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ORIGINAL ARTICLE

Phase I dose escalation study of temsirolimus in combination with metformin in patients with advanced/refractory cancers

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Abstract
Purpose Mammalian target of rapamycin (mTOR) inhibitors like temsirolimus may result in undesirable AKT upregulation. Metformin inhibits mTOR through different mechanisms and may enhance temsirolimus's antitumor activity. We conducted an open-label phase I dose escalation trial of this drug combination in patients with advanced/refractory cancers.
Methods Temsirolimus, 25 mg weekly, was combined with an escalating daily dose of metformin (level 1: 500; level 2: 1000; level 3: 1500; level 4: 2000 mg) by utilizing a standard 3 + 3 trial design. Treatment was administered in 28-day cycles following initial 2-week metformin titration during the first cycle.
Results Twenty-one patients (median age, 56 years) with sarcoma ($n = 8$), colorectal ($n = 3$), endometrial ($n = 4$), uterine carcinosarcoma ($n = 2$), ovarian ($n = 2$), and other ($n = 2$) cancers were enrolled. Patients had received median of four prior systemic treatments. Two dose-limiting toxicities were observed (grade 3 mucositis, grade 3 renal failure); both patients continued treatment after dose modification. Fifty-six percent patients had stable disease as best response; clinical benefit rate was 22 %. Patients continued treatment for median of 11 weeks.
Conclusions Combination temsirolimus/metformin was well tolerated with modestly promising effectiveness among this heavily pretreated patient cohort. We recommend a dose of temsirolimus 25 mg weekly and metformin 2000 mg daily for phase II study.

Keywords Metformin · Temsirolimus · mTOR · Advanced/refractory cancer

Introduction

Temsirolimus is an inhibitor of mammalian target of rapamycin (mTOR), which is normally regulated by upstream receptor tyrosine kinases, such as insulin-like growth factor-1 receptor (IGF-1R). mTOR controls initiation of protein translation and acts as a central regulator of pathways involved in cell growth, proliferation, and apoptosis [1]. Temsirolimus has established anticancer activity; it is approved for the treatment of advanced renal cell carcinoma in the USA and for treatment of relapsed or refractory mantle cell lymphoma in several countries.

mTOR inhibition can result in undesirable AKT activation through a positive feedback loop that results in upregulation of receptor tyrosine kinases such as IGF-1R [2]. Thus, inhibiting mTOR through more than one mechanism has generated interest. Metformin is an inexpensive, safe, and widely used oral hypoglycemic that also leads to inhibition of mTOR via upstream activation

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of 5′-AMP-activated protein kinase (AMPK) [3]. AMPK activation results in phosphorylation and activation of the tumor suppressor gene *TSC2*, which exerts an inhibitory effect on mTOR, thereby inhibiting cellular protein synthesis and growth [4, 5]. Metformin-induced activation of AMPK also disrupts crosstalk between insulin/IGF-1R and G protein-coupled receptor (GPCR) signaling in pancreatic cancer cells and inhibits the growth of these cells in xenograft models [6, 7].

Population studies have suggested decreased incidence of cancer among diabetic patients taking metformin and decreased cancer mortality among diabetic patients using metformin [8, 9]. Preclinical studies have also generated interest in an antineoplastic role of metformin. Cifarelli et al. [10] demonstrated that metformin and sirolimus, an mTOR inhibitor, inhibit pancreatic cancer growth in a mouse model.

In another study involving a number of breast cancer cell lines, metformin inhibited cellular proliferation, reduced colony formation, and caused partial cell cycle arrest at the G1 checkpoint among estrogen receptor-positive and receptor-negative as well as ERBB2-normal and ERBB2-overexpressing cell lines [11]. Metformin also inhibited MAP kinase, AKT, and mTOR in these cell lines. In a retrospective study of patients with early-stage breast cancer, the rate of pathologic complete response was higher in the diabetic patients taking metformin than in the diabetic patients not taking metformin or in the non-diabetic patients [12].

Given the complementary mechanisms of temsirolimus and metformin in inhibiting mTOR, this combination could potentially be effective for treating cancer. The current study was conducted to evaluate the safety and tolerability of this drug combination and to determine the maximum tolerated dose (MTD) of this combination among patients with advanced cancers refractory to standard therapy. Our secondary objective was to assess clinical tumor response to this combination.

Methods

This was an open-label phase I dose escalation trial conducted at the University of Texas MD Anderson Cancer Center between April 2012 and January 2014. Informed written consent was obtained from all patients, and the study was reviewed and approved by the institutional review board of MD Anderson Cancer Center. Patients were eligible if they were aged 14 years or older, had evaluable advanced or metastatic cancer refractory to standard therapies, and had significant organ function reserve, defined as absolute neutrophil count (ANC) ≥1000/mL, platelets ≥75,000/mL, creatinine ≤1.5 mg/dL in males or ≤1.4 mg/dL in females, total bilirubin ≤1.5 times the upper limit of normal (ULN), and AST (SGOT) and/or ALT (SGPT) ≤2 times the ULN. The Eastern Cooperative Oncology Group (ECOG) performance status had to be 0 or 1. Patients who had received metformin had to be at least five half-lives beyond such treatment and could not be taking metformin at the time of enrollment; they could be receiving other treatments for diabetes.

