Temsirolimus and rituximab in patients with relapsed or refractory mantle cell lymphoma: a phase 2 study

Stephen M Ansell, Hui Tang, Paul J Kurtin, Patricia A Koenig, David J Inwards, Keith Shah, Steven C Ziesmer, Andrew L Feldman, Radha Rao, Mamta Gupta, Charles Erlichman, Thomas E Witzig

Summary

Background **Temsirolimus is a mammalian target of rapamycin (mTOR) inhibitor with single-agent antitumour activity in patients with mantle cell lymphoma. We therefore tested its efficacy and toxicity in combination with rituximab (an antiCD20 antibody) in patients with relapsed or refractory mantle cell lymphoma.**

Methods **In a phase 2 study, patients (aged ≥18 years) at 35 centres in the USA were given temsirolimus 25 mg/week, and rituximab 375mg/m² per week for 4 weeks during the first cycle and thereafter a single dose of rituximab every other 28-day cycle. Both drugs were administered intravenously. Responding patients after six cycles could continue treatment for a total of 12 cycles, and were then observed without additional maintenance treatment. The primary endpoint was the proportion of patients with either rituximab-sensitive or rituximab-refractory disease who had at least a partial response. The analyses were done on all patients who were treated. The study was registered with ClinicalTrials.gov, number NCT00109967.**

Findings **71 patients with mantle cell lymphoma were enrolled and 69 were assessable and were included in the final analysis. The overall response rate (ORR) was 59% (41 of 69 patients)—13 (19%) patients had complete responses and 28 (41%) had partial responses. The ORR was 63% (30 of 48; 95% CI 47–76) for rituximab-sensitive patients, and 52% (11 of 21; 30–74) for rituximab-refractory patients. The most common treatment-related grade 3 or 4 adverse events in rituximab-sensitive and rituximab-refractory patients were thrombocytopenia (eight [17%] and eight [38%], respectively), neutropenia (ten [21%] and five [24%], respectively), fatigue (eight [17%] and two [10%], respectively), leucopenia (six [13%] and three [14%], respectively), pneumonia (five [10%] and two [10%], respectively), lymphopenia (five [10%] and two [10%], respectively), pneumonitis (four [8%] and none, respectively), oedema (four [8%] and none, respectively), dyspnoea (three [6%] and two [10%], respectively), and hypertriglyceridaemia (three [6%] and two [10%], respectively).**

Interpretation **mTOR inhibitors in combination with rituximab could have a role in the treatment of patients with relapsed and refractory mantle cell lymphoma.**

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Division of Hematology (Prof S M Ansell MD, D J Inwards MD, S C Ziesmer BS, M Gupta PhD, Prof T E Witzig MD), **Division of Biomedical Statistics and Informatics** (H Tang PhD), **Division of Hematopathology** (Prof P J Kurtin MD, A L Feldman MD), **and Department of Oncology** (Prof C Erlichman MD), **Mayo Clinic, Rochester, MN, USA; and North Central Cancer Treatment Group, Rochester, MN, USA** (P A Koenig RN, K Shah MD, R Rao MD)

Correspondence to:
Prof Stephen M Ansell, Division of Hematology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA
ansell.stephen@mayo.edu

Introduction

Mantle cell lymphoma is an aggressive, incurable B-cell malignancy that represents about 3–6% of lymphoma cases.[1–4] Patients with mantle cell lymphoma have a poorer prognosis than do those with other indolent lymphomas, despite an improvement in the median survival of younger patients (<60 years) treated aggressively.[5–7] Although most patients respond to initial treatment, many will subsequently progress and need further treatment.[7,8] Patients with relapsed mantle cell lymphoma have a poor prognosis with a median survival of less than 2 years[9] and are therefore candidates for treatment with novel drugs.

We have previously shown that temsirolimus is an active antitumour drug in mantle cell lymphoma.[10,11] Temsirolimus inhibits signalling though the phosphatidylinositol 3-kinase (PI3K) cellular pathway,[12,13] which is important in cell motility and survival.[14] PI3K catalyses the synthesis of phosphatidylinositol-3 phosphate (PIP3) from phosphatidylinositol-2 phosphate, resulting in activation of the serine-threonine kinase AKT.[15,16] AKT activates the mammalian target of rapamycin (mTOR), a key cellular regulator of mitosis, survival, and increased cellular size. mTOR activates p70 S6 kinase and inhibits 4EBP1, which enhance and inhibit, respectively, the translation of mRNAs.[17] Downregulation of p70 S6 kinase and 4EBP1 by mTOR inhibitors has been associated with responses to treatment.[18] AKT also induces accumulation of cellular cyclin D1. In our previous phase 2 trial, patients with mantle cell lymphoma had an overall response rate (ORR) of 38% with temsirolimus, when given alone at a dose of 250 mg/week, intravenously.[10] A similar response rate (41%) was noted in a subsequent study with a much lower dose of temsirolimus (25 mg/week, intravenously).[11] A randomised phase 3 trial was then undertaken to compare temsirolimus at two doses with the investigator's choice of treatment for patients with relapsed mantle cell lymphoma.[19] The progression-free survival (PFS) and ORR was significantly improved in patients given temsirolimus, although the response rate was lower and PFS was shorter than in the phase 2 studies.[10,11] In all studies,[10,11,19] the predominant toxicities were haematological and the time

