VOLUME 23 · NUMBER 23 · AUGUST 10 2005

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Phase II Trial of Single-Agent Temsirolimus (CCI-779) for Relapsed Mantle Cell Lymphoma

Thomas E. Witzig, Susan M. Geyer, Irene Ghobrial, David J. Inwards, Rafael Fonseca, Paul Kurtin, Stephen M. Ansell, Ronnie Luyun, Patrick J. Flynn, Roscoe F. Morton, Shaker R. Dakhil, Howard Gross, and Scott H. Kaufmann

From the Mayo Clinic College of Medicine and Mayo Foundation, Rochester, MN; Scottsdale CCOP, Scottsdale, AZ; Carle Cancer Center CCOP, Urbana, IL; Metro-Minnesota Community Clinical Oncology Program, St Louis Park, MN; Iowa Oncology Research Association CCOP, Des Moines, IA; Wichita Community Clinical Oncology Program, Wichita, KS; and Hematology & Oncology of Dayton Inc, Dayton, OH.

Submitted January 24, 2005; accepted April 7, 2005.

Supported in part by Public Health Service grants CA-25224, CA-37404, CA-15083, CA-63826, CA-35195, CA-35267, CA-35101, CS-35431, CA-35090, CA-35113, CA-35415, CA-60276, CA-35448, and CA-63848, and grant CA97274 from the National Cancer Institute, Department of Heath and Human Services, Bethesda, MD.

This study was conducted as a collaborative trial of the North Central Cancer Treatment Group and Mayo Clinic. Additional participating institutions include Medcenter One Health Systems, Bismarck, ND (Edward Wos, MD); Illinois Oncology Research Association CCOP, Peoria, IL (John W. Kugler, MD); Toledo Community Hospital Oncology Program CCOP, Toledo, OH (Paul L. Schaefer, MD); Scottsdale CCOP, Scottsdale, AZ (Tom R. Fitch, MD); Geisinger Clinic & Medical Center CCOP, Danville, PA (Albert Bernath, MD); and Ann Arbor Regional CCOP, Ann Arbor, MI (Philip J. Stella, MD).

Terms in blue are defined in the glossary, found at the end of this issue and online at www.jco.org.

Authors' disclosures of potential conflicts of interest are found at the end of this article.

Address reprint requests to Thomas E. Witzig, MD, Mayo Clinic, Stabile 628, 200 First St SW, Rochester, MN 55905; e-mail: witzig@mayo.edu.

0732-183X/05/2323-5347/$20.00

DOI: 10.1200/JCO.2005.13.466

ABSTRACT

Purpose
Mantle cell lymphoma (MCL) is characterized by a t(11;14) resulting in overexpression of cyclin D1 messenger RNA. This study tested whether temsirolimus (previously known as CCI-779), an inhibitor of the mammalian target of rapamycin kinase that regulates cyclin D1 translation, could produce tumor responses in patients with MCL.

Patients and Methods
Patients with relapsed or refractory MCL were eligible to receive temsirolimus 250 mg intravenously every week as a single agent. Patients with a tumor response after six cycles were eligible to continue drug for a total of 12 cycles or two cycles after complete remission, and were then observed without maintenance.

Results
Thirty-five patients were enrolled and were assessable for toxicity; one patient had MCL by histology but was cyclin D1 negative and was ineligible for efficacy. The median age was 70 years (range, 38 to 89 years), 91% were stage 4, and 69% had two or more extranodal sites. Patients had received a median of three prior therapies (range, one to 11), and 54% were refractory to the last treatment. The overall response rate was 38% (13 of 34 patients; 90% CI, 24% to 54%) with one complete response (3%) and 12 partial responses (35%). The median time-to-progression in all patients was 6.5 months (95% CI, 2.9 to 8.3 months), and the duration of response for the 13 responders was 6.9 months (95% CI, 5.2 to 12.4 months). Hematologic toxicities were the most common, with 71% (25 of 35 patients) having grade 3 and 11% (four of 35 patients) having grade 4 toxicities observed. Thrombocytopenia was the most frequent cause of dose reductions but was of short duration, typically resolving within 1 week.

Conclusions
Single-agent temsirolimus has substantial antitumor activity in relapsed MCL. This study demonstrates that agents that selectively target cellular pathways dysregulated in MCL cells can produce therapeutic benefit. Further studies of this agent in MCL and other lymphoid malignancies are warranted.

