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ORIGINAL REPORT

Phase II Study of Temsirolimus in Women With Recurrent or Metastatic Endometrial Cancer: A Trial of the NCIC Clinical Trials Group

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Clinical Trials repository link available on JCO.org.

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ABSTRACT

Purpose
Phosphatase and tensin homolog (*PTEN*) is a tumor suppressor gene, and loss of function mutations are common and appear to be important in the pathogenesis of endometrial carcinomas. Loss of *PTEN* causes deregulated phosphatidylinositol-3 kinase/serine-threonine kinase/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling which may provide neoplastic cells with a selective survival advantage by enhancing angiogenesis, protein translation, and cell cycle progression. Temsirolimus, an ester derivative of rapamycin that inhibits mTOR, was evaluated in this setting.

Patients and Methods
Sequential phase II studies evaluated single-agent activity of temsirolimus in women with recurrent or metastatic chemotherapy-naive or chemotherapy-treated endometrial cancer. Temsirolimus 25 mg intravenously was administered weekly in 4-week cycles.

Results
In the chemotherapy-naive group, 33 patients received a median of four cycles (range, one to 23 cycles). Of the 29 patients evaluable for response, four (14%) had an independently confirmed partial response and 20 (69%) had stable disease as best response, with a median duration of 5.1 months (range, 3.7 to 18.4 months) and 9.7 months (range, 2.1 to 14.6 months). Only five patients (18%) had progressive disease. In the chemotherapy-treated group, 27 patients received a median of three cycles (range, one to six cycles). Of the 25 patients evaluable for response, one (4%) had an independently confirmed partial response, and 12 patients (48%) had stable disease, with a median duration of 4.3 months (range, 3.6 to 4.9 months) and 3.7 months (range, 2.4 to 23.2 months). *PTEN* loss (immunohistochemistry and mutational analysis) and molecular markers of PI3K/Akt/mTOR pathway did not correlate with the clinical outcome.

Conclusion
mTOR inhibition with temsirolimus has encouraging single-agent activity in endometrial cancer which is higher in chemotherapy-naive patients than in chemotherapy-treated patients and is independent of *PTEN* status. The difference in activity according to prior therapy should be factored into future clinical trial designs.

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INTRODUCTION

Endometrial cancer is the most common gynecologic malignancy in women in North America and Europe.[1] Women with locally recurrent advanced or metastatic endometrial cancer are not curable and have few treatment options that incorporate hormones or chemotherapy. Hormonal agents such as progestins, antiestrogens, and aromatase inhibitors are commonly used, but efficacy is modest, and median survival is short at 7 to 12 months.[2-14] Chemotherapy is not curative and may be poorly tolerated in this group of often elderly women. The most active combination from randomized trials is cisplatin, doxorubicin and paclitaxel with filgrastim support (TAP), which produces objective responses in 57% of women with progression-free and overall survival of 8.3 and 15.3 months, respectively[15] The combination of carboplatin and paclitaxel is better tolerated and is currently undergoing formal randomized comparison with TAP.[16,17] A Cochrane collaboration meta-analysis[18] concluded that there was considerable variability in patient populations, and higher responses with combination chemotherapy

are associated with greater toxicity. The role of further systemic therapy following failure of chemotherapy remains limited with no recognized evidence-based standards.

There is a need to identify novel agents to improve survival, either as single agents or built into combination regimens. Attention has turned to drugs that target molecular pathways of relevance in malignancy.