to progression (TTP) was about 4–6 months in patients with relapsed or refractory disease.

Rituximab, a monoclonal antibody that targets the CD20 antigen, has also been used as a single drug to treat mantle cell lymphoma at the time of disease relapse. Response rates of 20–38% were reported in patients with this lymphoma[20–22] and were similar whether rituximab was given as a standard schedule or prolonged treatment.[21] Although rituximab alone induced responses in these trials in patients who had been treated previously, less than 10% of patients showed complete responses in all, but one,[20] studies.[21,22] Furthermore, the TTP after rituximab in relapsed patients with mantle cell lymphoma has been short, typically 6–8 months. Rituximab's mechanisms of action are complex and include induction of apoptosis,[23] complement-dependent cytotoxicity,[24] and antibody-dependent cellular cytotoxicity.[25] It has also been shown to inhibit the pathway of extracellular signal-regulated kinases 1 and 2 (ERK 1/2), and to interact with the PI3K pathway upstream of mTOR at the PIP3 level.[26]

Therefore, a strong rationale exists to combine rituximab with temsirolimus for patients with mantle cell lymphoma—both drugs have single-agent activity in this malignancy; both target, albeit differently, the PI3K pathway. Temsirolimus can mobilise tumour cells into the peripheral circulation and rituximab is known to deplete circulating lymphoma cells;[27] their toxicities when used alone do not overlap. Because similar efficacy with reduced toxicity has been noted with 25 mg temsirolimus,[11] we tested this dose in combination with rituximab in patients with previously treated mantle cell lymphoma.

Methods

Patients

For inclusion in this cooperative group phase 2 study, undertaken by the North Central Cancer Treatment Group at 35 centres in the USA, patients had to be aged 18 years or older and have relapsed or refractory mantle cell lymphoma, which was confirmed by central pathology review before enrolment. Other inclusion criteria were that the tumour cells had to be positive for cyclin D1 with immunohistochemistry or have the 11;14 translocation by use of cytogenetic or interphase fluorescence in-situ hybridisation analysis. All patients had to be previously treated and there was no restriction on the number of previous treatments. All patients had measurable disease. Patients had to have adequate organ and bone marrow function defined as an absolute neutrophil count (ANC) greater than 1000 cells per μL, platelet count greater than 75 000 per μL, total bilirubin less than 1·5 times the upper limit of normal (ULN), aspartate aminotransferase less than three times the ULN (<5×ULN if liver involvement with mantle cell lymphoma), serum creatinine less than two times the ULN, and serum concentrations of cholesterol less than 9 mmol/L, and fasting triglycerides less than 4·5 mmol/L. Additionally, patients had to have a life expectancy of more than 3 months and an Eastern Cooperative Oncology Group performance status of 0, 1, or 2. Women of childbearing potential were required to have a negative pregnancy test done at least 7 days before registration.

Exclusion criteria included patients who were HIV positive; had CNS involvement; had used investigational drugs, corticosteroids, chemotherapy, immunotherapy, biological treatment, or radiation therapy in the previous 1 month; did not fully recover from previous chemotherapy irrespective of time since previous treatment; or had previous treatment with an mTOR inhibitor. Pregnant and nursing women were not eligible.

All patients provided written informed consent. The study was approved by the institutional review boards of the Mayo Clinic, and each North Central Cancer Treatment Group treatment site approved the study.

