Camptothecin Analogs (Irinotecan or Topotecan) plus High-Dose Cyclophosphamide as Preparative Regimens for Antibody-Based Immunotherapy in Resistant Neuroblastoma

Brian H. Kushner, Kim Kramer, Shakeel Modak, and Nai-Kong V. Cheung
Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, New York

ABSTRACT

Purpose: We used high-dose cyclophosphamide plus topotecan/vincristine (CTV) or irinotecan (C/I) in patients with resistant neuroblastoma. The aim was to use a regimen with little risk to major organs to (a) achieve or consolidate remission in heavily treated patients and to (b) induce an immunological state conducive to passive immunotherapy with the murine 3F8 antibody.

Experimental Design: CTV and C/I included cyclophosphamide 140 mg/kg (~4200 mg/m²), with CTV, topotecan 2 mg/m² was infused i.v. (30 min) on days 1–4 (total, 8 mg/m²), and vincristine 0.067 mg/kg was injected on day 1. With C/I, irinotecan, 50 mg/m² was infused i.v. (1 h) on days 1–5 (total, 250 mg/m²). Mesna and granulocyte colony-stimulating factor were used.

Results: Twenty-nine patients received 38 courses of CTV, and 26 patients received 38 courses of C/I. All patients had previously received topotecan, a hematopoietic stem-cell transplant, and/or high-dose cyclophosphamide. CTV and C/I caused myelosuppression of comparably prolonged duration as follows: absolute neutrophil counts <500/µl lasted 5–12 days in patients who had not previously received transplant and 7–21 days in patients who were post-transplant. Other significant toxicities included typhlitis (two CTV-treated patients, one C/I-treated patient) and hemorrhagic cystitis (one C/I-treated patient). Major responses were seen in 4 (15%) of 26 CTV-treated patients and in 4 (17%) of 24 C/I-treated patients with assessable disease. Bone marrow disease resolved in 5 (28%) of 18 CTV-treated patients and in 4 (27%) of 15 C/I-treated patients. 3F8 after CTV or C/I was not blocked by neutralizing antibodies, consistent with the desired immunosuppressive effect of high-dose cyclophosphamide.

Conclusions: CTV and C/I require transfusional and antibiotic support but otherwise entail tolerable morbidity. They have modest antineuroblastoma activity in heavily treated patients and are good preparative regimens for passive immunotherapy with monoclonal antibodies.

INTRODUCTION

The camptothecin analogs topotecan and irinotecan hold great promise in the treatment of neuroblastoma (NB) for several reasons. First, they have impressive activity against NB cell lines resistant to standard anti-NB agents (1–5); this finding implies that they may provide a novel non-cross-resistant chemotherapeutic addition to the standard drug armamentarium for NB. Second, their nonhematological toxicity (mucositis/topotecan, Refs. 6 to 11; diarrhea/irinotecan, Refs. 12 to 16) is manageable and transient, major consideration in NB patients who often undergo nephrectomy and who are treated with cardiotoxic (doxorubicin) and nephrotoxic (platinum compounds, ifosfamide) agents, as well as with total body or local irradiation. Third, cytotoxicity occurs at relatively nonmyelosuppressive dosages (6–8, 13–15, 17, 18), which suggests that they can be safely given with other anticancer drugs.

Camptothecin analogs target topoisomerase I, which is involved in DNA replication; hence, their cytotoxicity may be mainly S-phase specific (19). The mechanism of action suggests that their optimal use might be with alkylators, which also obstruct DNA activity. In fact, combined use of topotecan 3.75 mg/m² and cyclophosphamide 1250 mg/m² (relatively low dosages of each agent) has become standard treatment for resistant NB (20). In contrast, there have been no detailed reports on irinotecan used with other agents in children or with cyclophosphamide in any age group (21).

