

## Overview



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**Title:** A Phase I Study of Temozolomide and Bryostatins in Patients With Metastatic Renal Cell Carcinoma and Soft Tissue Sarcoma

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**IRB Approved:** Yes

### Disclosures

The authors indicated no financial relationships.

## Author Summary: Abstract and Brief Discussion

### Background

Temozolomide, an inhibitor of mammalian target of rapamycin (mTor) complex 1, is approved for the treatment of metastatic renal cell carcinoma (RCC). Bryostatins inhibit protein kinase C, a downstream effector of mTor complex 2. We observed antitumor effects with the combination of temozolomide and bryostatins in RCC cell lines.

### Methods

Four cohorts of patients received weekly bryostatin-1 ( $20 \mu\text{g}/\text{m}^2$ ) with temozolomide (10, 15, 25, or 37.5 mg) in 28-day cycles.

### Results

Thirty patients received a total of 138 cycles across four dose levels. Twenty-five patients had RCC (17 clear cell, 7 papillary, and 1 unclassified). Two sarcoma patients with prior cytotoxic therapy experienced dose-limiting toxicity at 15 mg of temozolomide (grade 3 neutropenia and grade 3 hypophosphatemia). Subsequently, patients with prior cytotoxic therapy were excluded. Two additional dose-limiting toxicities were noted with 37.5 mg of temozolomide (grade 3 neutropenia and grade 3 creatinine elevation). Consequently, the maximum tolerated dose was defined as temozolomide at 25 mg and bryostatin-1 at  $20 \mu\text{g}/\text{m}^2$  every 28 days. Of the 25 RCC patients, 3 patients had partial responses that lasted for 14 months, 28 months, and  $\geq 80$  months, respectively. Partial responses were seen in both clear cell and papillary histology.

### Conclusion

This combination of 37.5 mg of temozolomide with  $20 \mu\text{g}/\text{m}^2$  of bryostatin-1 was reasonably safe and well tolerated. Durable responses were observed in 3 of 25 patients with RCC.

## Discussion

Temsirolimus is a selective inhibitor of mTOR kinase that was approved in the U.S. for advanced renal cell carcinoma (RCC). One suggested mechanism of resistance to temsirolimus is its inability to block mTOR complex 2 and, in turn, phosphorylation and activation of downstream AKT. We observed that three patients who had received bryostatin-1 approximately 2 months prior to temsirolimus demonstrated durable partial responses. This finding prompted further preclinical studies in which bryostatin-1 and temsirolimus demonstrated at least additive antitumor effects in RCC cell lines. These observations suggested that the combination of temsirolimus with a protein kinase C (PKC) inhibitor such as bryostatin-1 may produce greater antitumor activity in RCC through more complete inhibition of mTOR signaling by inhibiting both p70 S6 kinase (mTOR complex 1) and mTOR complex 2.

We report the results of a phase I clinical trial of temsirolimus and bryostatin-1. Four successive cohorts of patients received weekly bryostatin-1 at a fixed dose of 20  $\mu\text{g}/\text{m}^2$ , which was previously established as a safe single-agent dose on this schedule, together with weekly temsirolimus at 10, 15, 25, or 37.5 mg in 28-day cycles. At the time of analysis, all 30 patients had discontinued treatment because of disease progression (22 patients), treatment toxicity (5 patients), concomitant illness (2 patients), or withdrawal of consent (1 patient). The most common adverse events of any grade noted were fatigue (83%) and anemia (77%). The most commonly reported grade 3 or 4 toxicities related to therapy were thrombosis (10%) and neutropenia (10%). Two dose-limiting toxicities were noted with temsirolimus at 37.5 mg: grade 3 neutropenia in a non-RCC patient with prior radiation and grade 3 creatinine elevation in an RCC patient. Consequently, the maximum tolerated dose was defined as temsirolimus at 25 mg and bryostatin-1 at 20  $\mu\text{g}/\text{m}^2$  every 28 days.

