Phase I Trial of Oral Irinotecan and Temozolomide for Children With Relapsed High-Risk Neuroblastoma: A New Approach to Neuroblastoma Therapy Consortium Study

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ABSTRACT

Purpose

Irinotecan and temozolomide have single-agent activity and schedule-dependent synergy against neuroblastoma. Because protracted administration of intravenous irinotecan is costly and inconvenient, we sought to determine the maximum-tolerated dose (MTD) of oral irinotecan combined with temozolomide in children with recurrent/resistant high-risk neuroblastoma.

Patients and Methods

Patients received oral temozolomide on days 1 through 5 combined with oral irinotecan on days 1 through 5 and 8 through 12 in 3-week courses. Daily oral cefixime was used to reduce irinotecan-associated diarrhea.

Results

Fourteen assessable patients received 75 courses. Because neutropenia and thrombocytopenia were initially dose-limiting, temozolomide was reduced from 100 to 75 mg/m²/d for subsequent patients. Irinotecan was then escalated from 30 to 60 mg/m²/d. First-course grade 3 diarrhea was dose-limiting in one of six patients treated at the irinotecan MTD of 60 mg/m²/d. Other toxicities were mild and reversible. The median SN-38 lactone area under the plasma concentration versus time curve at this dose was 72 ng · hr/mL. One patient with bulky soft tissue disease had a complete response through six courses. Six additional patients received a median of seven courses (range, three to 22 courses) before progression.

Conclusion

This all-oral regimen was feasible and well tolerated in heavily pretreated children with resistant neuroblastoma, and seven (50%) of 14 assessable patients had response or disease stabilization for three or more courses in this phase I trial. SN-38 lactone exposures were similar to those reported with protracted intravenous irinotecan. The dosages recommended for further study in this patient population are temozolomide 75 mg/m²/d plus irinotecan 60 mg/m²/d when given with cefixime.

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INTRODUCTION

Current regimens for high-risk neuroblastoma cure fewer than half of all patients, with many experiencing relapse from minimal residual disease remaining after consolidation with high-dose chemotherapy and autologous hematopoietic stem-cell transplantation.¹ Although some success has been achieved using post-transplantation maintenance therapy with the differentiating agent 13-*cis*-retinoic acid¹ or with monoclonal antibodies targeting neuroblastoma-specific proteins,² further advances are needed. Ideal characteristics of a maintenance therapy regimen include proven activity against residual neuroblastoma, as well as safety, fea-

sibility, and convenience for this population of heavily pretreated children.

With these features in mind, we initiated a clinical trial to explore the combination of orally administered irinotecan and temozolomide. Both drugs have single-agent activity against mouse models of neuroblastoma,³⁻⁶ and irinotecan induces differentiation in *MYCN*-amplified xenografts unresponsive to 13-*cis*-retinoic acid.⁷ Responses have been seen in all eight published trials of single-agent irinotecan that have included neuroblastoma patients,⁸⁻¹⁵ as well as in a phase II trial of single-agent temozolomide.¹⁶ Further, there is schedule-dependent synergy of this combination against preclinical tumor models,¹⁷ including

neuroblastoma.³ Mechanistically, temozolomide-induced methylation of DNA helps recruit topoisomerase I molecules, thereby potentiating irinotecan cytotoxicity.¹⁸ Consistent with these findings, activity is greatest when temozolomide is given at least 1 hour before irinotecan.¹⁷ This two-drug combination has been well tolerated and active in patients with Ewing sarcoma¹⁹ and high-grade glioma,²⁰ and a Children's Oncology Group (COG) phase II trial of temozolomide and irinotecan is now underway to define activity in first relapse of neuroblastoma. That trial uses a protracted irinotecan schedule with low dosages administered intravenously for 5 days/wk for 2 consecutive weeks, based on preclinical experiments showing superiority for protracted scheduling.²¹

Although protracted irinotecan may have less myelosuppression compared with single doses given every 1 to 3 weeks,²² this schedule is inconvenient and expensive when administered intravenously. In contrast, oral administration is attractive for protracted scheduling, provided that acceptable systemic drug exposures can be achieved. Previous trials of oral irinotecan have also shown potential pharmacokinetic advantages of this approach,²³⁻³¹ such as more efficient conversion of the parent drug to the active metabolite SN-38 as a result of presystemic metabolism mediated by intestinal carboxylesterases.³² Because diarrhea is a dose-limiting toxicity of protracted irinotecan regardless of route of administration, in this trial we used prophylactic daily cephalosporins to ameliorate this toxicity.³³

We now report the clinical and pharmacologic findings of an all-oral phase I trial of irinotecan, temozolomide, and cefixime in heavily pretreated children with relapsed or refractory high-risk neuroblastoma.