J Clin Oncol 23:5347-5356. © 2005 by American Society of Clinical Oncology

INTRODUCTION

Mantle cell lymphoma (MCL) is an incurable, aggressive B-cell non-Hodgkin's lymphoma (NHL) that represents approximately 8% of cases of NHL. The disease usually presents in an advanced stage (III or IV), and involvement of extranodal sites such as the gut, bone marrow, and peripheral blood are common. There is a male predominance, and most patients are older adults. The characteristic tumor cell immunophenotype is CD20+, CD10−, CD5+, and CD23−, with monoclonal light chain expression on the cell surface. MCL is a unique subtype in that the tumor cells have a t(11;14)(q13;q32) chromosomal translocation that juxtaposes the *cyclin D1*

gene on chromosome 11 to the immunoglobulin heavy chain enhancer region on chromosome 14.[1-3] The transcription enhancers on 14q32, now linked to the cyclin D1 gene, result in the characteristic overexpression of cyclin D1 in the MCL tumor cells.

There is currently no standard therapy for newly diagnosed or relapsed MCL. Many regimens have been demonstrated to be highly active in producing responses,[4-17] but relapse typically occurs, and patients usually die of their disease, with a median survival of 3 to 4 years. It is clear that new treatments are needed for MCL.

Even though cyclin D1 mRNA is constitutively expressed in MCL, it is potentially subject to translational regulation by a pathway (Fig 1) involving the mammalian target of rapamycin (mTOR).[18,19] Activated receptor tyrosine kinases and activated ras proteins enhance the catalytic activity of the lipid kinase phosphatidylinositol-3 kinase (PI3K), which converts phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphoate (PIP3). PIP3 activates the protein kinase phosphoinositide-dependent kinase 1 (PDK1), which, along with a second kinase such as integrin-linked kinase (ILK), contributes two phosphorylations required for maximal Akt activity. Akt then phosphorylates a number of substrates, including tuberous sclerosis (TSC) protein 2 (TSC2), which in its unphosphorylated state is complexed with TSC protein 1 (TSC1) and acts as a GTPase activating protein that diminishes activation of the small guanine nucleotide binding protein Rheb. When the TSC1/TSC2 complex is inactivated by Akt, Rheb remains in a GTP-bound state that activates mTOR, a protein kinase that regulates mRNA translation by phosphorylating two critical substrates, eukaryotic initiation factor (eIF) 4E (eIF4E) binding protein (4E-BP1) and p70S6 kinase.[20,21] Previous studies have shown that eIF4E is a component of a helicase complex that binds to the cap structure at the 5′ end of mRNAs and enhances the ability of ribosome-eIF complexes to scan the mRNA in search of a translation initiation site.[22] The ability of eIF4E to bind to and participate in this helicase complex is inhibited when 4E-BP1 is bound. This inhibitory interaction is possible only when 4E-BP1 is unphosphorylated and is abrogated when 4E-BP1 is sequentially phosphorylated by mTOR and other kinases.[22,23] At the same time, mTOR-mediated phosphorylation activates p70S6K, enabling its phosphorylation of ribosomal protein S6 and possibly other substrates, thereby enhancing the translation of messages with 5′ terminal oligopyrimidine tracts.[18,22]