Study design

Eligible patients were given a fixed dose of temsirolimus 25 mg/week, and four doses of rituximab 375 mg/m² per week during the first 28-day cycle of treatment. For subsequent 28-day cycles, patients were given a fixed dose of temsirolimus 25 mg/week, and one dose of rituximab 375 mg/m² on day 1 of every other cycle (cycles 3, 5, 7, 9, and 11). Both drugs were administered intravenously. Patients underwent a restaging assessment after every three cycles of treatment that included CT scanning of the chest, abdomen, and pelvis, and flow cytometry of the peripheral blood. A repeat bone marrow biopsy and aspirate were done only if the pretreatment bone marrow showed involvement by mantle cell lymphoma and the patient was in complete remission by use of CT scanning. Patients with a tumour response after six cycles were eligible to continue treatment for a total of 12 cycles, or two cycles after complete remission, and were then observed without additional maintenance treatment. Patients with stable disease after six cycles and those who progressed at any time went off study. Treatment responses and disease progression were assessed by use of the international standard response criteria for non-Hodgkin lymphoma.[28]

The patients were stratified according to their previous response to rituximab into rituximab sensitive (group 1) or rituximab refractory (group 2). Rituximab refractory was defined as no response (stable disease or progression) or a response that lasted less than 6 months the previous time the patient was given rituximab alone or with chemotherapy. Rituximab sensitive was defined as a response lasting 6 months or more the previous time the patient was given rituximab alone or with chemotherapy. A two-stage design was used for group 1, and a one-stage design with an interim analysis was used for group 2. The first stage of the design for group 1 required the enrolment of 24 assessable patients and a follow-up for at least

24 weeks. Seven or fewer patients showing a response in this timeframe was judged to be early evidence that this treatment regimen was not sufficiently active. If eight or more patients showed a response then accrual would continue in the second stage of this trial. If 15 or more responses were noted in the first 24 assessable patients, then the treatment regimen would be judged to be promising. In the second stage for group 1, an additional 17 assessable patients would be enrolled. 20 or fewer successes in the first 41 assessable patients would be judged to be evidence that this treatment regimen was not sufficiently active in this patient population. If 21 or more successes were noted in the first 41 assessable patients then this treatment regimen would be regarded as promising in this patient population and would be assessed further in future studies. For group 2, the study regimen would be judged to be promising in the patient population if five or more of the first 25 assessable patients had a response. Otherwise, the regimen would be judged to be inactive. When the first 16 patients had been accrued and followed up for 24 weeks, an interim analysis would be undertaken. The regimen would be judged to be inactive if no responses were noted.

Toxicity and adverse events

As per the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 3), toxicity was defined as adverse events that were classified as either possibly, probably, or definitely related to study treatment by the treating physician. Administration of temsirolimus was stopped if the ANC was fewer than 1000 per µL or the platelet count was less than 50 000 per µL. On recovery to ANC of at least 1000 per µL and platelets to at least 50 000 per µL, the dose of temsirolimus was reduced by 5 mg. The administration of temsirolimus was also stopped for any grade 3 or 4 non-haematological adverse events and restarted once the toxicity had resolved to at least grade 2. No dose modifications were made for rituximab, and rituximab was given even if temsirolimus was stopped for toxicity. Overall (both groups), if three of the first 22 patients, or if at any time seven or more patients developed grade 4 non-haematological toxicity (ie, adverse events judged to be at least possibly related to treatment) then protocol accrual would be suspended pending review by the study chair and the Cancer Therapy Evaluation Program of the National Cancer Institute. Infusion reactions related to rituximab were not included in the stopping rule. The toxicity data were regularly reviewed by the data safety monitoring board to ensure compliance with the safety stopping rules.

Immunohistochemistry

5 µm sections of whole tissue were cut from paraffin-embedded pretreatment biopsy samples. Proteins implicated in the mTOR pathway and those expressed on cells in the microenvironment were assessed by use of immunohistochemistry. The primary antibodies used for staining recognised the following antigens: GATA3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), FoxP3 (Abcam, Cambridge, MA, USA), Tbet (Santa Cruz Biotechnology), CD8 (DAKO North America, Carpinteria, CA, USA), CD11c (Abcam), CD68 (DAKO North America), raptor (Novus Biologicals USA, Littleton, CO, USA), rictor (Novus Biologicals USA), phospho-4EBP1 (Thr70, Cell Signaling, Danvers, MA, USA), phospho-Akt (Novus), and phospho-S6 ribosomal protein (Ser235/236, Cell Signaling). Immunohistochemical staining was judged to be high when more than 15% of cells showed staining in the appropriate cellular compartment. Slides were viewed with an Olympus BX51 microscope (20× objective; Olympus America, Melville, New York), and pictures were taken with an Olympus DP71 camera (Olympus America). Olympus BSW with DP Controller software (02.01) was used for image acquisition and storage.