PATIENTS AND METHODS

Eligibility

Patients with high-risk neuroblastoma ≤ 30 years of age at diagnosis were eligible if they had either recurrent/progressive disease or residual tumor documented by histology after front-line therapy. All patients were required to have measurable or nonmeasurable but assessable tumor documented by computed tomography (CT), magnetic resonance imaging (MRI), metaiodobenzylguanidine (MIBG) scanning, or bone marrow morphology. Other eligibility criteria included Eastern Cooperative Oncology Group performance status score of ≤ 2 , hemoglobin ≥ 8.0 g/dL, absolute neutrophil count $\geq 1,000/\mu\text{L}$, platelet count $\geq 100,000/\mu\text{L}$, and adequate renal and hepatic function. Patients with bone marrow disease were eligible if they met hematologic criteria. Prior treatment with autologous stem-cell transplantation was allowed. Exclusion criteria included use of enzyme-inducing anticonvulsants, cephalosporin allergy, active infection, or $\geq$ grade 2 diarrhea. Sixteen patients were enrolled from May 2004 to July 2006, through the New Approaches to Neuroblastoma Therapy (NANT) consortium (www.nant.org). Local institutional review board approval and informed consent were obtained.

Drug Formulation and Administration

The injectable formulation of irinotecan (Camptosar; Pfizer, New York, NY) was obtained commercially in 20-mg/mL vials. One course (10 doses) was drawn up in oral syringes and dispensed with instructions to refrigerate until administration. Irinotecan was mixed with cranberry-grape juice immediately before administration to mask the bitter flavor and administered once daily on days 1 through 5 and 8 through 12 of each 3-week course. Temozolomide (Temodar; Schering-Plough, Kenilworth, NJ) was obtained commercially, and patients were allowed to open capsules and mix in apple sauce or juice if unable to swallow whole capsules. Temozolomide was administered on days 1 through 5 of each course and given at least 1 hour before irinotecan, based on preclinical experiments.^{3,17} Cefixime 8 mg/kg (maximum daily dose, 400 mg)

was started 5 days before chemotherapy and continued daily.²³ Patients were instructed to begin the antidiarrhea agent loperamide with the first loose stool.³⁴

Study Design

The starting dose of oral irinotecan was 30 mg/m²/d (75% of the maximum-tolerated dose [MTD] of oral irinotecan without cefixime), with planned escalation to 60 mg/m²/d, which is the single-agent MTD of oral irinotecan when using cefixime.²³ The starting dose of temozolomide was 100 mg/m²/d, based on a previous trial in combination with intravenous irinotecan.³⁵ Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria version 3.0. Hematologic dose-limiting toxicity (DLT) was defined as grade 4 neutropenia for more than 7 days, platelets less than 10,000/ μL , or the need for more than one platelet transfusion per week. Nonhematologic DLT included any grade 3 to 4 toxicity excluding grade 3 nausea or vomiting, grade 3 diarrhea lasting less than 72 hours, grade 3 fever or infection, and grade 3 transaminase elevation resolving to eligibility criteria before next course. Any grade 3 to 4 toxicity not resolving to eligibility criteria within 35 days after the start of chemotherapy was considered DLT. A standard three-plus-three phase I design was used. The MTD was defined as the highest dose in which no more than one of six patients experienced first-course DLT.

Additional courses were started every 21 days, provided that toxicity resolved to eligibility criteria and there was no disease progression. Patients not meeting eligibility criteria within 35 days from the start of a course were taken off protocol therapy. Patients with stable or responding disease who had reversible DLT were offered further modified treatment as specified in the protocol, depending on the DLT. For example, patients with hematologic DLT had daily temozolomide reduced by 25 mg/m² and received prophylactic filgrastim with subsequent courses while remaining on study. In contrast, for patients with dose-limiting diarrhea, the irinotecan dose was reduced one dose level.