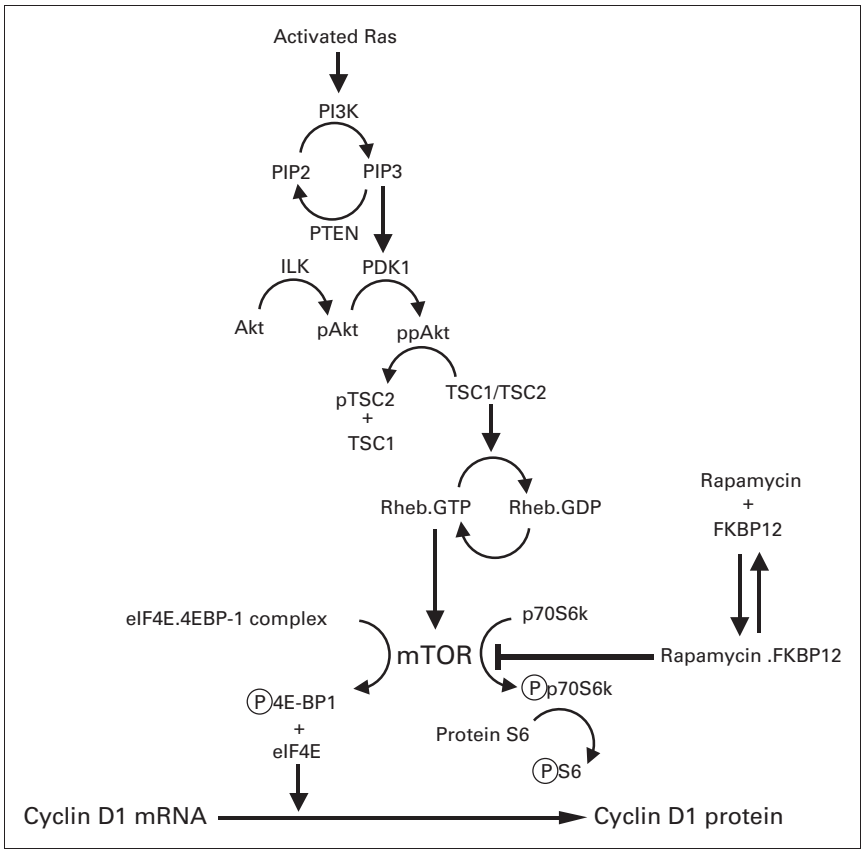

Fig 1. Current understanding of the mammalian target of rapamycin (mTOR) pathway and mechanism of action of rapamycin. In this diagram, arrows pointing downward or curved arrows pointing to the right indicate activation, whereas curved arrows pointing leftward or lines ending in crossbars indicate inhibition. The phosphorylation of S6 was studied before and after temsirolimus on clinical samples from patients in this study (see Fig 4). PI3K, phosphatidylinositol-3 kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphoate; PDK1, phosphoinositide-dependent kinase 1; ILK, integrin-linked kinase; TSC, tuberous sclerosis; 4E-BP1, eIF4E binding protein; eIF4E, eukaryotic initiation factor 4E.

Collectively, these events markedly enhance translation of a small but important group of messages, including those encoding c-myc, ornithine decarboxylase, and cyclin D1, as well as ribosomal proteins themselves.[18,22,24,25]

mTOR activity is modulated by mitogenic signals, which are transmitted through a signal transduction pathway involving PI3K, Akt, and TSC1 and TSC2 (Fig 1).[18,19,26,27] In addition, mTOR-mediated signaling is also subject to modulation by the macrocyclic lactone rapamycin and its derivatives.[19,26,27] Once these agents bind to the 12 kDa cytosolic FK506-binding protein FKBP12, the resulting rapamycin-FKBP12 complexes bind to a specific site near the catalytic domain of mTOR and inhibit phosphorylation of mTOR substrates by a mechanism that remains somewhat poorly understood.[27] As a consequence, translation of messages that require mTOR signaling is inhibited. This mechanism is thought to be responsible for the immunosuppressive effects of rapamycin as well as its putative antineoplastic activity.

Temsirolimus (also known as CCI-779), a dihydroester of rapamycin that is suitable for intravenous use, is currently undergoing testing in solid tumor patients as a potential antineoplastic agent.[28-31] In view of the role of cyclin D1 in MCL, we conducted a phase II trial of single-agent temsirolimus for patients with relapsed MCL to learn if therapy that specifically targeted this pathway could result in tumor responses.

PATIENTS AND METHODS

A single-stage phase II study with an interim analysis was conducted to assess the proportion of previously treated MCL patients who achieved a partial response (PR) or better after treatment with temsirolimus. This study was conducted through the North Central Cancer Treatment Group (NCCTG) cooperative group and was approved by the institutional review boards of each treatment site. Patients were eligible for this trial if they had previously received therapy and had relapsed or were refractory to their last treatment. There was no limit on the number of prior therapies. Central pathology review confirmed the diagnosis of MCL based on morphology and phenotype. In addition, all tumors were positive for cyclin D1 by immunohistochemistry or demonstrated t(11;14)(q13;q32)/immunoglubulin H fusion by fluorescence in situ hybridization. Patients were required to have measurable disease with a lymph node or tumor mass $\geq$ 2 cm or malignant lymphocytosis with an absolute lymphocyte count $\geq$ 5,000; a life expectancy of $\geq$ 3 months; Eastern Cooperative Oncology Group performance status of 0, 1, or 2; absolute neutrophil count (ANC) $\geq$ 1,000; platelets $\geq$ 75,000; hemoglobin $\geq$ 8 g/dL; serum creatinine $\leq$ 2$\times$ the upper limit of normal (ULN); serum bilirubin $\leq$ 1.5 ULN; serum cholesterol $\leq$ 350 mg/dL; and triglycerides $\leq$ 400 mg/dL. Patients could not have had CNS involvement or HIV infection.