We combined these camptothecin analogs with high-dose cyclophosphamide, which has well-known anti-NB and immunosuppressive activity, spares hematopoietic stem cells, and has modest nonhematological toxicity. The aim was to use a regimen that poses little risk to major organs to (a) achieve or consolidate remission in heavily treated NB patients and to (b) induce an immunological state conducive to passive immunotherapy with the murine anti-GD2 monoclonal antibody 3F8 (22–24). Optimal use of 3F8 involves multiple two-week cycles, but that is not possible if there is early development of human antimouse antibody.

MATERIALS AND METHODS

Patients with relapsed or refractory (i.e., incompletely responding) NB were assessed in this Memorial Sloan-Kettering...
Cancer Center retrospective study. Patients needed to have satisfactory cardiac (normal echocardiogram), hepatic (normal serum bilirubin, serum liver enzymes 1–1.5× the upper limits of normal), and renal (normal serum creatinine) status. Informed written consents for treatments were obtained in accordance with hospital policy after guardians understood the investigational nature of the treatments, the potential life-threatening risks to be expected, the known side effects of each agent, and the possibility of unforeseen life-threatening toxicities.

Both CTV and C/I included 70 mg/kg cyclophosphamide infused i.v. over 6 h on days 1 and 2 (total = 140 mg/kg, i.e., ∼4200 mg/m²), with hydration using D5/1/2 normal saline plus 30 meq/l potassium chloride and 10 mg/l furosemide at 150 ml/m²/h. Mesna (70 mg/kg) was started on days 1 and 2 along with the cyclophosphamide but was infused over 24 h.

With CTV, 2 mg/m² topotecan was infused i.v. over 30 min on days 1–4 (total: 8 mg/m²) and 0.067 mg/kg vincristine was infused i.v. over 6 h on days 1 and 2 (total = 0.267 mg/kg vincristine) on day 1.

With C/I, 50 mg/m² irinotecan was infused i.v. over 1 h on days 1–5 (total = 250 mg/m²).

Granulocyte colony-stimulating factor was used after completion of chemotherapy.

Disease status was assessed by computed tomographic scans, ⁹⁹ᵐTc-bone scan, ¹³¹I- or ¹²³I-metaiodobenzylguanidine scan, urine catecholamine measurements, histological examinations of bone marrow biopsy specimens and aspirates from bilateral iliac crests.

Disease status was defined by the International Neuroblastoma-Response Criteria (25) as follows: complete response, no evidence of disease; very good partial response, volume of primary mass reduced by 90%–99%, no evidence of distant disease (including normal metaiodobenzylguanidine) except for skeletal residua, catecholamines normal; partial response (PR), >50% decrease in measurable disease and ≤1 positive bone marrow site; mixed response, >50% decrease of any lesion with <50% decrease in any other; no response, <50% decrease but >25% increase in any lesion; and progressive disease, new lesion or >25% increase in an existing lesion.

Human antimouse antibody was measured using an ELISA as described previously (22).

Toxicity was graded using the National Cancer Institute Common Toxicity Criteria.

Eligibility criteria to start treatment with 3F8 included previous treatment with CTV in 34% and 45% of the CTV-treated patients, respectively, versus 88% and 77% of the C/I-treated patients. Two-thirds of the patients had prior exposure to high-dose cyclophosphamide. Fifteen (52%) of the 29 CTV-treated patients had NB that was refractory to, or progressed during, induction that included topotecan (n = 5) and/or high-dose cyclophosphamide combined with doxorubicin and vincristine (n = 12). In contrast, only 4 (15%) of the 26 C/I-treated patients were treated for a poor response to induction, whereas 22 (85%) were treated after at least one relapse.

**Toxicity.** CTV and C/I were routinely given on an outpatient basis because acute toxicities such as emesis, hematuria, and hyponatremia were readily preventable. There was one toxic death (from infection).

CTV and C/I caused grade 4 myelosuppression of comparably prolonged duration. In patients who had not received hematopoietic stem-cell transplants previously, absolute neutrophil counts <500/µL lasted 5–12 days, and platelet count recovery to >75,000/µL occurred by day 21–28. Five previously transplanted patients with poor bone marrow reserve received CTV (n = 2) or C/I (n = 3), which was followed 48 h later by infusion of hematopoietic stem cells to minimize myelosuppression. In the other 27 post-transplant patients, absolute neutrophil counts <500/µL lasted 7–21 days and platelet count recovery to >75,000/µL occurred by day 17–40+. Blood-borne bacterial infections were documented in eight patients, one of whom died of septic shock. Two other patients had clinical sepsis and pneumonia, respectively, but no organisms were identified.