Activation of the phosphatidylinositol-3 kinase/serine-threonine kinase (PI3K/AKT) pathway occurs frequently in endometrial carcinoma. Loss of tumor suppressor genes phosphatase and tensin homolog *(PTEN)*, tuberous sclerosis complex 1,2 (*TSC1* and *TSC2*), and *LKB* as well as activation mutations and/or amplification in oncogenes *PI3K* and *AKT* have been reported.[19-23] The *PTEN* gene encodes a phosphoinositide-3-phosphatase that antagonizes PI3K signaling through dephosphorylation of its intracellular second messenger, phosphoinositidyl-3 phosphate (PIP3) and serves as a major negative regulator of the PI3K/AKT signaling pathway[24] Loss of PTEN expression leads to deregulated activation of protein kinase B (PKB)/Akt signaling, an event that is thought to provide cells with a selective survival advantage by enhancing angiogenesis, protein translation, and cell cycle turnover.[25] *PTEN* knock-out mice develop endometrial neoplasia which can be delayed by administration of pathway inhibitors.[26,27] Often *PTEN* mutations present as a loss of PTEN protein expression and are more common in endometrioid endometrial cancers (26% to 80%) than nonendometrioid subtypes.[24,25,28] *PTEN* inactivation may be associated with adverse prognosis, but data are conflicting. Some studies suggest that the presence of *PTEN* mutation predicts earlier disease stage and better survival in patients with endometrial cancer;[29-31] however, a recent study demonstrated that approximately 60% of patients with recurrent/metastatic disease showed PKB/Akt phosphorylation and presumably activation of that pathway.[13]

Temsirolimus (CCI-779), an ester of the macrocyclic immunosuppressive agent sirolimus (rapamycin [Rapamune]; Wyeth, Madison, NJ), is a cytostatic cell cycle inhibitor with antitumor properties. Temsirolimus inhibits the mammalian target of rapamycin (mTOR), a serine-threonine kinase involved in the initiation of mRNA translation,[32] and has been shown to inhibit the growth of a wide range of histologically diverse tumor cells. Temsirolimus improves progression-free and overall survival in patients with renal cell carcinoma.[33] Intermittent schedules of administration have been evaluated to minimize the agent's immunosuppressive effects while maintaining antitumor activity. Dose dependence was not seen in early studies, and therefore the standard dosing schedule is 25 mg weekly.

Because *PTEN* loss is common in endometrial carcinoma and seems to be associated with constitutional activation of PI3K/Akt/mTOR signaling, targeting this pathway with temsirolimus is appropriate. This pathway and the level of inhibition by temsirolimus is illustrated in Figure 1.

This trial (NCIC Clinical Trials Group [NCIC CTG] IND160) assessed the activity of temsirolimus in women with endometrial cancer and explored possible molecular predictors of efficacy in archival tumor samples. The first cohort, IND160A (group A), enrolled women with no prior chemotherapy. Following observations of activity in this group, the protocol was amended to include a second cohort of women, IND160B (group B), who had received prior chemotherapy. Both were evaluated separately for response to treatment.