Of the 25 evaluable RCC patients who had received a median of one prior systemic therapy, 3 (12%) had partial responses (PRs) and 13 (52%) had stable disease, as defined by RECIST. Median progression-free survival was 4.3 months. Two patients had prolonged duration of PR until disease progression—one with papillary type II RCC for 14 months and one with clear cell RCC for 28 months. A third clear cell patient who had a PR discontinued treatment after 12 cycles because she required prednisone for interstitial lung disease, perhaps due to temsirolimus-induced pneumonitis. She remained in PR as of the last follow-up, for a total of 80 months.

Our study suggests that the combination of bryostatin-1 and temsirolimus was reasonably well tolerated for multiple cycles at phase II doses of each agent on a weekly schedule. Unfortunately, development of bryostatin-1 has been discontinued and the agent is no longer available for clinical study. The development of potent PKC inhibitors, potentially with isoform specificity, may facilitate further study of the interaction between mTOR and PKC inhibition.

## Trial Information

<b>Disease:</b>	Advanced cancer/Solid Tumor Only
<b>Disease:</b>	Sarcomas – Adult
<b>Disease:</b>	Renal cell carcinoma – clear cell
<b>Disease:</b>	Renal cell carcinoma – not clear cell
<b>Stage of disease / treatment:</b>	Metastatic / Advanced
<b>Prior Therapy:</b>	No designated number of regimens
<b>Type of study - 1:</b>	Phase I
<b>Type of study - 2:</b>	Other
<b>Primary Endpoint:</b>	Maximum Tolerated Dose
<b>Additional Details of Endpoints or Study Design:</b>	RECIST response and progression free survival
<b>Investigator’s Analysis:</b>	Drug Tolerable, Efficacy Indeterminate

## Drug Information

<b>Drug 1:</b>	
<b>Generic/Working name:</b>	Temsirolimus
<b>Trade name:</b>	Torisel
<b>Company name:</b>	Pfizer
<b>Drug type:</b>	Small molecule
<b>Drug class:</b>	m-TOR

<b>Dose:</b>	milligrams (mg) per flat
<b>Route:</b>	IV
<b>Schedule of Administration:</b>	Days 1, 8, and 15 of each 28-day treatment cycle.
<b>Drug 2:</b>	
<b>Generic/Working name:</b>	Bryostatin-1
<b>Trade name:</b>	None
<b>Company name:</b>	None
<b>Drug type:</b>	Small molecule
<b>Drug class:</b>	Other
<b>Dose:</b>	micrograms (mcg) per squared meter (m2)
<b>Route:</b>	IV
<b>Schedule of Administration:</b>	Bryostatin-1 was administered alone on day 1 (week 1) for cycle 1 only. Both bryostatin-1 and temsirolimus were administered days 8, 15, and 22 of cycle 1, followed by 1-week rest (no treatment day 29). For cycle 2 and all subsequent cycles both bryostatin-1 and temsirolimus were administered days 1, 8, and 15 of each 28-day treatment cycle.

## Patient Characteristics

Number of patients, male:	20
Number of patients, female:	11
Stage:	IV
Age:	Median (range): 55 (39-76)
Number of prior systemic therapies:	Median (range): 1 (0-5)
Performance Status:	ECOG 0 — 7 1 — 24 2 — 0 3 — 0 unknown — 0
Other:	Not Collected
Cancer Types or Histologic Subtypes:	renal cell carcinoma clear cell type 18 renal cell carcinoma papillary type 7 renal cell carcinoma unclassified type 1 synovial sarcoma 1 fibrosarcoma 1 desmoplastic sarcoma 1 leiomyosarcoma 1 paraganglioma 1

## Primary Assessment Method

### Experimental Arm: Total Patient Population

Number of patients screened:	33
Number of patients enrolled:	31
Number of patients evaluable for toxicity:	30
Number of patients evaluated for efficacy:	30
Evaluation method:	RECIST 1.0

Response assessment CR:	0%
Response assessment PR:	10%
Response assessment SD:	47%
Response assessment PD:	43%
Response assessment other:	0%
(Median) duration assessments PFS	4.3 months
(Median) duration assessments TTP	months
(Median) duration assessments OS	months
(Median) duration assessments response duration	months
(Median) duration assessments duration of treatment	months