	Rituximab-sensitive patients (n=48)	Rituximab-refractory patients (n=21)	Total (n=69)	p value
Age (years; median, range)	68 (51–86)	66 (44–85)	67 (44–86)	0·79*
Sex				0·90†
Female	13 (27%)	6 (29%)	19 (28%)	..
Male	35 (73%)	15 (71%)	50 (72%)	..
ECOG performance score				0·81‡
0	32 (67%)	12 (57%)	44 (64%)	..
1	14 (29%)	8 (38%)	22 (32%)	..
2	2 (4%)	1 (5%)	3 (4%)	..
Previous treatments				<0·0001‡
Mean (SD)	2·1 (1·51)	3·6 (1·66)	2·5 (1·70)	..
Median (range)	2·0 (1–9)	3·0 (1–9)	2·0 (1–9)	..
Previous initial treatment				0·83‡
R-CHOP	33 (69%)	18 (86%)	51 (74%)	..
R-HyperCVAD	5 (10%)	1 (5%)	6 (9%)	..
R-DHAP	1 (2%)	0	1 (1%)	..
Cladribine + rituximab	5 (10%)	1 (5%)	6 (9%)	..
Other	4 (8%)	1 (5%)	5 (7%)	..
Previous stem cell transplant				0·43†
Yes	16 (33%)	5 (24%)	21 (30%)	..
No	32 (67%)	16 (76%)	48 (70%)	..
Previous radioimmunotherapy				0·58‡
Yes	2 (4%)	2 (10%)	4 (6%)	..
No	46 (96%)	19 (90%)	65 (94%)	..
Previous radiation treatment				0·06†
Yes	10 (21%)	9 (43%)	19 (28%)	..
No	38 (79%)	12 (57%)	50 (72%)	..

Data are number (%), unless otherwise indicated. *Kruskal-Wallis. †χ^2. ‡Fisher's exact. R-CHOP=rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone. R-HyperCVAD=rituximab, cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate, and cytarabine. R-DHAP=rituximab, dexamethasone, cytarabine, and cisplatin. ECOG=Eastern Cooperative Oncology Group.

Table 1: **Characteristics of patients**

	Rituximab-sensitive patients (n=48)	Rituximab–refractory patients (n=21)	Total (n=69)*
Complete response + partial response	30 (63%; 47–76)	11 (52%; 30–74)	41 (59%)
Complete response	8 (17%; 8–30)	5 (24%; 8–47)	13 (19%)
Partial response	22 (46%; 31–61)	6 (29%; 11–52)	28 (41%)

Data are number (%; 95% CI) or number (%). *95% CIs are not appropriate statistically for the whole group because patients in the two cohorts were analysed separately and with different designs.

Table 2: **Response rates**

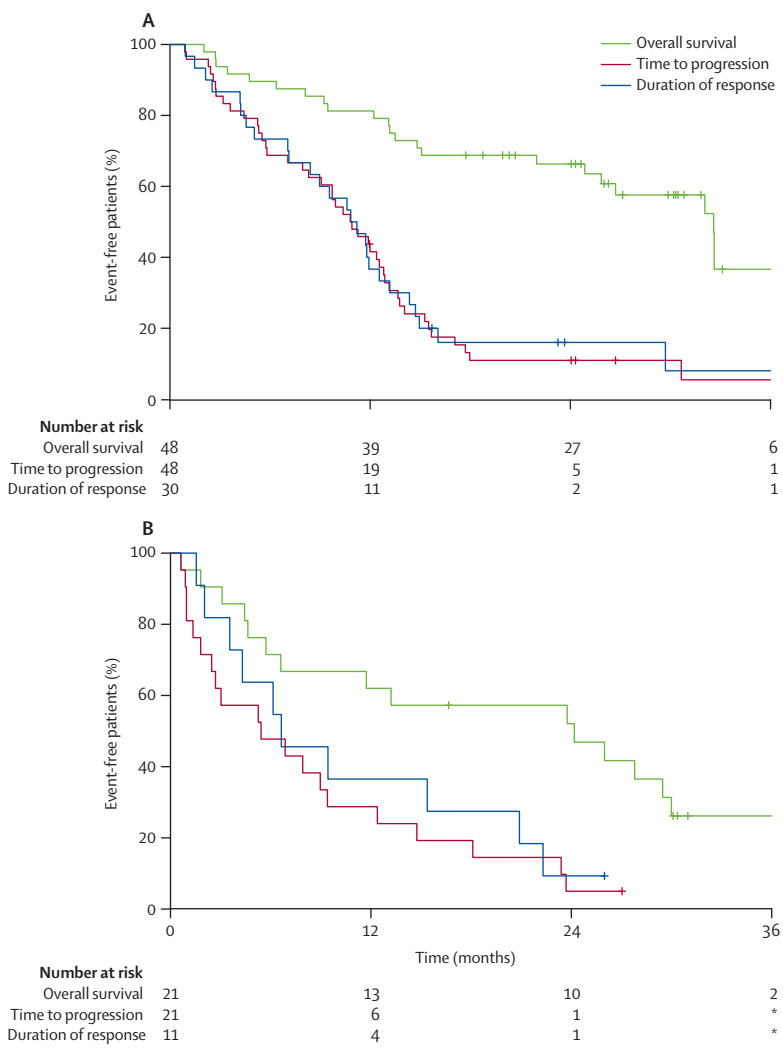

Figure 1: **Distribution of time-to-event endpoints in patients with mantle cell lymphoma who were treated with temsirolimus and rituximab**