Tumor Response

Tumor response was evaluated after courses 2, 4, and 8. Overall response was graded according to NANT criteria, modified from the International Neuroblastoma Response Criteria using the Response Evaluation Criteria in Solid Tumors method for measurable tumor on CT/MRI³⁶ and Curie score for MIBG response.³⁷ Complete disappearance of tumor by morphology was required to be considered response of bone marrow disease. CT/MRI and MIBG responses were evaluated by two central reviewers.

Clinical Pharmacology Evaluation

Irinotecan, SN-38 lactone, and SN-38 glucuronide (SN-38G) lactone pharmacokinetics were assessed during course 1 in consenting patients. The lactone form of SN-38 was measured because this is the form with antitumor activity. Blood samples were collected before irinotecan and at 0.25, 1.5, 2.5, 5, 7, and 24 hours after administration and were processed as previously described.^{23,38} A multicompartment model was fit to plasma concentration-time data of the lactone forms of irinotecan, SN-38, and SN-38G using maximum likelihood estimation as implemented in ADAPT II.³⁹ Area under the plasma concentration versus time curves (AUC) for irinotecan, SN-38, and SN-38G from the beginning of the infusion to 24 hours were calculated by integration of the simulated concentration-time data from model estimates.

The pharmacokinetics of temozolomide and active metabolite 3-methyl-(triazene-1-yl)imidazole-4-carboxamide (MTIC) were also evaluated during course 1 in consenting patients. Blood was collected before and at 0.25, 1.5, and 5 hours after temozolomide administration and processed as previously described.⁴⁰ Temozolomide and MTIC plasma concentration-time data were modeled using a posteriori (MAP) Bayesian estimation as implemented in ADAPT II.³⁹ The parameter estimates were derived from a pediatric population.⁴¹ A first-order absorption, one-compartment linear model, which included first-order MTIC formation and elimination, was used to simultaneously describe temozolomide and MTIC disposition. The AUC was calculated from the model parameters. These estimates then allowed for calculation of temozolomide systemic clearance.

UGT1A1 Genotyping

For adults receiving irinotecan as a single dose every 1 to 3 weeks, toxicity is associated with polymorphisms of the *UGT1A1* gene, which codes for a

protein critical in the metabolism of the active metabolite SN-38.⁴² To investigate whether a similar relationship exists with protracted low-dose irinotecan, we prospectively collected blood samples for *UGT1A1* genotyping in consenting patients. Genomic DNA was extracted using standard molecular procedures, and 10 ng of DNA was used for genotyping. The *UGT1A1**28 promoter polymorphism was genotyped by polymerase chain reaction amplification followed by fragment size analysis as previously described.⁴³ Genotypes were denoted as 6/6, 6/7, or 7/7 depending on the number of TA repeats found in each allele.

RESULTS

Patient Characteristics

Sixteen patients were enrolled from nine NANT institutions. All patients had disease progression and were eligible at time of enrollment, although one patient had unexpected intracranial disease progression and died before receiving protocol therapy. A second patient developed autoimmune encephalitis not attributed to study treatment and died before completing course 1. The histopathology and immunologic evaluation was consistent with autoimmune encephalitis caused by anti-Hu antibodies, which is a paraneoplastic complication previously reported in patients with neuroblastoma.⁴⁴ No direct link with protocol therapy was identified, as illness developed during the first week of treatment, and in retrospect, subtle neurologic deficits may have been present even before study treatment. Anti-Hu antibodies were retrospectively documented in serum archived before study enrollment. The remaining 14 patients (Table 1) were assessable for toxicity and response. One patient withdrew after course 1 without DLT or disease progression to pursue other treatments.

Table 1. Assessable Patient Characteristics (n = 14)

Characteristic	No.
Sex	
Male	11
Female	3
Age, years	
Median	7
Range	3-22
<i>MYCN</i>	5
Amplified	
Nonamplified	6
Unknown	3
No. of prior chemotherapy regimens	
Median	3.5
Range	1-7
Prior therapy with autologous stem-cell transplantation	11
Prior therapy with cyclophosphamide/topotecan	11
Measurable tumor on CT/MRI	9
MIBG avid lesions	14
Bone marrow metastases by morphology	6
No. of courses assessable for toxicity	75
No. of courses per patient	
Median	20.5
Range	1-22
Abbreviations: CT, computed tomography; MRI, magnetic resonance imaging; MIBG, metaiodobenzylguanidine.	