Patients were treated with a flat dose of 250 mg of temsirolimus diluted in 250 mL of normal saline and delivered intravenously (IV) over 30 minutes. Patients were pretreated with diphenhydramine 25 to 50 mg IV. Treatment was weekly, and 4 weeks was considered to be one cycle. A CBC was performed each week, and the full dose of temsirolimus was delivered if the platelet count was $\geq$ 50,000 and the ANC $\geq$ 1,000, and if there were no grade 3 or 4 nonhematologic toxicities (National Cancer Institute Common Toxicity Criteria, version 2). Patients who did not meet the retreatment criteria had the dose held until recovery, followed by a stepwise dose modification to 175, 125, 75, or 50 mg. Patients were not to receive prophylactic WBC growth factors to maintain dosing but could receive them at physician discretion if neutropenia developed. Erythropoietin treatment for anemia was also permitted.

Patients were restaged after one cycle and every three cycles thereafter or at physician discretion. Responses were categorized using the International Workshop Criteria.[32] Patients who progressed anytime or those patients with stable disease after six cycles went off study. Patients who had a complete remission (CR) or PR at 6 months were to receive two cycles after CR or for a total of 12 months if there was a PR and they were then observed without further therapy.

Statistical Design

This trial was designed to test the null hypothesis that the true overall response rate (ORR) was at most 5%. The smallest ORR that would indicate that this regimen was worth further study in this relapsed MCL patient population was 20%. The design was generated based on the parameters and assumptions of a two-stage Simon min max design, but where accrual was not suspended for the interim analysis. This study design required a maximum of 32 assessable patients, where the interim analysis was performed after 18 patients had been accrued and followed up for at least 24 weeks for response. An additional three patients were accrued to this cohort (for a maximum of 35 patients overall) to account for the possibilities of ineligibility, withdrawal from study before drug administration, or major violations. However, only the first 32 assessable patients were used to evaluate the decision criteria for this design. At least one response in the first 18 assessable patients needed to be observed in the interim analysis to continue accrual. At the time of the final analyses, a total of four or more responses were required to indicate that this regimen warrants further evaluation in this patient population. The proportion of responses was calculated, and the 90% exact binomial CI for the true ORR was calculated (with all eligible patients accrued), assuming that the number of responses was binomially distributed.

Duration of response (DR) was defined as the time from the date of documented response to the date of progression. Patients who went off treatment due to other reasons (eg, adverse reactions, refusal of further treatment) were censored at that time. Time to progression (TTP) was defined as the time from registration to the date of progression. Patients who died without disease progression were censored at the date of their last evaluation. If a patient died without documentation of disease progression, the patient was considered to have had disease progression at the time of death unless there was sufficient documented evidence to conclude that progression did not occur before death. Time to discontinuation of active treatment was defined as the time from registration to the date the decision was made to take the patient off active treatment. Patients who were still receiving treatment at the time of these analyses were censored at the date of their last evaluation. Overall survival (OS) was defined as the time from registration to death resulting from any cause. The distributions of these time-to-event end points were each estimated using the Kaplan-Meier method.[33]

Tissue Culture and Exposure to Rapamycin in Vitro

The MO2058 human line, which was established from a patient with prolymphocytic leukemia and which contains the t(11;14)(q13;q32) translocation associated with *Cyclin D1* activation,[34] was propagated at 37°C in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 2 mmol/L L-glutamine, 100 units/mL penicillin G, and 100 μg/mL streptomycin. To establish conditions for detecting an effect of rapamycin on downstream signaling of mTOR, cells were treated with various rapamycin (Sigma, St Louis, MO) concentrations for 24 hours, washed three times with ice-cold serum-free RPMI 1640 10 mmol/L HEPES (pH 7.4), solubilized in buffered 6M guanidine hydrochloride under reducing conditions, and prepared for electrophoresis, as previously described.[35]

To determine whether mTOR signaling was inhibited in MCL tumor cells in situ, circulating mantle cells were purified from the peripheral blood of eight patients at four to five time points, which typically included: before therapy, 24 hours after administration of dose 1, 48 hours after dose 1, before dose 5, and before dose 12. At each time point, 1 to 2 $\times$ 10^6 $CD19^+$ cells were purified by magnetic bead selection, washed and solubilized under strongly denaturing conditions as describe above. Further characterization of an additional aliquot of these cells by flow cytometry confirmed that they were typically $>$ 90% $CD19^+$.