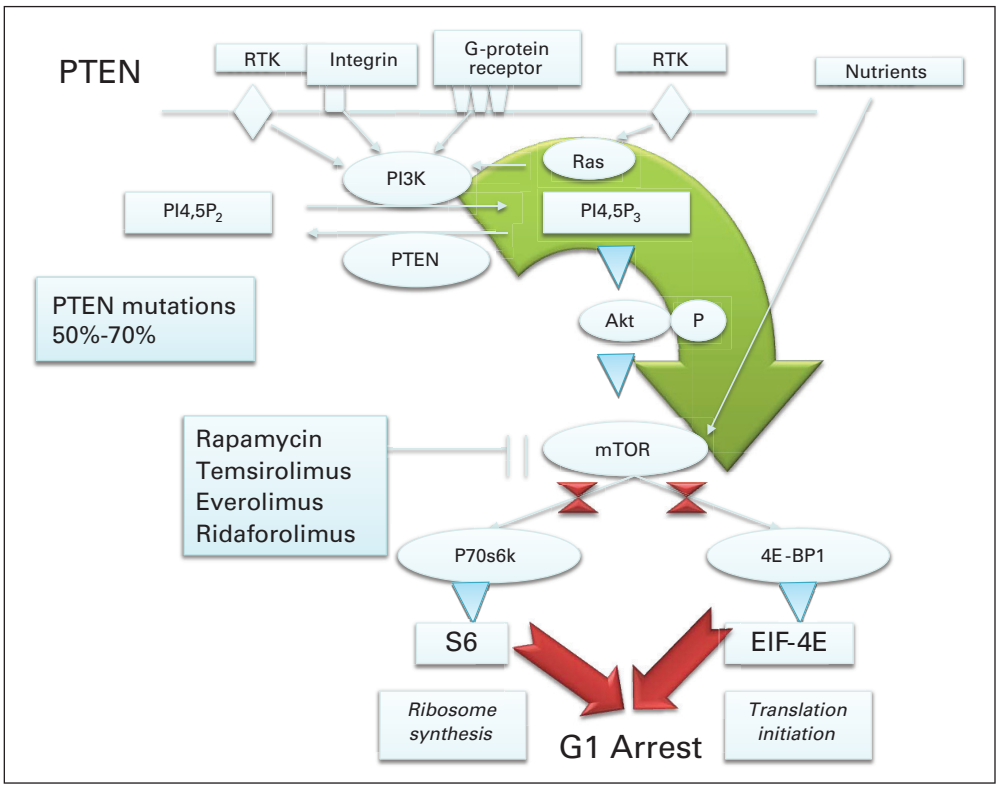

Fig 1. Representation of PTEN/Akt/mTOR (phosphatase and tensin homolog/Akt/mammalian target of rapamycin) pathway. CCI-779, temsirolimus; EIF-4E, eukaryotic translation initiation factor 4E; P13K, phosphatidylinositol 3-kinase; P70s6k, phosphorylated s6 kinase; Rad 001, everolimus; RTK, receptor tyrosine kinase.

PATIENTS AND METHODS

This was a nonrandomized, nonblinded, multicenter phase II trial to investigate the efficacy of temsirolimus in patients with locally advanced, recurrent, and/or metastatic carcinoma of the endometrium conducted by the NCIC CTG. The study was conducted according to Good Clinical Practice guidelines, with full research ethics board approval at each of the participating institutions. All patients signed written informed consent before study entry.

Eligibility

Patients with histologically confirmed metastatic or locally advanced adenocarcinoma or adenosquamous carcinoma of the endometrium, incurable by standard therapies, were eligible for this trial. Group A (IND160A, chemotherapy-naive) patients could have had up to one prior hormonal treatment (progestational or aromatase inhibitor) for their disease but no prior chemotherapy. Group B (IND160B, chemotherapy-treated) patients had received one prior line of chemotherapy. All patients were required to have archival tumor specimens available for exploration of molecular correlative measures in the PTEN/AKT/mTOR pathway.

Study Design and Treatment Plan

Temsirolimus was administered once per week in 4-week cycles at a dose of 25 mg intravenously over 30 minutes on days 1, 8, 15, and 22. Cycle length was not changed if doses were missed. Temsirolimus was administered via an automatic dispensing pump that used tubing that did not contain polyvinyl chloride with the appropriate filter. Treatment was continued until disease progression, intercurrent toxicities, unacceptable adverse events, patient's decision to withdraw from the study, or inability to continue treatment.

Management of Toxicity

Suspension of the drug was generally recommended for any grade 2 or higher toxicity. Drug was recommenced if toxicity recovered to grade ≤ 1 within 2 weeks at a one-dose-level reduction. Dose reductions were level -1, 20 mg; level -2, 15 mg; and level -3, 10 mg. Evidence of symptomatic interstitial pneumonitis warranted withholding drug and permanent discontinuation if the diagnosis was confirmed and thought to be related to temsirolimus.

On-Study Evaluation

Chest x-ray and computed tomography was performed at baseline and after every two cycles at 8 weeks or at any time there was clinical suspicion of progressive disease (PD). Tumor response was evaluated by using Response Evaluation Criteria in Solid Tumors (RECIST) criteria.[34] Stable disease (SD) required a minimum of 4 weeks duration. All claimed responses were reviewed by independent radiologists, and only those responses confirmed by radiology review were included in the final analysis of results. Hematology was evaluated on days 1, 8, 15, and 22 of each cycle. Biochemistry, patient review, and assessment of toxic effects were carried out on day 1 of each cycle and graded according to the National Cancer Institute Common Toxicity Criteria Version 3.0.