Toxicity

Hematologic DLT was identified at dose level 1 in two patients, including one who came off study after course 3 because of persistent neutropenia despite temozolomide dose reduction and filgrastim (Table 2). Because myelosuppression is more closely linked with temozolomide,^{14,45} the temozolomide dose was decreased from 100 to 75 mg/m²/d (dose level 1a). Three additional patients were treated, with no further first-course DLT. Subsequent patients were then treated with irinotecan 60 mg/m²/d and temozolomide 75 mg/m²/d (dose level 2). No further dose-limiting hematologic toxicity was observed after reducing temozolomide. Overall, grade 4 neutropenia and grade 4 thrombocytopenia were each observed in only five (7%) of 75 courses. Only one patient received blood product transfusions, and one patient received filgrastim.

Regarding nonhematologic toxicity, one patient treated at dose level 2 had dose-limiting grade 3 diarrhea during course 1. A second patient developed similar symptoms with grade 3 dehydration during the third course, but went on to receive three additional courses at a reduced irinotecan dose. This was not considered DLT because it was not first-course toxicity. However, because two of six patients experienced grade 3 diarrhea at some point during their treatment at dose level 2, and because excessive gastrointestinal toxicity had been previously reported at oral irinotecan dosages more than 60 mg/m²/d despite cefixime prophylaxis,²³ we did not pursue further dose escalation and instead defined dose level 2 as the MTD.

An important finding for this all-oral regimen was the absence of any grade 3 vomiting attributable to therapy. In addition, no grade 4 toxicities were noted in six patients receiving 29 courses at the MTD. Three patients had infections, all without neutropenia. No patients were hospitalized for therapy-associated complications. Compliance with cefixime was confirmed by a medication diary, and there were no recognized complications of using continuous antibiotics, despite six patients receiving at least 18 or more weeks of treatment. Specifically, there were no reports of *Clostridium difficile* enteritis or fungal or cephalosporin-resistant infections.

Pharmacokinetics and UGT1A1 Analysis

Twelve patients consented to pharmacokinetic studies. In general, drug and metabolite exposures for both agents were increased at larger dosages (Table 3). SN-38 lactone exposures at the MTD of 60 mg/m²/d were similar to those reported with the single-agent intravenous irinotecan MTD of 20 mg/m²/d.⁴⁶ The median apparent temozolomide clearance was 6.7 L/h/m² (range, 2.1 to 12.3 L/h/m²). For oral irinotecan, the median apparent clearance was 580.1 L/h/m² (range, 99.3 to 2,992.3 L/h/m²).

Thirteen patients consented to *UGT1A1* pharmacogenetic studies. DLT occurred in two of nine patients with the 6/7 genotype and one of three patients with the 6/6 genotype and did not occur in the one patient with the 7/7 genotype. No association was seen between *UGT1A1* genotype and toxicity in this small study.

Antitumor Activity

Of 14 assessable patients, one had a centrally reviewed complete response at dose level 1a (oral irinotecan 30 mg/m²/d and temozolomide 75 mg/m²/d), consisting of resolution of biopsy-proven soft tissue disease in the axilla, neck, and chest. This patient had prior multiagent induction and tandem stem-cell transplantation. After

Table 2. Toxicities Reported to Be Possibly, Probably, or Definitely Attributable to Chemotherapy

Dose Level	TEM Daily Dose (mg/m ²)	IRN Daily Dose (mg/m ²)	No. of Assessable Patients	No. of Assessable Courses	First-Course DLT		Grade 3		Grade 4	
					No. of Patients	Toxicity	No. of Patients	Toxicity	No. of Patients	Toxicity
1	100	30	5	10	2	Neutropenia, thrombocytopenia	1	Transaminitis	1	Neutropenia
									2	Thrombocytopenia
1a	75	30	3	36	0		1	Neutropenia	1	Neutropenia
							1	Infection		
2	75	60	6	29	1	Diarrhea	2	Diarrhea		None
							1	Neutropenia		
							1	Dehydration		

Abbreviations: TEM, temozolomide; IRN, irinotecan; DLT, dose-limiting toxicity.

relapsing post-transplantation, there was continued disease progression despite treatment with cyclophosphamide/topotecan as well as single-agent temozolomide. The response to oral irinotecan/temozolomide was maintained for six courses before progression.