Major nonhematological toxicities included suspected typhlitis in two CTV-treated patients (concomitant with bacterial sepsis) and in one C/I-treated patient. Grade 2 mucositis occurred in three CTV-treated patients but not with those treated with C/I. Diarrhea was grade ≤2. One CTV-treated patient had grade 3 generalized pain attributable to vincristine, and one C/I-treated patient developed hemorrhagic cystitis. No significant renal, cardiac, or hepatic dysfunction was seen.

3F8 was used after CTV in 13 patients and after C/I in seven patients. These patients received a median of three cycles of 3F8 (range, 1–8) over a median period of 4.5 months (range, 1–12 months) without developing persistent human antimouse antibody, consistent with the desired immunosuppressive effect of high-dose cyclophosphamide, except in one patient who was treated with CTV followed by infusion of autologous stem cells that had been collected after relatively low-dose chemotherapy.

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>CTV (n = 29)</th>
<th>C/I (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at study entry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1 y 10 m–14 y 6 m</td>
<td>3 y 5 m–11 y 8 m</td>
</tr>
<tr>
<td>Median</td>
<td>5 y 9 m</td>
<td>7 y 3 m</td>
</tr>
<tr>
<td>Male/female</td>
<td>14:15</td>
<td>19:7</td>
</tr>
<tr>
<td>Stage 4 disease</td>
<td>26 (90%)</td>
<td>25 (96%)</td>
</tr>
<tr>
<td>Prior therapy with:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Topotecan</td>
<td>3 (10%)</td>
<td>4 (16%)</td>
</tr>
<tr>
<td>Stem-cell transplantation</td>
<td>10 (34%)</td>
<td>23 (88%)</td>
</tr>
<tr>
<td>High-dose cyclophosphamide</td>
<td>13 (45%)</td>
<td>20 (77%)</td>
</tr>
<tr>
<td>Disease status at study entry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 st refractory/PD</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>1 st relapse</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>2 nd refractory/PD</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>≥2nd relapse</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>CR2</td>
<td>2</td>
<td>2</td>
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</tbody>
</table>

Notes: CTV, cyclophosphamide plus topotecan/vincristine; C/I, cyclophosphamide plus irinotecan; PD, progressive disease; CR, complete remission.
Table 2  Responses in patients with assessable disease

<table>
<thead>
<tr>
<th>Response</th>
<th>CTV (n = 26)</th>
<th>CI (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR/VGPR/PR</td>
<td>4 (15%)</td>
<td>4 (17%)</td>
</tr>
<tr>
<td>MR</td>
<td>2 (8%)</td>
<td>5 (19%)</td>
</tr>
<tr>
<td>NR</td>
<td>15 (58%)</td>
<td>11 (46%)</td>
</tr>
<tr>
<td>PD</td>
<td>5 (19%)</td>
<td>4 (17%)</td>
</tr>
</tbody>
</table>

*a Three CTV and two CI patients were not assessable for response.
*b CTV, cyclophosphamide plus topotecan/vincristine; CI, cyclophosphamide plus ironotecan; CR, complete response; VGPR, very good partial response; PR, partial response; MR, mixed response; PD, progressive disease; NR, no response.

Responses. Bone marrow disease resolved in 5 (28%) of 18 CTV-treated patients and in 4 (27%) of 15 CI-treated patients. These bone marrow responses left three patients in complete response/very good partial response, two patients in PR with improved but still abnormal 123I-metaiodobenzylguanidine scans, one patient in PR with slightly high urine catecholamine levels, and three patients with mixed response attributable to persistence of 123I-metaiodobenzylguanidine uptake in a single skeletal site or soft tissue.