All patients were seen 4 weeks after completion of protocol therapy. Continued follow-up was not required for patients off protocol treatment with PD, except to document late toxicities and death. Patients who went off protocol treatment with complete response, partial response (PR), or SD required ongoing follow-up every 3 months until disease progression or death.

Molecular Analysis of PTEN and Gene Status

Archival tissue was obtained from all patients to explore the relationship between expression of PTEN and other proteins in the PI3K/AKT/mTOR pathway with outcome. These translational correlations are illustrated in Figure 1.

Immunohistochemistry

Archival paraffin slides were stained immunohistochemically by using primary antibodies to PTEN, AKT, pS6, and phosphorylated mTOR (pmTOR; Appendix Table A1, online only), and biotin-conjugated secondary antibodies, followed by incubation with horseradish peroxidase–streptavidin labeling reagent (ID Labs, London, Ontario, Canada). The slides were scored independently by two pathologists (M.-S.T. and W. Chapman), and final

Table 1. Patient Characteristics

Characteristic	Group A (chemotherapy naive) No.	Group A (chemotherapy naive) %	Group B (chemotherapy treated) No.	Group B (chemotherapy treated) %
No. of evaluable patients	33		27	
Age, years				
Median	66		60	
Range	52-80		41-81	
Performance status				
0	14		13	
1	17		14	
2	2			
Prior therapy				
Hormonal therapy	13		6	
Radiation	21		13	
Chemotherapy	0		27	
Other	2		1	
Histology				
Adenocarcinoma	4			
Adenosquamous carcinoma	3		2	
Endometrioid endometrial cancer	19		10	
Serous carcinoma	6		9	
Mixed	1		5	
Clear			1	
Grade				
1	6		2	
2	12		3	
3	12		18	
Unknown	3		4	
Number of disease sites				
1	8		8	
2	11		6	
≥ 3	14		13	
Common disease sites (> five patients)				
Nodes	23		21	
Lung	18		12	
Pelvis	6		5	
Ascites	7		3	
Peritoneum	3		4	
Abdomen	6		3	
Liver	5		6	
Dose delivered				
No. of cycles				
Median	4		3	
Range	1-23		1-6	
0-2	11		11	
3-4	7		13	
5-6	5		3	
7-8	5		—	
≥ 12	5		—	
Dose intensity, mg				
Median	23.4		21.9	
Range	6.3-25		9.1-25	
Percentage of patients at > 90% dose intensity		69.7		48.1

NOTE. There was a higher proportion of patients with grade 3 (67% *v* 36%) and serous/clear cell disease (37% *v* 18%) in the chemotherapy-pretreated group (group B, IND160B) than in the chemotherapy-naive group (group A, IND160A). In group A, 70% of patients received 90% or more of the planned dose intensity of temsirolimus. The total number of cycles administered was 183 and ranged from 1 to 23. The median duration of treatment was 16 weeks, and five patients (15%) received temsirolimus for more than 48 weeks. In group B, 48% of patients received 90% or more of the planned dose intensity of temsirolimus. The total number of cycles administered was 81 and ranged from 1 to 6, with median duration of treatment being 12 weeks and two patients receiving 24 or more weeks of therapy.

scores were based on consensus. The tumor cells were assessed for the predominant staining intensity from 0 (absent) to 3 (strong) and for the percentage of stained cells with the predominant staining intensity. For PTEN, positive staining of the stromal cells served as an internal control. PTEN-negative tumors showed complete absence of cytoplasmic staining of the malignant cells while maintaining the stromal cell staining. For the other markers, the median of H-scores (predominant staining intensity × percentage of tumor cells stained) was used to dichotomize the tissue samples when correlating with response. Details of the methods are provided in the Appendix (online only).