(A) Rituximab-sensitive patients (group 1). (B) Rituximab-refractory patients (group 2). *All patients relapsed at this timepoint and none remained at risk.

Statistical analysis

The primary endpoint was the proportion of patients with rituximab-sensitive and rituximab-refractory mantle cell lymphoma who had at least a partial response. The secondary endpoint was the duration of response, TTP, and overall survival in this cohort of patients. All patients who were given treatment were analysed. Duration of response was defined as the time from the date of documented response to the date of progression. Patients who went off treatment for other reasons (eg, adverse reactions, refusal of further treatment) were censored at that time. Time to progression 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 assessment. Patients who were still receiving treatment at the time of these analyses were censored at the date of their last assessment. Overall survival was defined as the time from registration to death resulting from any cause. The distributions of these time-to-event endpoints were each estimated with the Kaplan-Meier method. SAS software (version 9.2) was used for the statistical analyses.

The study was registered with ClinicalTrials.gov, number NCT00109967.

Role of the funding source

The sponsors of the study were not involved in the collection, analysis, or interpretation of the data. The Cancer Therapy Evaluation Program of the National Cancer Institute was involved in the design and monitoring of the trial. The Predolin Foundation and grant funding from the National Cancer Institute funded the correlative studies. The sponsors were not involved in the writing of the report but reviewed the report on submission. SMA, HT, and PAK had access to the raw data. The corresponding author had full access to all the data and the final responsibility to submit for publication.

Results

71 patients were enrolled between May 6, 2005, and March 6, 2009. Two patients dropped out before starting treatment, and therefore 69 patients were included in the analysis (table 1). 23 patients had previously been given cytarabine as part of the salvage chemotherapy regimens.

In the planned interim analysis, 13 responses were noted in the first 24 assessable patients in group 1. Therefore, the criteria were met for continuation to the second stage for the rituximab-sensitive cohort, and a total of 48 patients were enrolled in group 1. Eight responses were noted in the first 16 assessable patients in group 2. We therefore closed the study early because the accrual goal was met per the protocol, which stated that "The study regimen will be considered promising in this patient population if 5 or more of the first 25 evaluable patients have a treatment success". Table 2 shows the response rates after the accrual goals of the study had been met. The median duration of response for all patients in complete or partial remission was

10·6 months (95% CI 6·6–12·5). The median duration of response was 11·0 months (7·1–13·2) in the rituximab-sensitive patients, and 6·6 months (2·0–20·9) in the rituximab-refractory patients (figure 1). The median duration of complete response in the rituximab-sensitive patients was 12·5 months (10·6–not reached), and 6·6 months (3·5–not reached) in the rituximab-refractory patients. The median overall survival for all patients was 29·5 months (23·8–32·6) and the median TTP was 9·7 months (5·8–12·0). The overall survival was 32·6 months (24·9–39·7) in the rituximab-sensitive group and 24·2 months (5·7–30·0) in the rituximab-refractory group (figure 1). The TTP was 10·9 months (8·0–12·8) in the rituximab-sensitive patients and 5·4 months (1·8–9·4) in the rituximab-refractory patients (figure 1).