CTV achieved no major responses of soft tissue disease present in 11 patients, although gross total resections were subsequently performed in five of these patients. CI produced PR of retroperitoneal and/or thoracic NB in three of nine patients with assessable soft tissue disease and was followed by gross total resections in seven of nine patients.

Overall, among patients with assessable disease, major responses (PR or better) were achieved in 4 (15%) of 26 CTV-treated patients and in 4 (17%) of 24 CI-treated patients (Table 2).

DISCUSSION

Based on past experience with each agent individually in the doses and schedules applied, combined use of camptothecin analogs and high-dose cyclophosphamide in a large series of heavily treated NB patients had few unexpected side effects. Neutropenia was profound, with predictable infectious events; thrombocytopenia was prolonged, especially in post-transplant patients; nonhematological toxicity was insignificant aside from typhlitis (three patients); and immunosuppression allowed subsequent treatment with the murine 3F8 monoclonal antibody. The 15–17% rate of major responses (Table 2) should be weighed in the context of the prior prolonged and/or high-dose (including myeloablative) therapy of the study patients. The absence of toxicity to major nonhematological organs in this very high-risk patient population met a key study aim, although aggressive supportive care was required in some patients to control acute problems related to myelosuppression.

CTV is the first reported use of topotecan with two other chemotherapeutic agents for a pediatric solid tumor. We previously tried high-dose cyclophosphamide/topotecan against various resistant pediatric solid tumors (11). That regimen did not include vincristine and also differed from CTV in terms of infusion times (48 h for cyclophosphamide, 72 h for topotecan) and topotecan dosage (6 mg/m²). We added vincristine to the high-dose cyclophosphamide/topotecan combination based on preclinical data that showed a greater than additive cytotoxic effect when topotecan and vincristine were used together (26). The choice of topotecan daily dosage and infusion time was based on the absence of undue toxicity with 30-minute daily infusions ×5 days of topotecan 2–2.4 mg/m² (i.e., 10–12 mg/m² per cycle; Refs. 17 and 27) and the fact that daily dosages of 1–2 mg/m² correlated with efficacy against topotecan-sensitive malignancies (17, 26–29). We increased the dosage of topotecan to 8 mg/m² because of the lack of nonhematological toxicity in the earlier study with 6 mg/m² (11).

Uncertainty exists regarding the optimal dosage and schedule of irinotecan. Preclinical studies using xenografts of pediatric solid tumors, including NB, favor 5- or 10-day schedules, and the few published clinical reports support that timing. Thus, in pediatric phase I studies, (12–14) and in small pilot studies (15, 18), irinotecan administered at 50–200 mg/m²/day × 3 days (14), at 30–65 mg/m²/day × 5 days, (12, 18), or at 20–29 mg/m²/day × 10 days (13, 15) produced favorable results in patients with NB or other solid tumors. In contrast, the sole phase II trial of irinotecan for NB reported to date used a single dose of 600 mg/m² every 3 weeks but, despite the higher dosage than in the 3-, 5-, or 10-day regimens, yielded “no clinically useful activity” (16). For CI, we chose the 5-day schedule with irinotecan 50 mg/m²/day because myelosuppression from irinotecan would coincide with that caused by the high-dose cyclophosphamide.

CTV and CI have modest anti-NB activity in heavily treated patients, immunomodulatory effects conducive to anti-body-mediated immunotherapy, and modest toxicity to major nonhematological organs. Other aspects of CTV and CI worthy of note include the activity of camptothecins as radiation sensitizers, (30) evidence that the camptothecins may be less affected by P-glycoprotein multidrug resistance mechanisms when compared with doxorubicin and etoposide, (7, 31) the excellent penetration into the central nervous system of topotecan (32) though not of irinotecan (33), and the absence of altered pharmacokinetics with combined use of cyclophosphamide and topotecan (Refs. 34 and 35; no report to date on this issue regarding irinotecan). Furthermore, because cyclophosphamide, camptothecin analogs, and vincristine are active against a variety of solid tumors, CTV and CI might have applicability beyond the NB patient population.

REFERENCES
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