## Secondary Assessment Method

### Experimental Arm: Total Patient Population

Evaluation Method Other

## Adverse Events

Name	NC/NA*	1	2	3	4	5	All Grades
Glucose, serum-high (hyperglycemia)	0%	50%	40%	10%	0%	0%	100%
ALT, SGPT (serum glutamic pyruvic transaminase)	0%	100%	0%	0%	0%	0%	100%
Diarrhea	0%	75%	25%	0%	0%	0%	100%
Anorexia	0%	76%	23%	0%	0%	0%	100%
Vomiting	0%	50%	50%	0%	0%	0%	100%
Phosphate, serum-low (hypophosphatemia)	0%	0%	77%	22%	0%	0%	100%
Dermatology/Skin - Other (Specify, __)	0%	0%	50%	50%	0%	0%	100%
Dry skin	0%	100%	0%	0%	0%	0%	100%
Ocular/Visual - Other (Specify, __)	0%	50%	50%	0%	0%	0%	100%
Weight loss	0%	50%	50%	0%	0%	0%	100%
Pain - Other (Specify, __)	0%	83%	16%	0%	0%	0%	100%
Fever (in the absence of neutropenia, where neutropenia is defined as ANC <1.0 × 10e9/L)	0%	100%	0%	0%	0%	0%	100%
Dyspnea (shortness of breath)	0%	0%	0%	100%	0%	0%	100%
Cough	0%	50%	25%	25%	0%	0%	100%
Pneumonitis/pulmonary infiltrates	0%	33%	0%	66%	0%	0%	100%
AST, SGOT(serum glutamic oxaloacetic transaminase)	0%	83%	16%	0%	0%	0%	100%
Potassium, serum-low (hypokalemia)	0%	0%	100%	0%	0%	0%	100%
Pruritus/itching	0%	60%	20%	20%	0%	0%	100%
Infection with normal ANC or Grade 1 or 2 neutrophils	0%	0%	100%	0%	0%	0%	100%
Nasal cavity/paranasal sinus reactions	0%	100%	0%	0%	0%	0%	100%
Hemorrhage/Bleeding - Other (Specify, __)	0%	83%	16%	0%	0%	0%	100%
Alkaline phosphatase	0%	85%	14%	0%	0%	0%	100%
Renal failure	0%	80%	0%	20%	0%	0%	100%
Lymphopenia	0%	33%	66%	0%	0%	0%	100%
Hypoxia	0%	0%	0%	100%	0%	0%	100%
Edema: limb	0%	100%	0%	0%	0%	0%	100%
Arthritis (nonseptic)	0%	66%	33%	0%	0%	0%	100%
Left ventricular systolic dysfunction	0%	0%	0%	100%	0%	0%	100%

Thrombosis/thrombus/embolism	0%	0%	0%	33%	66%	0%	100%
Pain - Other (Specify, __)	0%	100%	0%	0%	0%	0%	100%
Infection - Other (Specify, __)	0%	0%	50%	50%	0%	0%	100%
Rash: acne/acneiform	0%	77%	22%	0%	0%	0%	100%
Dehydration	0%	100%	0%	0%	0%	0%	100%
Infection - Other (Specify, __)	0%	0%	0%	100%	0%	0%	100%
Nail changes	0%	100%	0%	0%	0%	0%	100%
Phlebitis (including superficial thrombosis)	0%	0%	100%	0%	0%	0%	100%
Fatigue (asthenia, lethargy, malaise)	0%	28%	64%	8%	0%	0%	100%
Hemoglobin	0%	34%	56%	8%	0%	0%	100%
Nausea	0%	84%	15%	0%	0%	0%	100%
Neutrophils/granulocytes (ANC/AGC)	0%	60%	10%	30%	0%	0%	100%
Leukocytes (total WBC)	0%	77%	5%	16%	0%	0%	100%
Platelets	0%	57%	28%	14%	0%	0%	100%
Cholesterol, serum-high (hypercholesteremia)	0%	73%	21%	0%	5%	0%	100%
Triglyceride, serum-high (hypertriglyceridemia)	0%	50%	37%	6%	6%	0%	100%
Heartburn/dyspepsia	0%	50%	50%	0%	0%	0%	100%
Mucositis/stomatitis (clinical exam)	0%	68%	31%	0%	0%	0%	100%
Gastrointestinal - Other (Specify, __)	0%	80%	20%	0%	0%	0%	100%
Musculoskeletal/Soft Tissue - Other (Specify, __)	0%	36%	63%	0%	0%	0%	100%