All 69 treated patients were assessed for adverse events. The most common treatment-related toxicities in the rituximab-sensitive patients are shown in table 3, and those in the rituximab-refractory patients are shown in table 4. Two (4%) patients in the rituximab-sensitive group and one (5%) in the rituximab-resistant group died during the study as a result of disease progression, and their deaths were judged to be unrelated to treatment. Thrombocytopenia (five patients with eight adverse events) and neutropenia (three patients with five adverse events) were the only grade 4 haematological adverse events reported that were related to treatment (tables 3 and 4). Overall, serious adverse events (at least grade 4) were thrombocytopenia (five [7%]), neutropenia (three [4%]), dyspnoea (one [1%]), haemorrhage (one [1%]), ischaemia (one [1%]), and sexual dysfunction (one [1%]). Seven (15%) patients in group 1 and two (10%) in group 2 had grade 3 infections (at least possibly related to treatment). No grade 4 or 5 infections were noted. Only one (1%) patient had grade 3 febrile neutropenia in the rituximab-sensitive group. Initiation of temsirolimus was delayed in 47 patients (33 in group 1, 14 in group 2) because of toxicity during treatment. The median number of cycles per patient was six (range one to 12). 21 patients completed the study per protocol and 14 of these had temsirolimus dose adjustments during treatment. 42 patients had a total of 89 dose adjustments—dose adjustments for rituximab were not allowed, and one patient had his dose of rituximab delayed by 1 week on one occasion. The median proportion of time that the full-dose treatment was administered on time as per protocol was similar between the responding (80%, 15–100) and non-responding patients (75%, 10–100). Patients stopped treatment because they had disease progression (24 [35%] of 69), completed study per protocol (21 [30%]), adverse events (13 [19%]), refused further treatment (five [7%]), died during study (three [4%]), other medical problems (two [3%]), alternate treatment (one [1%]), and symptomatic deterioration (one [1%]).

	Grade 1	Grade 2	Grade 3	Grade 4
Hypertriglyceridaemia	27 (56%)	7 (15%)	3 (6%)	..
Fatigue	16 (33%)	12 (25%)	8 (17%)	..
Thrombocytopenia	17 (35%)	11 (23%)	4 (8%)	4 (8%)
Hypercholesterolaemia	24 (50%)	9 (19%)	1 (2%)	..
Anaemia	14 (29%)	13 (27%)	2 (4%)	..
Leucopenia	12 (25%)	10 (21%)	6 (13%)	..
Rash	11 (23%)	11 (23%)	..	..
Neutropenia	3 (6%)	8 (17%)	7 (15%)	3 (6%)
Hyperglycaemia	12 (25%)	5 (10%)	2 (4%)	..
Oral ulcers	6 (13%)	13 (27%)	..	..
Diarrhoea	7 (15%)	8 (17%)	1 (2%)	..
Oedema	6 (13%)	4 (8%)	4 (8%)	..
Anorexia	4 (8%)	8 (17%)	..	..
Dysgeusia	8 (17%)	4 (8%)	..	..
Weight loss	9 (19%)	2 (4%)	1 (2%)	..
Nausea	8 (17%)	3 (6%)	..	..
Sensory neuropathy	6 (13%)	4 (8%)	1 (2%)	..
Nail changes	7 (15%)	2 (4%)	1 (2%)	..
Dyspnoea	2 (4%)	4 (8%)	2 (4%)	1 (2%)
Pneumonitis	3 (6%)	2 (4%)	4 (8%)	..
Elevated alkaline phosphatase	7 (15%)	..	..	..
Cough	7 (15%)	..	..	..
Hypocalcaemia	6 (13%)	..	1 (2%)	..
Lymphopenia	..	2 (4%)	5 (10%)	..
Arthralgia	5 (10%)	1 (2%)	..	..
Constipation	4 (8%)	2 (4%)	..	..
Elevated creatinine	4 (8%)	1 (2%)	1 (2%)	..
Headache	5 (10%)	1 (2%)	..	..
Hypoalbuminaemia	4 (8%)	2 (4%)	..	..
Pruritus	6 (13%)	..	..	..
Myalgia	3 (6%)	2 (4%)	..	..
Pneumonia	..	..	5 (10%)	..

Data are number (%).

Table 3: **Treatment-related adverse events reported in more than 10% of patients with rituximab-sensitive mantle cell lymphoma (group 1, n=48)**

In an exploratory analysis, immunohistochemistry was done with the pretreatment biopsy samples from 33 patients who had adequate biopsy specimens available. Specimens were not available for all patients because many had core-needle biopsies to confirm recurrent disease, resulting in insufficient tissue for immunohistochemistry. Expression of established markers of T-cell subsets, including T-helper-1 (Th1), Th2, Treg, and cytotoxic T cells (Tbet, GATA3, FoxP3, and CD8), monocytes and dendritic cells (CD68 and CD11c), and the relevant proteins involved in the mTOR signalling pathway (raptor, rictor, phospho-4EBP1, phospho-AKT, and p70S6K) were tested and correlated with response to treatment and TTP. None of these proteins were associated with response to treatment. The median TTP for patients with low phospho-4EBP1 expression was 10·0 months (95% CI 5·4–15·5)