PTEN Mutational Analysis

Tumor tissue was micro- or macrodissected from paraffin-embedded tissue slides guided by a hematoxylin and eosin–stained serial section, and DNA was extracted and amplified with polymerase chain reaction primers to assess *PTEN* gene mutations. The primer sequences and methodology are provided in the Appendix.[35,36]

Design, End Points, and Statistical Considerations

The study was conducted as two sequential phase II studies; group A and group B patients were enrolled and evaluated for outcome separately. The major end points of this study were objective clinical response and progression by RECIST criteria.[34] As noted earlier, only responses confirmed by independent review were included in the final report. Maximum change in tumor size, based on investigator measures, was also plotted in the form of a waterfall plot. Secondary end points included the determination of molecular markers of mTOR activity in primary tumor samples and objective response. The protocol planned to accrue up to 30 patients who could be evaluated for response in group A and 25 in group B. Group B was added to the protocol through amendment after responses were seen in group A to determine the level of response in previously treated patients. Additional details of patient eligibility and statistics are provided in the Appendix.

RESULTS

This phase II study enrolled 62 patients with recurrent and/or metastatic endometrial cancer from 10 participating centers across Canada between May 2004 and June 2007. One patient withdrew from the trial before beginning treatment (Part A) and one patient was ineligible (Part B), leaving 60 patients treated, 33 in group A (chemotherapy-naive) and 27 in group B (chemotherapy-treated). At the time of this

Table 2. Drug-Related Toxicities Seen in ≥ 10% Patients

Variable	Group A (n = 33)						Group B (n = 27)					
	CTCAE Grade				Patients		CTCAE Grade				Patients	
	1	2	3	4	Total No.	%	1	2	3	4	Total No.	%
Adverse event												
Fatigue	6	10	4		20	61	5	9	3		17	63
Fever	2	2			4	12						
Acne	4	1			5	15						
Dry skin	6	1			7	21	2	1			3	11
Nail changes	4	1	1		6	18						
Pruritis	3	9			12	36	1	2			3	11
Rash/desquamation	7	7	1		15	45	4	6			10	37
Anorexia	3	2	1		6	18	3	7	1		11	41
Diarrhea	3	1	2		6	18	6	2	3		11	41
Mouth dryness	5	1			6	18	2	1			3	11
Mucositis (functional/symptomatic), oral cavity	10	9			19	58	2	1			3	11
Nausea	6	2	1		9	27	9	8	1		18	67
Taste alteration	10	3			13	39	5	3			8	30
Vomiting	3	3	1		7	21	4	3			7	26
Epistaxis	5				5	15						
Edema, limb	2	2	1		5	15	1	2			3	11
Neuropathy, sensory	4				4	12						
Pain, muscle	4				4	12						
Pain, abdomen NOS							1	2			3	11
Pain, head/headache							1	1	1		3	11
Cough	8	1			9	27	4				4	15
Dyspnea	1	4			5	15	1	3	2		6	22
Pneumonitis	9	4	2		15	45	4	3	3		10	37
Hematology												
White cells	11	9			20	63	7	6			13	50
Neutropenia	13	3	1		17	53	4	5			9	35
Thrombocytopenia	13				13	41	11				11	42
Anemia	17	7			25	78	10	10			20	77
Biochemistry												
Creatinine	9	4		1	15	47	4	2			6	22
Hypokalemia	3		1		4	12	5		2	1	8	30
AST	15	3			18	58	13	3	1		17	63
Bilirubin							1					4

NOTE. For Group A (IND160A), n = 32 for all hematologic, creatinine, and hypokalemia toxicity; for Group B (IND160B), n = 26 for hematologic toxicity. Grade 3 hyperglycemia, hypertriglyceridemia, hypoposphatemia, hyponatremia, and hypokalemia were seen in fewer than 5% of patients.
Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events, version 3.0; NOS, not otherwise specified.

report, all patients were off study, 32 with documented progression, five with symptomatic progression, 16 because of toxicity, four having completed treatment, one because of treatment refusal, and two for other reasons. Sixty patients were eligible and evaluable for toxicity and 54 were evaluable for response. Reasons for patients not being evaluable were that they discontinued treatment early for toxicity without repeat imaging,[4] lacked a baseline scan,[1] or left the trial after a single dose of therapy without repeat imaging.[1]

Patient Characteristics

Table 1 summarizes the pretreatment patient and disease characteristics and treatment delivery in the 60 eligible and treated patients.