Immunoblotting

Aliquots containing protein from 5 $\times$ 10^5 immunopurified B cells were subjected to electropheresis on sodium dodecyl sulfate (SDS)-polyacrylamide gels containing 5% to 12% acrylamide, transferred to nitrocellulose, and probed under previously described conditions[36] with polyclonal antibodies that recognize the following antigens: phospho-$Ser^{235/236}$ ribosomal protein S6, S6, phospho-Thr^{389} p70S6K and p70S6K (all from Cell Signaling Technology, Beverly, MA). Antigen-antibody complexes were detected using peroxidase-coupled secondary antibodies (KPL, Gaithersburg, MD) and enhanced chemiluminescence reagents (Amersham Pharmacia Biotech, Piscataway, NJ) as described.[36] Blots were reprobed with antibody to heat shock protein 90 (David Toft, Mayo Clinic, Rochester, MN) as a loading control.

RESULTS

Patient Characteristics

A total of 35 patients were enrolled onto this trial by the NCCTG sites from April 2002 to October 2003. One patient was declared ineligible after pathology review indicated that although the histology was consistent with MCL, the cyclin D1 stain was negative. The patients tended to be older adults with a median age of 70 years (range, 38 to 89 years). Most patients (91%) had stage IV disease and were heavily pretreated with a median number of three prior therapies (mean, four therapies; range, one to 11 therapies). The majority of patients had failed to improve on rituximab, an alkylator agent such as cyclophosphamide, and an anthracycline such as doxorubicin. More than half of the patients had received a purine nucleoside analogue. Twenty-nine percent of patients (10 of 35) had an elevated lactate dehydrogenase at baseline. Additional baseline characteristics of these patients are presented in Table 1.

Clinical Outcomes

The ORR was 38% (13 of 34 patients; 90% CI, 24% to 54%) with one CR and 12 PR. The tumor responses occurred rapidly, with a median time to response of 1 month (range, 1 to 8 months) (Fig 2). Eight responses occurred after one cycle, three were documented after three cycles, and one each after the evaluations at 4 and 8 months, respectively. In addition, the patient who was ineligible obtained a PR with temsirolimus.

The patient who achieved a CR received a total of six cycles; three patients who achieved a PR completed 12 cycles; and one patient completed six cycles and went to observation with stable disease. The other nine patients who achieved PR received a mean of six cycles (median, 6.5; range, 3 to 10). One patient with a PR remains on treatment; the other eight patients with PR stopped drug before 12 cycles for various reasons: progression on temsirolimus (two patients), adverse events (three patients), and refusal of further treatment (three patients).

Table 1. Patient Characteristics

Characteristic	No. of Patients	%
Age, years		
Median	70	
Range	38-89	
Sex, male	26	74
Performance status		
0	19	54
1	12	34
2	4	12
Tumor stage		
1	1	3
2	1	3
3	1	3
4	32	91
Bone marrow involvement	27	77
"B" symptoms	5	14
No. of extranodal sites		
0	3	9
1	8	23
2	12	34
3	8	23
4	3	9
5	0	0
6	1	3
0-1	11	31
$\geq$ 2	24	69
Disease status		
Relapsed	16	46
Refractory	19	54
No. of prior therapy treatments		
Mean	4	
Median	3	
Range	1-11	
Type of prior therapy		
Rituximab	31	89
Alkylator	33	94
Anthracycline	29	83
Purine nucleoside analog	20	57
Platinum analog	10	29
Radiotherapy	8	23
Stem-cell transplantation	4	11