\*No Change from Baseline/No Adverse Event

## Assessment, Analysis, and Discussion

### Completion:

Study completed

### Pharmacokinetics / Pharmacodynamics:

Not Collected

### Investigator's Assessment:

Drug Tolerable, Efficacy Indeterminate

## Discussion

Mammalian target of rapamycin is a critical component of the intracellular signal transduction cascades that mediate cell proliferation and tumor angiogenesis. It is found in two functionally distinct multiprotein complexes: mTOR complex 1 (mTORC1) and mTORC2. The p70 S6 kinase (p70S6K) and 4E-BP1 are the two main effectors of mTORC1. Temsirolimus is a selective inhibitor of mTOR kinase that exclusively binds to and inhibits mTORC1 but not mTORC2. This inhibition compromises mRNA translation of key regulatory proteins of the cell cycle leading to G1/S arrest. Temsirolimus was approved in the U.S. for advanced renal cell carcinoma based on a pivotal phase III trial showing that temsirolimus monotherapy was statistically superior to interferon alone [1]. One suggested mechanism of resistance to temsirolimus is its inability to block mTORC2 and, in turn, phosphorylation and activation of downstream AKT. Consequently, mTOR inhibitors targeting both mTORC1 and mTORC2 could provide greater antitumor activity, and clinical trials of dual mTORC1 and mTORC2 inhibitors are ongoing [2].

PKC is a downstream effector of mTORC2. When activated, PKC acts to phosphorylate potent activators of transcription and thereby leads to increased expression of oncogenes, promoting cancer progression. In addition, earlier studies suggested that certain isoforms of PKCs are regulators of p70S6K, a main effector of mTORC1 [3]. Bryostatin-1, a macrocyclic lactone isolated from the marine bryozoan *Bugula neritina*, is a potent modulator of PKC [4, 5]. Prolonged exposure to bryostatin-1 downregulates PKC activity, leading to inhibition of cell growth and angiogenesis. Bryostatin-1 has demonstrated modest activity as a single agent in phase II trials for advanced RCC, including one conducted at our institution [6, 7].

We observed that three patients who had received bryostatin-1 approximately 2 months prior to temsirolimus demonstrated durable partial responses. This finding prompted further preclinical studies in which bryostatin-1 and temsirolimus demonstrated at least additive antitumor effects in ACHN (papillary) and A498 (clear cell) RCC cell lines. These observations suggested that the combination of temsirolimus with a PKC inhibitor such as bryostatin-1 may produce greater antitumor activity in RCC through more complete inhibition of mTOR signaling by inhibiting both p70S6K (mTORC1) and mTORC2.