Toxicity

The most common adverse events were fatigue, rash, mucositis, and pneumonitis. Asymptomatic pneumonitis was common (42%) but was grade 3 in only five patients (8%). Table 2 displays the nonhematologic adverse events considered to be at least possibly drug related in 10% or more of the patients. Hematologic adverse events were generally mild in severity in both groups (Table 2).The most common hematologic toxicity was lymphopenia, with grade 3 lymphopenia being seen in 16 of 58 patients. The spectrum of toxicities seemed similar between the two cohorts, with perhaps the exception of rash, anorexia, nausea, and diarrhea being reported more frequently in the previously treated cohort and mucositis being more common in chemotherapy-naive patients. Median dose delivered was 23 mg in the chemotherapy-naive and 22 mg in the chemotherapy-treated cohorts, with 70% and 48%, respectively, receiving more than 90% of the planned dose intensity. Dose reductions seemed to be more frequent and occur earlier in patients who had previously been treated with chemotherapy.

Objective Tumor Response

Twenty-nine patients from the chemotherapy-naive group and 25 from the chemotherapy-treated group were evaluable for response. Objective RECIST responses assessed by investigators were centrally rereviewed and the reported PRs are the ones that have been confirmed independently.

In chemotherapy-naive patients (group A), no CRs were reported. Investigators reported PRs in seven patients (24%) but only four were confirmed on independent radiology review (14%; median duration, 5.1 months; range, 3.7 to 18.4 months). Twenty group A patients (69%) had a best response of SD with a median duration of 9.7 months (range, 2.1 to 14.6 months), and five patients had PD. Median progression-free survival was 7.33 months (95% CI, 3.61 to 9.86 months).

In chemotherapy-treated patients (group B), investigators reported PRs in two patients, and one was confirmed by independent radiology review (4%; duration 4.9 months). Twelve patients (48%) had a best response of SD with a median duration of 3.8 months (range, 2.4 to 23.2 months), and 12 (48%) had PD on study. Median progression-free survival was 3.25 months (95% CI, 1.97 to 3.84 months). Temsirolimus did not meet the predefined primary efficacy parameters in this cohort of women previously treated with chemotherapy.

Responses and SD were seen in patients with all grades of disease, as well as in patients with serous histology (Table 3). The maximal decreases in tumor measurements based on investigator measurements during the course of the study for patients in both groups are illustrated in waterfall plots in Figure 2. These plots include only patients with repeated assessments of all disease (three patients with new lesions who went off study with PD but with no reassessment of baseline lesions could not be included). Antitumor activity estimated by waterfall plots may appear to be superior to that using RECIST criteria since only the best change in sum of diameters, without need for confirmation, is plotted.

Correlative Analyses

Archival tissue from the original histologic diagnosis was available and was analyzed for 58 of the 62 enrolled patients. All specimens were stained for assessment of PTEN, pmTOR, cytoplasmic and nuclear phosphorylated AKT (pAKT), and phosphorylated S6 (pS6). PTEN loss by immunohistochemistry was seen in 20 of 33 chemotherapy-naive patients and in 11 of 27 chemotherapy-treated patients. Immunohistochemistry was indeterminate/not done in four patients from group A and five from group B. PTEN loss by immunohistochemistry did not correlate with response or SD in either chemotherapy-naive or chemotherapy-treated patients. There was no correlation of response or progression with pAKT, pmTOR, or pS6. We were unable to demonstrate any patient subset that benefitted more than any other with the measurements performed.