	Grade 1	Grade 2	Grade 3	Grade 4
Anaemia	6 (29%)	10 (48%)	..	..
Fatigue	4 (19%)	10 (48%)	2 (10%)	..
Hypertriglyceridaemia	10 (48%)	4 (19%)	2 (10%)	..
Leucopenia	4 (19%)	9 (43%)	3 (14%)	..
Thrombocytopenia	6 (29%)	2 (10%)	7 (33%)	1 (5%)
Hypercholesterolaemia	11 (52%)	3 (14%)	..	..
Neutropenia	4 (19%)	3 (14%)	5 (24%)	..
Hyperglycaemia	6 (29%)	3 (14%)	1 (5%)	..
Nausea	5 (24%)	4 (19%)	1 (5%)	..
Rash	5 (24%)	4 (19%)	1 (5%)	..
Anorexia	5 (24%)	4 (19%)	..	..
Weight loss	4 (19%)	2 (10%)	1 (5%)	..
Diarrhoea	3 (14%)	2 (10%)	1 (5%)	..
Elevated alkaline phosphatase	5 (24%)	..	..	..
Dyspnoea	3 (14%)	..	2 (10%)	..
Sensory neuropathy	4 (19%)	1 (5%)	..	..
Constipation	2 (10%)	2 (10%)	..	..
Oedema	4 (19%)	..	..	..
Hypokalaemia	3 (14%)	..	1 (5%)	..
Oral ulcers	4 (19%)	..	..	..
Elevated aspartate aminotransferase	3 (14%)	..	..	..
Cough	2 (10%)	1 (5%)	..	..
Elevated creatinine	1 (5%)	2 (10%)	..	..
Lymphopenia	..	1 (5%)	2 (10%)	..
Muscle weakness	1 (5%)	1 (5%)	1 (5%)	..
Nail changes	1 (5%)	2 (10%)	..	..
Pneumonia	..	1 (5%)	2 (10%)	..
Pruritus	2 (10%)	..	1 (5%)	..

Data are number (%).

Table 4: **Treatment-related adverse events in more than 10% of patients with rituximab-refractory mantle cell lymphoma (group 2, n=21)**

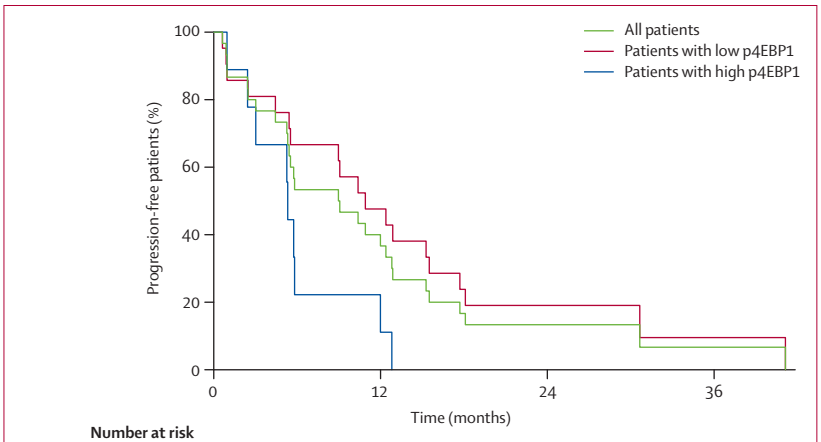

Figure 2: **Clinical outcome of patients with mantle cell lymphoma by immunohistochemical staining for phospho-4EBP1 (p4EBP1)**
Time to progression after treatment with temsirolimus and rituximab in patients with mantle cell lymphoma with high or low expression of p4EBP1.*All patients relapsed at this timepoint and none remained at risk.

Panel: Research in context

Systematic review

A comprehensive systematic review of the literature was done before this clinical trial was undertaken. We systematically reviewed studies reported in PubMed, and abstracts from national and international meetings for clinical trials and preclinical studies about the role of mammalian target of rapamycin (mTOR) inhibitors in mantle cell lymphoma and combination treatments with mTOR inhibitors in haematological malignancies. The results of trials with temsirolimus alone or rituximab alone in mantle cell lymphoma were reviewed thoroughly. Based on the results of the clinical trials of single-agent mTOR inhibitors in mantle cell lymphoma, we sought to test the hypothesis that the combination of temsirolimus and rituximab would have activity in patients with relapsed or refractory mantle cell lymphoma.

Interpretation

The results of this phase 2 study show that the combination of temsirolimus and rituximab is active in mantle cell lymphoma and results in overall response rates and complete response rates that are very promising. The efficacy of the combination needs to be confirmed and compared with other commonly used drugs in a randomised controlled trial.

compared with 5·2 months (1·0–12·0) for patients with high phospho-4EBP1 expression (p=0·02; figure 2).