Using the standard 3 + 3 trial design, patients received a fixed dose of temsirolimus, 25 mg, intravenously (IV) every week in combination with an escalating dose of metformin administered orally over four levels (Table 1). The first cycle included a 2-week period for metformin titration to limit side effects; thereafter, treatment was administered in 4-week cycles (Fig. 1). Three patients were enrolled at a dose level; if none of them experienced dose-limiting toxicity (DLT), the next cohort of three patients was treated at the next higher dose level. If one of the three patients treated at a dose level experienced DLT, three more patients were enrolled at the same dose level. If no other patient experienced DLT, the next cohort of three patients was enrolled at the next higher dose level. If two or more patients treated at a dose level experienced DLT, the MTD

Table 1 Treatment plan

Dose level	Metformin			Temsirolimus
	Week 1	Week 2	Week 3 and onwards	
1	500 mg QD	500 mg QD	500 mg QD	25 mg QW
2	500 mg QD	500 mg BID	500 mg BID	25 mg QW
3	500 mg QD ×5 days, then 500 mg BID ×5 days, then 500 mg TID ×4 days		500 mg TID	25 mg QW
4	500 mg QD ×4 days, then 500 mg BID ×4 days, then 500 mg TID ×3 days, then 1000 mg BID ×3 days		1000 mg BID	25 mg QW

QD daily, *BID* twice daily, *TID* three times daily, *QW* weekly

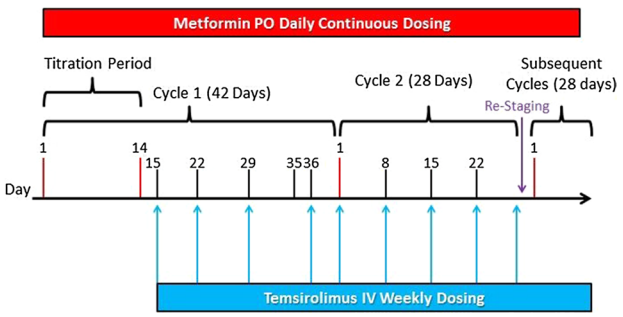

Fig. 1 Dosing schema

was considered to have been exceeded. In that case, three more patients (for a total of six) were enrolled at the next lower dose level unless six patients had already been treated at that dose. In summary, the MTD was defined as the highest dose studied in which the incidence of DLT was less than 33 %. For patients who received treatment for at least four cycles, intra-patient dose escalation was allowed at the investigator's discretion if higher doses had been found to be safe.

No patients were enrolled in the next dose level until three patients enrolled at the previous dose level had completed at least one cycle of therapy. If a DLT was observed in a patient, dose escalation did not proceed until a total of six patients in the cohort had been assessed for toxicity after one cycle. A patient must have completed at least 75 % of planned doses of both drugs to be evaluable for DLT.

DLTs were defined as adverse events related to the study agents during the first cycle of treatment and included grade 4 neutropenia or thrombocytopenia lasting more than 7 days; documented grade ≥3 infection with ANC $<1.0 \times 10^9$/L; febrile neutropenia (defined as ANC $<1.0 \times 10^9$/L and fever ≥38.5 °C); any non-hematologic grade 3 toxicity except for nausea, vomiting or diarrhea unless persistent in spite of adequate symptomatic management; or any grade 4 or 5 non-hematologic toxicity. Alopecia, asymptomatic hypothyroidism on replacement therapy, and clinically non-significant toxicities were not considered DLTs. All toxicities were graded according to Common Terminology Criteria for Adverse Events, version 4.0 (CTCAE version 4.0).

Once the MTD was determined or dose level 4 was found to be safe, another 14 patients were enrolled for additional safety analysis and response evaluation. Patients were re-staged after every two cycles and allowed to continue treatment in absence of disease progression or significant toxicity. Radiographic response or progression was evaluated on the basis of Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST version 1.1). Metformin was held for 48 h before and after any evaluation involving IV iodine contrast. Descriptive statistics were performed to report patient characteristics, adverse events, and responses observed. Clinical benefit was defined as no evidence of progression for six or more cycles.

Results

Twenty-one patients were enrolled in this dose escalation study. Table 2 shows the patients' characteristics. The median age of the patients was 56 (range 18–81) years. The most common diagnoses were sarcoma ($n = 8$) and endometrial cancer ($n = 4$). The patients had received a median of 4 (range 2–11) lines of prior systemic treatments. Fourteen patients had undergone prior surgery, and 11 patients had received prior radiation therapy. Six patients had previously received treatment on a phase I clinical trial.