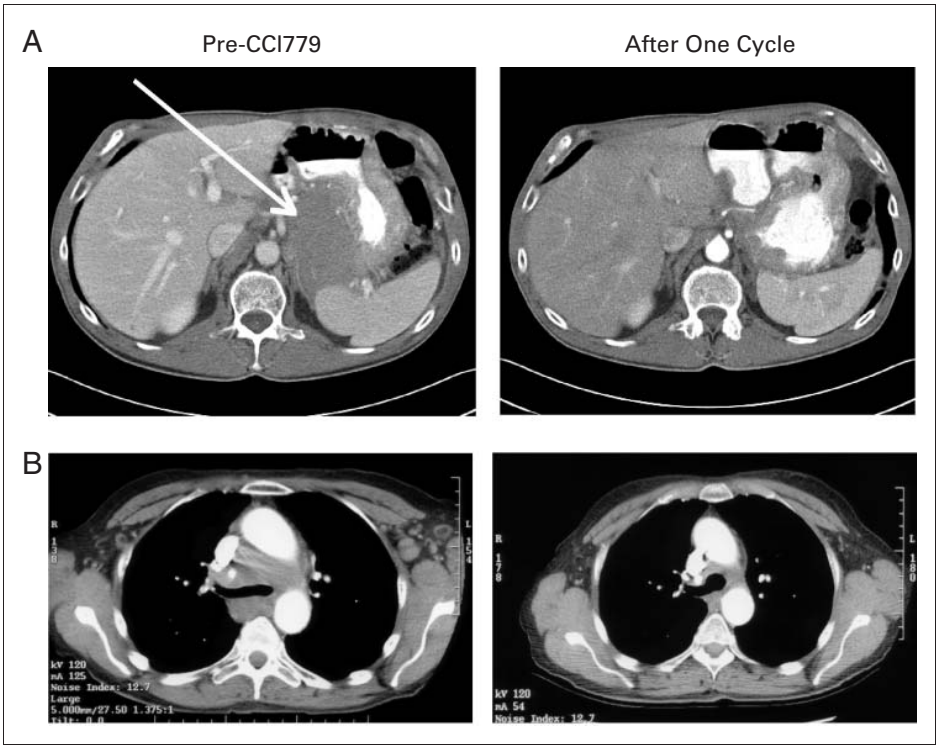

Fig 2. Computed tomography scans from two patients who had marked tumor response after one cycle (four doses) of temsirolimus (CCI779). (A) Patient with a large perigastric lymphomatous mass; (B) patient with bulky paratracheal and left axillary adenopathy.

Fourteen additional patients progressed on temsirolimus without ever achieving a response. Six patients went off study without tumor response or progression due to adverse reactions (three patients), refusal of further treatment (one patient), treatment with alternative therapy for MCL (one patient), and other medical problems (one patient). Those patients who refused further treatment or who went off for other medical problems discontinued this treatment regimen largely due to low-grade adverse events and a perceived decline in quality of life. The median time to discontinuation of treatment was 3.7 months (95% CI, 3 to 6.2 months).

Dose reductions were necessary in all but four patients. Overall, nine patients were able to receive 250 mg weekly for at least the first cycle of treatment, with a median of 2.5 cycles at this full dose (range, 1 to 8 cycles); the other patients required dose reductions in the first cycle. Of the six patients who received more than one cycle at the full dose level, two eventually required a dose reduction in subsequent cycles. Across all patients, the median dose received per month on study was 525 mg, with 564 mg in responding patients and 525 mg in nonresponders.

The median time to progression (Fig 3) was 6.5 months (95% CI, 2.9 to 8.3 months). The median overall survival was 12 months (95% CI, 6.7 months to not yet reached). The median duration of response for the 13 responders was 6.9 months (95% CI, 5.2 to 12.4 months). The median follow-up on living patients was 11 months (range, 6.7 to 24.6+). Overall, 30 patients have had disease progression, and 22 patients have died. No patients have had documented death without disease progression.

Safety and Tolerability

All 35 patients were included in the analysis of safety and tolerability. Patients tolerated the 30-minute infusion of temsirolimus without significant toxicity. All severe (grade 3 or greater) toxicities experienced by these patients

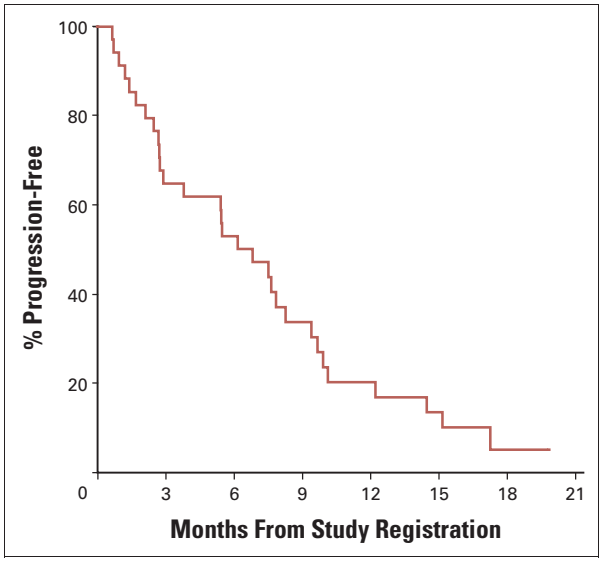

Fig 3. Time to progression after temsirolimus in all 34 patients.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.