Discussion

In this phase 2 trial, in which temsirolimus was used in combination with rituximab in patients with mantle cell lymphoma, the ORR was 59%, consisting of 19% complete responses and 41% partial responses. This ORR is promising, and the complete response rate is higher than that reported with either drug alone. The much higher ORR in this study than in the phase 3 trial of temsirolimus alone might not only be attributable to the addition of rituximab but could also result from patients in the randomised phase 3 trial having received a greater median number of previous treatments.[19] However, compared with a TTP of 3·4–7·0 months in previous studies of temsirolimus alone,[10,11,19] the TTP for all patients and for those with rituximab-sensitive disease in this study suggests an additive effect when rituximab is used in combination with temsirolimus.

Consistent with the results of previous studies showing that temsirolimus can cause thrombocytopenia and neutropenia,[10,11,19,29] haematological toxicity was the most common side-effect in the present trial (panel). The frequency of grades 3 and 4 haematological toxicities when rituximab was added to temsirolimus did not seem to be different to that in the previous studies of temsirolimus alone. Aside from haematological toxicity,

the other more frequent grades 3 and 4 toxicities included increased serum concentrations of cholesterol and triglycerides, hyperglycaemia, fatigue, and dyspnoea. The frequencies of these toxicities also seemed similar to those in previous studies of temsirolimus given as a single drug, suggesting that rituximab can be safely added to temsirolimus without much increase in toxicity.

To identify prognostic markers of clinical outcome, we undertook immunohistochemistry on pretreatment tumour biopsy samples to measure the expression of proteins associated with mTOR signalling or expressed on intratumoural cells in the tumour microenvironment. Increased expression of phospho-4EBP1, one of the targets of mTOR, was associated with a much shorter TTP, suggesting that the expression of phospho-4EBP1 might be useful in identifying patients most likely to benefit from temsirolimus-containing regimens. The importance of 4EBP1 as a potential prognostic marker for patients treated with a temsirolimus-containing regimen is supported by the findings that the clinical efficacy of the drug was associated with maximum reduction in phospho-4EBP1 in peripheral blood mononuclear cells in a phase 2 trial of patients with multiple myeloma.[18] Additionally, in in-vitro experiments with lymphoma cells, we noted a substantial reduction in phospho-S6, but not phospho-4EBP1, when the cells were treated with rapamycin, suggesting that rapamycin and rapalogues such as temsirolimus might not substantially affect 4EBP1.[30] The high expression of phospho-4EBP1 in the tumour cells before treatment might not be adequately or durably suppressed by temsirolimus and therefore predicts an increased likelihood of progression after treatment. This analysis however was done on a small subset of the patients in this study and therefore needs to be confirmed in other studies.

Our data suggest that mTOR inhibitors in combination with rituximab could have a role in the treatment of patients with relapsed and refractory mantle cell lymphoma.Since no clear treatment of choice exists for patients with relapsed mantle cell lymphoma and traditional salvage treatments commonly provide little clinical benefit, novel combinations are clearly needed for these patients. The combination of temsirolimus and rituximab has substantial antitumour activity in patients with relapsed mantle cell lymphoma who were treated with either temsirolimus or rituximab alone, with little increase in toxicity compared with either agent drug. The expression of p4EBP1 in pretreatment biopsy specimens correlated with the TTP and could potentially be used to identify patients who are most likely to benefit from this combination. To clearly assess the role of this effective and well tolerated combination in the management of patients with relapsed mantle cell lymphoma, randomised studies of a dose-dense schedule of rituximab in combination with temsirolimus are planned for comparison of temsirolimus and rituximab with other salvage treatments.

Contributors

SMA was the principal investigator, participated in the study design, oversaw the conduct of the study, analysed the data, and wrote the report. HT participated in the study design, did the statistical analysis, and reviewed the report. PJK did the pathology review and reviewed the report. PAK managed the data collection and reviewed the report. DJI, KS, and RR did the accrual of patients and reviewed the report. SCZ, ALF, and MG undertook and analysed the correlative studies, and reviewed the report. CE participated in the design of the study and reviewed the report. TEW participated in the design of the study, accrued patients to the study and reviewed the report.

Conflicts of interest

TEW is listed as one of the inventors on a patent application assigned to Mayo Foundation that claims methods for treating mantle cell lymphoma by use of temsirolimus. Mayo Foundation licensed the patent application and through the Mayo Foundation license-revenue-sharing policy TEW received a single remuneration from an upfront consideration payment made to Mayo from Wyeth in 2004, and a final milestone payment in 2009. There is no further remuneration. This remuneration was not tied to accrual in the clinical trial reported herein. The other authors declared no conflicts of interest.

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