Five patients had stable disease by NANT criteria, with stabilization maintained for a median of seven courses (range, three to 22 courses). An additional patient received eight courses before disease progression, but response assessment was limited by inadequate contrast on CT scan at study entry, preventing the confirmation of stable disease on central review. There was no clear difference in disease site(s) between patients treated with fewer than three courses versus three or more courses. The median progression-free survival for the 15 patients who began treatment was 4.2 months (95% CI, 1.5 to 4.9 months).

DISCUSSION

This study is the first to report use of an all-oral regimen of temozolomide and irinotecan. This strategy capitalizes on the known antineuroblastoma effects of these agents, the potential for schedule-dependent synergy and nonoverlapping toxicities, the more efficient conversion of oral irinotecan to SN-38 through first-pass metabolism, and the convenience and reduced cost of oral administration.

Previous attempts to administer oral irinotecan have been limited by relatively poor bioavailability, necessitating higher doses that resulted in dose-limiting diarrhea. Furman et al²³ reported this toxicity may be ameliorated with daily cefixime, which allowed for a 50% increase in the MTD of protracted oral irinotecan, corresponding to an 87% increase in median SN-38 exposure. It is theorized that cefixime eradicates enteric Gram-negative bacteria, which produce glucuronidases that prolong gut exposure to the toxic metabolites of irinotecan. As seen previously,²³ cefixime allowed for the achievement of SN-38 exposures similar to those seen with protracted intravenous irinotecan at its single-agent MTD.⁴⁶ Importantly, we did not observe any infectious problems related to prolonged antibiotic therapy. Such theoretical complications could be further minimized if prophylaxis could be targeted to those at risk. However, our results did not show any correlation between *UGT1A1* genotype and toxicity, consistent with a recent pediatric study.⁴³

Other trials of oral irinotecan using different schedules are summarized in Table 4. Irinotecan for those studies was supplied as either a powder-filled capsule,^{24,27,30} a semi-solid matrix,^{26,28,29} or, as done in this study, with the intravenous formulation diluted in cranberry-grape juice.^{23,25} There have been no direct comparisons of pharmacokinetic properties between these formulations. Comparison of drug exposures between studies is difficult because of interpatient variability and the use of different assays and sampling schedules. However, because SN-38 exposures with protracted administration generally increase with higher dosages,^{23,30} and because preclinical antitumor activity of oral irinotecan also increases in a dose-dependent fashion,⁴⁷ it is intuitive that higher irinotecan doses may be optimal. Only our study and that of Furman et al²³ used cephalosporin prophylaxis against irinotecan-associated diarrhea, and both regimens were able to achieve doses providing up to 140% more oral irinotecan per 21-day course. A caveat to this finding is that these two trials were performed

Table 3. Results of Pharmacokinetic Studies for Parent Drugs and Metabolites

Drug/Metabolite	Lower Dose	Higher Dose
Irinotecan		
Dose, mg/m ² /d	30	60
No. of patients	7	5
Irinotecan AUC, ng · hr/mL		
Median	29.6	72.5
Range	6.4-86.1	19.5-375.3
SN-38 lactone AUC, ng · hr/mL		
Median	25.0	69.5
Range	6.9-119.2	35.2-86.5
SN-38G lactone AUC, ng · hr/mL		
Median	27.9	45.4
Range	2.4-133.3	21.8-238.9
Temozolomide		
Dose, mg/m ² /d	75	100
No. of patients	7	5
Temozolomide AUC, ng · hr/mL		
Median	7.0	15.4
Range	5.9-15.4	10.9-27.0
MTIC AUC, ng · hr/mL		
Median	0.36	0.75
Range	0.26-4.14	0.26-73.82

Abbreviations: AUC, area under the plasma concentration versus time curve; MTIC, 3-methyl-(triazene-1-yl)imidazole-4-carboxamide.