Table 3. Response by Grade and Histology in Chemotherapy-Naive (Group A) and Chemotherapy-Treated (Group B) Patients

	Group A (n = 29)				Group B (n = 25)			
	PR		SD		PR		SD	
Response	No.	%	No.	%	No.	%	No.	%
RECIST response (investigator assessed)	7	24	20	69	2	4	12	46
Grade								
1	2/6		4/6		0/2		2/2	
2	1/12		7/12		0/3		0/3	
3	1/12		8/12		1/18		7/18	
Histology								
Endometrioid	2/19		12/19		1/10		5/10	
Serous/clear cell	2/6		3/6		0/10		6/10	

NOTE. The No. column indicates the number of patients with outcome in each group; for example, for Grade 1, 2 of 6 patients in Group A demonstrated PR.
Abbreviations: PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

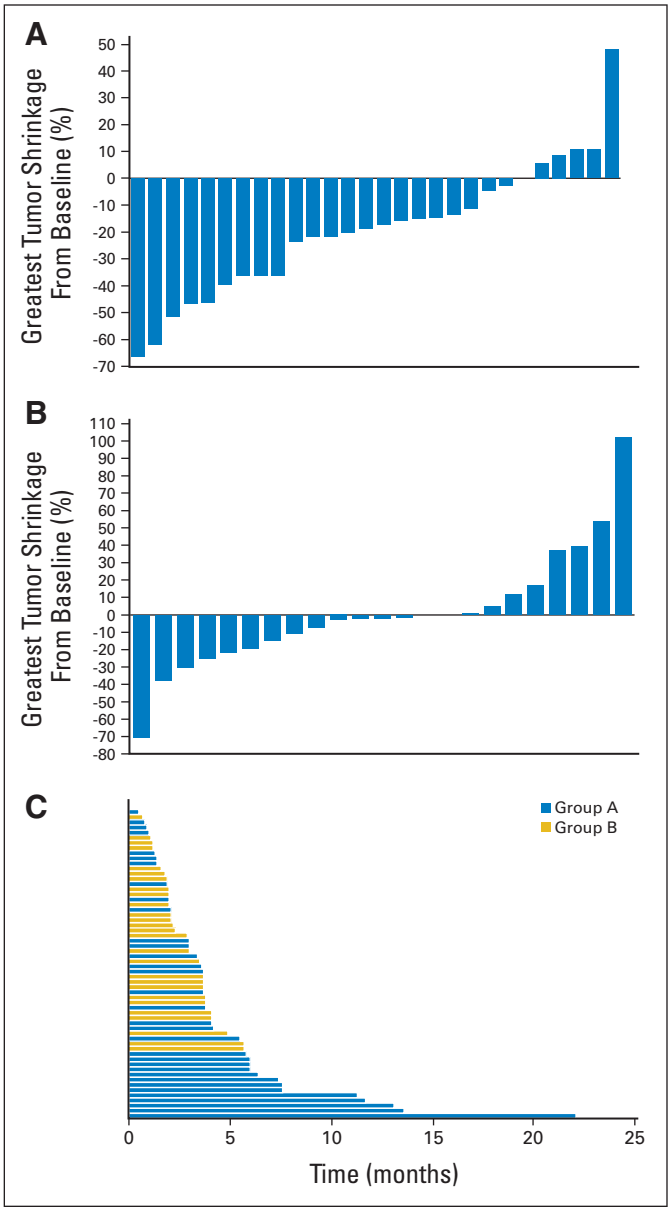

Fig 2. Waterfall plots demonstrating best radiologic response for (A) Group A (IND160A) and (B) Group B (IND160B) of the NCIC Clinical Trials Group and (C) duration of therapy for Group A and Group B.

PTEN Mutational Analyses

Mutational analysis was performed on all chemotherapy-naive patients to explore whether the results were similar to immunohistochemistry and also correlated with response outcome data. *PTEN* mutations were detected in 17 of 31 patients. The presence or absence of *PTEN* mutations did not correlate with response (Appendix Table A2, online only).