We report the results of a phase I clinical trial of temsirolimus and bryostatin-1. Four successive cohorts of patients received weekly bryostatin-1 at a fixed dose of 20  $\mu\text{g}/\text{m}^2$ , which was previously established as a safe single-agent dose on this schedule, together with weekly temsirolimus at 10, 15, 25, or 37.5 mg in 28-day cycles. Between April 11, 2005, and March 30, 2010, 31 patients were enrolled. Thirty patients received a total of 138 cycles across the four dose levels, with a median of 3 cycles (range: 1–29 cycles) per patient. Fifteen patients received  $\geq 4$  cycles, and four received  $> 10$  cycles. The study initially allowed non-RCC patients to enroll, and five were treated on the study. Two of these patients with prior cytotoxic therapy experienced dose-limiting toxicity with temsirolimus at 15 mg (grade 3 neutropenia and grade 3 hypophosphatemia), a dose that was otherwise well tolerated by RCC patients who were less likely to have had prior cytotoxic treatments. Subsequently, patients with prior cytotoxic therapy were excluded. At the time of analysis, all 30 patients had discontinued treatment because of disease progression (22 patients), treatment toxicity (5 patients), concomitant illness (2 patients), or withdrawal of consent (1 patient). The most common adverse events of any grade noted were fatigue (83%) and anemia (77%). The most commonly reported grade 3 or 4 toxicities related to therapy were thrombosis (10%) and neutropenia (10%). Two dose-limiting toxicities were noted with temsirolimus at 37.5 mg: grade 3 neutropenia in a non-RCC patient with prior radiation and grade 3 creatinine elevation in an RCC patient. Consequently, the maximum tolerated dose was defined as temsirolimus at 25 mg and bryostatin-1 at 20  $\mu\text{g}/\text{m}^2$  every 28 days.

Of the 25 evaluable RCC patients who had received a median of one prior systemic therapy, 3 (12%) had partial responses (PRs) and 13 (52%) had stable disease (SD), defined as in the Response Evaluation Criteria in Solid Tumors. Median progression-free survival was 4.3 months. Two patients had prolonged duration of PR until disease progression, one with papillary type II RCC for 14 months (temsirolimus dose of 37.5 mg) and one with clear cell RCC for 28 months (temsirolimus dose of 25 mg). A third clear cell patient who had a PR discontinued treatment after 12 cycles (temsirolimus dose of 15 mg) because she required prednisone for interstitial lung disease, perhaps due to temsirolimus-induced pneumonitis. She remained with PR as of the last follow-up, for a total of 80 months. Among the 25 evaluable RCC patients, there were 17 with clear cell histology, 7 with papillary histology (1 type I, 5 type II, and 1 unspecified), and 1 unclassified. Among the seven papillary patients, one achieved PR (14.3%) and four achieved SD (57.1%) as best response. Among the 17 RCC patients with clear cell histology, 2 patients achieved PR (11.8%) and 9 achieved SD (52.9%). Overall, the response proportions in this small phase I study in patients with RCC were not distinguishable from those seen in large phase III studies of mTOR inhibitors in RCC [1, 8].

Our study suggests that the combination of bryostatin-1 and temsirolimus was reasonably well tolerated for multiple cycles at phase II doses of each agent on a weekly schedule. Tissue correlates to define mechanism were not included in this early phase study. Unfortunately, development of bryostatin-1 has been discontinued, and the agent is no longer available for clinical study; however, there is much to be learned regarding the interaction of mTOR inhibition and PKC inhibition. Additional laboratory studies are needed to define which of the numerous PKC isoforms are most likely to affect mTOR signaling in human tumors and through which molecular mechanism this occurs in RCC and other tumors. In addition, the development of potent PKC inhibitors, potentially with isoform specificity, may facilitate further study of the interaction between mTOR and PKC inhibition.

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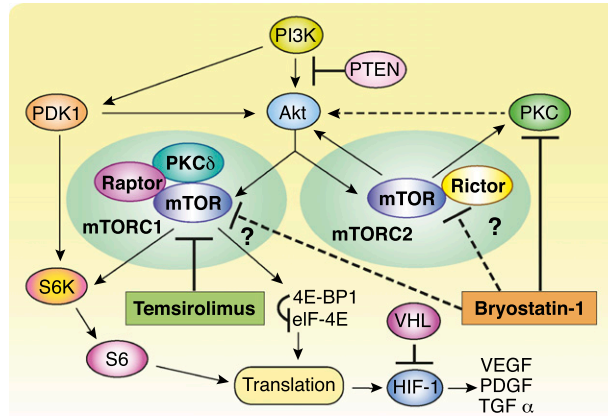
## ACKNOWLEDGMENT

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## Figures and Tables



**Figure 1.** Proposed mechanism of action of temsirolimus plus bryostatin-1.

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