Table 4. Previous Phase I Trials Using Oral Irinotecan in 3-Week Courses

First Author	Schedule	Formulation	Other Drugs	No. of Patients	No. of Courses	Irinotecan MTD (mg/m ² /d)	DLT	Irinotecan (mg/m ² per course)
Drengler ²⁵	Daily × 5	IV with juice	—	28	156	66 for patients < 65 years of age; 50 for patients > 65 years of age	D	330/250
Pitot ²⁷	Daily × 5	PFC	—	20	87	50	N, V, D, FN	250
Soepenberg ²⁸	Daily × 5	SSM	—	25	101	70	D	350
Dumez ²⁴	Daily × 5	PFC	—	47	171	80	D, FN, V, colitis	400
Schoemaker ³⁰	Daily × 14	PFC	—	45	179	30	N, V, D	420
Kuppens ²⁶	Daily × 14	SSM	—	17	50	30	N, V, D	420
Kuppens ²⁶	Daily × 14	SSM	Capecitabine	24	67	30	N, V, D	420
Soepenberg ²⁹	Daily × 5	SSM	Capecitabine	28	155	50	D, anorexia, colitis	250
Furman ²³	Daily × 5 × 2	IV with juice	Cefixime	19	63	60	V, D	600
Current study	Daily × 5 × 2	IV with juice	Temozolomide Cefixime	14	75	60	D	600

Abbreviations: MTD, maximum-tolerated dose; DLT, dose-limiting toxicity; IV, intravenous; PFC, powder-filled capsules; SSM, semi-solid matrix; D, diarrhea; N, nausea; V, vomiting; FN, febrile neutropenia.

in children, who may be able to tolerate higher doses of chemotherapy compared with adults, many of whom had colon cancer.

Toxicity attributable to therapy on this trial was minimal. There was no grade 4 toxicity in six patients receiving 29 courses of therapy at the MTD. In addition, there were minimal infections or requirements for transfusions or filgrastim. The relative tolerance of this drug combination has also been reported in other studies.^{19,20,35} Considering the favorable pharmacokinetic exposures achieved and the likelihood of greater toxicity with higher irinotecan dosages,²³ we recommend 60 mg/m²/d of irinotecan for further study of this combination. It is possible the temozolomide dose of 75 mg/m²/d, which compares to 200 mg/m²/d in single-agent trials,^{16,45} could be increased in less heavily pretreated patients or by using filgrastim.⁴⁸ However, pre-clinical synergy is seen even with relatively low temozolomide dosages,³ and higher dosages combined with irinotecan are of unknown additional benefit and may result in treatment delays and dose reductions.^{15,48}

In this trial, six (43%) of 14 patients remained progression-free through at least six courses, including one patient with a complete response. The ongoing COG phase II trial of temozolomide and intravenous irinotecan for first relapse high-risk neuroblastoma should provide a more accurate estimate of the activity of this regimen, as well as a comparison to historical controls treated with cyclophosphamide and topotecan, which is standard first-line salvage therapy that had already been administered to 79% of patients in this study. Although both are camptothecin agents, topotecan and irinotecan may have different mechanisms of resistance.⁴⁹ Because cyclophosphamide/topotecan is now used in front-line COG induction regimens, potentially non-cross-resistant agents like temozolomide and irinotecan are attractive for either maintenance or salvage therapy.

A recent randomized trial in patients with rhabdomyosarcoma suggested that a 5-day schedule of irinotecan combined with vincristine was as efficacious and tolerable as the more protracted schedule used in our study.⁵⁰ On the basis of these data, the COG is now evaluating a 5-day course of oral irinotecan together with temozolomide and vincristine. If feasible, this schedule would be more convenient. Results from a recent study using temozolomide concurrent with 5 days of intravenous irinotecan support a shorter irinotecan schedule, because complete and partial responses were seen in patients with relapsed or refractory high-risk neuroblastoma.⁴⁸

In conclusion, this all-oral regimen was safe and had activity in heavily pretreated patients with relapsed/refractory high-risk neuroblastoma. Oral antibiotic prophylaxis allowed for SN-38 systemic exposures similar to that observed after intravenous irinotecan administration. This combination could serve as a backbone for adding other synergistic drugs such as vincristine, bortezomib, or O⁶-benzylguanine,⁵¹⁻⁵³ and may be explored in other cancers as well. For patients with neuroblastoma, this all-oral regimen is attractive because of convenience and tolerability, particularly if activity is confirmed in the ongoing COG phase II trial.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Appendix

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