10 (30%)
Relapse/Progressive disease	23 (70%); median number of prior relapses: 2 (range 1–8)
Prior irinotecan and/or temozolomide therapy	25 (76%)

Abbreviations: INSS: International Neuroblastoma Scoring System; Stdev.: standard deviation

Table 2

Related Toxicities

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Total (n=33)
Thrombocytopenia	11	1	11	8	31
Neutropenia	2	7	9	11	29
Lymphopenia	1	5	19	3	28
Diarrhea	16	10	1	0	27
Elevated AST	19	4	3	0	26
Elevated ALT	11	5	5	0	21
Vomiting	13	6	0	0	19
Epistaxis	11	2	0	0	13
Hyponatremia	8	0	0	0	8
Hypokalemia	3	0	1	0	4
Proteinuria	0	0	3	0	3
Hyperkalemia	0	0	1	0	1
Hypertension	0	1	0	0	1

Table 3

Responses

Response	CR	PR	NR	PD
By INRC at end of treatment (n=33)	3	0	18	12
By INRC after 4 cycles (n=23)	1	0	13	9
Best site-specific responses				
Bone marrow disease (involved pre-BIT in 18 patients)	5	N/A	12	1
MIBG scan (n = 32)	3	3 *	20 *	7
Soft tissue disease (n= 10 patients)	1	0	5	4

* PR for MIBG scans was defined as >50% but <100% reduction in MIBG score while NR was <50% reduction but no increase in MIBG score.

Abbreviations: CR: Complete response; INRC: International Neuroblastoma Response Criteria; NR: No response; PD: progressive disease; PR: Partial response