DISCUSSION

To the best of our knowledge, this is the first report demonstrating promising clinical activity in endometrial cancer by targeting mTOR, with activity seen in all subgroups and grades, apparently influenced by prior chemotherapy but not limited to patients with PTEN loss. This may be because of the frequency of alterations in other pathway components such as mutations and/or amplification in PI3K, mutations in AKT, or loss of tumor suppressors on LKB1. These observations provide guidance for future development of mTOR inhibitors in this disease and clinical trial design.

Temsirolimus administered intravenously at a dose of 25 mg on a weekly basis was well tolerated and demonstrated encouraging clinical activity in chemotherapy-naive women with recurrent or metastatic endometrial cancer. Toxicities were generally grade 1 or 2. Women who had previously been treated with chemotherapy required more frequent and earlier dose adjustments, and it is possible this was because of prior treatment. Eighty-three percent of patients who were chemotherapy-naive had SD or objective response compared with 52% of patients who had received prior chemotherapy. Objective disease regression was confirmed with PRs in 14% of women who had metastatic/recurrent disease but were chemotherapy-naive. In addition, 69% of women in the same chemotherapy-naive group had SD with a median duration of more than 9 months. Activity was not confined to low-grade or nonserous tumors but did seem to be influenced by prior chemotherapy, with lower response rates, SD, and shorter duration of therapy observed in this group. The frequency of SD was high, and duration seemed longer in the chemotherapy-naive cohort. Only five chemotherapy-naive patients (17%) exhibited primary disease progression on temsirolimus; this is a lower proportion than seen with any single-agent hormonal or chemotherapeutic agent and underscores the need for further randomized prospective evaluation of mTOR inhibitors in this disease to fully assess the relevance of disease stability. The difference in activity between chemotherapy-naive and previously treated cohorts strongly suggests that prior therapy should be considered in future clinical trial design when evaluating the use of temsirolimus in endometrial cancer. Restricting the patient population to chemotherapy-naive patients will likely improve the chances of demonstrating therapeutic benefit.

At the time these studies were planned, the high frequency of *PTEN* mutations in endometrial cancer was known, and our hypothesis that mTOR inhibition would be a potentially active therapeutic intervention was based on this observation. Our results suggest that temsirolimus is an active agent but not because of PTEN loss per se, as determined by archival specimen analysis. At the time the study was being designed, preclinical activity data and clinical data on PTEN prevalence suggested evaluating mTOR inhibitors in patients with PTEN null tumors. Recognizing the limitations of preclinical data to predict clinical activity of cancer drugs and the limited molecular characterization of endometrial carcinoma available at that time, we deliberately chose to evaluate the agent's activity in patients with and without PTEN loss and in patients with high-grade or serous carcinomas. The decision to allow patients with and without PTEN loss and all histologic subtypes proved to be appropriate and has important implications for the design of other studies that are testing a targeted therapy on the basis of a putative biologic hypothesis. Unless the evidence for limiting eligible patient populations is compelling, trial design must allow the hypothesis to be adequately tested clinically.

A limitation of our correlative study is that the correlative studies were based on archival tissue and not fresh biopsies. The concordance of *PTEN* mutations between primary and recurrent/metastatic disease has not been adequately studied. It is also possible that *PTEN* mutations may underestimate the overall addiction to the PI3K/Akt/mTOR

pathway in endometrial cancer, and mTOR inhibition may be effective when the PI3K/AKT pathway is activated. We did assess pAKT, pmTOR, and pS6 to probe the cascade and correlate any activity with response or treatment failure, but again no correlation was evident. The ability to accurately assess phosphorylated proteins in archived tissue to predict outcome in patients with recurrent/metastatic disease may be limited because of variations in processing, antibody specificity, or changes in cancer biology.

The level of activity seen with single-agent temsirolimus is encouraging for a single targeted agent in this disease and warrants additional studies to explore mTOR inhibition in endometrial cancer by using temsirolimus as a single agent or in conjunction with or following chemotherapy. Several other mTOR inhibitors are currently being evaluated in single-agent and combination phase II studies. PI3K and AKT inhibitors are also being investigated, given the importance of this pathway.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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Final approval of manuscript: All authors

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