Table 2 Patient characteristics

	N
Sex	
Male	7
Female	14
ECOG performance status	
0	6
1	14
2	1
Diagnosis	
Sarcoma	8
Endometrial cancer	4
Colorectal cancer	3
Ovarian cancer	2
Uterine carcinosarcoma	2
Non-small cell lung cancer	1
Pancreatic cancer	1
Prior therapies	
2 or fewer	4
3–6	14
7 or more	3

The MTD was not reached during the study, and the dose was escalated to level 4. Table 3 shows the adverse events attributable to the study drugs observed during follow-up. Fatigue was the most common adverse event, followed by mucositis and rash. Only three grade 2 and two grade 3 adverse events were noted. Two DLTs were observed which included grade 3 mucositis in a patient at dose level 1 and grade 3 renal failure in a patient at dose level 4. Both of these patients were able to continue treatment after dose modification. No grade 4 or 5 toxicity was observed.

After excluding three patients who stopped participation during the first cycle because of decline in performance status ($n = 1$), withdrawal of consent ($n = 1$), or development of myelodysplasia ($n = 1$), 18 patients were evaluated for treatment response. Eight patients had progression and 10 (56 %) had stable disease as their best response. The clinical benefit rate was 22 %. Among four patients who had stable disease for six or more cycles, one had lung adenocarcinoma with *STK11* mutation, one had dedifferentiated liposarcoma, one had chondrosarcoma, and one had alveolar soft part sarcoma.

Patients continued treatment for a median of 11 (range 1–99; interquartile range 8–25) weeks. Eleven patients received one or two cycles, six patients continued treatment for four cycles and four patients received eight or

Table 3 Drug-related adverse events observed

	Any grade	Grade 1	Grade 2	Grade 3
Mucositis	4	2	1	1
Rash	3	3		
Fatigue	5	4	1	
Dyspnea	1	1		
Anorexia	1	1		
Nausea	1	1		
Vomiting	1	1		
Dyspepsia	1	1		
Diarrhea	1	1		
Pneumonitis	2	1	1	
Renal insufficiency	1			1
Anemia	1	1		

more cycles. Two patients received 20 or more cycles of treatment.

Discussion

This open-label phase I trial studied the safety and tolerability of the temsirolimus plus metformin combination among patients with advanced cancer refractory to several lines of therapy. The combination was well tolerated, and the dose was successfully escalated to the highest level (level 4) without exceeding the MTD. Toxicities observed were attributable to temsirolimus and consistent with previously published literature [13].

MacKenzie et al. [14] have previously studied this combination in the phase I setting, with different findings. Although they used a dose escalation plan similar to ours, all three patients experienced DLT at dose level 1 (temsirolimus 25 mg IV weekly plus metformin 500 mg orally twice a day). Among the next three patients enrolled at dose level 1, one experienced a DLT; this led to enrollment of five more patients at that dose level, of whom one experienced a DLT. In contrast to this report, current study found the combination of temsirolimus and metformin to be well tolerated. We utilized a titration period for metformin before first dose of temsirolimus to limit toxicity, as suggested by a consensus statement from the American Diabetes Association and the European Association of Study of Diabetes [15]. The 2-week titration period for metformin may have contributed to better tolerance of this combination in our study.

Although there are no prospective data on the combination of temsirolimus and metformin, some preclinical and retrospective analyses have supported the rationale for combining these drugs. Liu et al. [16] demonstrated synergistic inhibition of proliferation in breast cancer cell lines with the combination of metformin, everolimus (another mTOR inhibitor), and chemotherapy.

In a combined analysis of three phase II trials by National Cancer Institute of Canada Clinical Trials Group (trials IND160A, IND160B, and IND192) involving use of mTOR inhibitors for recurrent or metastatic endometrial cancer, self-reported use of metformin was associated with a higher objective response rate (17.7 vs. 6.5 %) and a lower rate of disease progression (11.8 vs. 32.5 %) [17]. Although these differences did not reach statistical significance given the small sample size, the numerical differences were impressive and warrant further investigation. Similarly, in another phase II clinical trial of the combination of everolimus and letrozole for recurrent endometrial carcinoma, patients taking metformin for preexisting or protocol-related hyperglycemia had a higher response rate (56 vs. 23 %, p value <0.05) [18]. This finding has led to an ongoing clinical trial of the combination of everolimus, letrozole, and metformin for patients with advanced or recurrent endometrial carcinoma (NCT01797523) [19].

Although we did not see radiographic responses to this combination in this trial, several patients had a clinical benefit, as 22 % had stable disease for six or more cycles. This rate is encouraging in the setting of advanced cancers that had progressed on several previous lines of chemotherapy. Among four of 18 response-evaluable patients with stable disease after six or more cycles, three had sarcoma and one had non-small cell lung cancer.

In conclusion, the combination of temsirolimus and metformin was safe and had modestly promising effectiveness in this cohort of heavily pretreated patients with advanced cancers. Toxicities observed were attributable to temsirolimus and consistent with previously published literature. For phase II studies, we recommend a dose of 25 mg temsirolimus IV weekly and 2000 mg metformin orally daily administered in 28-day cycles.

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Compliance with ethical standards

Conflict of interest This work was funded by the National Cancer Institute at the National Institute of Health (Award Number P30CA016672). The authors declare that they have no relevant conflict of interest.

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