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Experience with everolimus (RAD001), an oral mammalian target of rapamycin inhibitor, in patients with systemic mastocytosis

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Abstract

KIT D816V mutation has been observed in more than 90% of patients with systemic mastocytosis (SM). This mutation constitutively activates the mammalian target of rapamycin (mTOR) signaling pathway. We tested the efficacy of everolimus (RAD001), a novel oral mTOR inhibitor, at a dose of 10 mg daily in an open label, non-comparative Phase II trial for patients with SM. Ten patients were enrolled from April 2007 to October 2008. Median age was 55 years, four were males, seven had indolent and three aggressive SM, and six were previously treated with other agents. Median duration of therapy was 4 months (range 0.2–18). No objective responses were noted. Four patients had a short-lasting subjective improvement in symptoms for a median duration of 3 months (range 3–15). Grade 1–3 diarrhea, mucositis, and neutropenia were the most common adverse effects. No Grade 4 toxicity was noted. In conclusion, everolimus does not result in appreciable clinical activity in patients with SM.

Keywords

KIT mutation; mTOR; mast cell disease; D816V mutation

Background

Systemic mastocytosis (SM) is an uncommon condition resulting from the clonal expansion of abnormal mast cell progenitors. Abnormal mast cells may propagate in any organ including the bone marrow, skeletal system, spleen, liver, and lymph nodes [1]. Symptoms can range from flushing, diarrhea and urticaria in patients with indolent SM (ISM) to severe life threatening anaphylactic reactions and organ dysfunction causing death in patients with aggressive SM (ASM). To counteract the symptoms related to the disease, patients are treated with non specific agents including antihistamines, mast cell stabilizers, corticosteroids, and antileukotriene drugs. Patients with ASM may also receive interferon- α

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or cladribine as disease ‘debulking’ agents. However, with the advent of molecular studies and better insight into the pathogenesis of SM, more specific, mechanism-driven, targeted drugs are being sought.

More than 90% of patients with SM have an activating *KIT* point mutation, D816V, resulting from substitution of asparagine by valine, that contributes to disease development and abnormal clustering of neoplastic cells in tissues [2,3]. Mutated *kit* tyrosine kinase constitutively activates the mammalian target of rapamycin (mTOR) signaling pathway [4]. mTOR is a key regulator of cell growth, protein synthesis, and progression through the cell cycle. It phosphorylates p70S6kinase (p70S6k) and the eukaryotic initiation factor 4E-binding protein-1 (4E-BP1), both of which regulate mRNA translation. Rapamycin is an immunosuppressive macro-cyclic lactone that inhibits the activation of mTOR and induces apoptosis in mast cells bearing the D816V mutation isolated from patients with SM (but not in normal mast cells) [4]. Rapamycin is not approved for clinical use due to its toxicity. Everolimus (RAD001®, Novartis, East Hanover, NJ) is a novel macrolide derivative of rapamycin formulated for oral administration. Everolimus acts on interleukin and growth-factor-dependent proliferation of cells through high affinity for an intracellular receptor protein, the immunophilin FKBP-12. The resulting FKBP-12/everolimus complex then binds with mTOR to inhibit downstream signaling events, including the p70S6k and 4E-BP1 pathways.

In vitro studies have shown that everolimus potently inhibits the growth of numerous human tumor cell lines, with 50% inhibition of growth in the femtomolar range [5]. *In vivo* studies have established the activity of everolimus in experimental tumor models, both as an anti-proliferative and an anti-angiogenic agent [6,7]. Everolimus has also been tested in numerous Phase II/III trials in the treatment of melanoma, breast and renal cancer [8–11]. Here, we present the results of a prospective open-label phase II study of everolimus conducted in patients with SM at our institution.

Methods

Study design and patients

The primary objective was to determine the clinical activity of everolimus in patients with SM, regardless of the SM subtype and the *KIT* mutational status. The protocol was approved by the Institutional Review Board of The University of Texas M.D. Anderson Cancer Center. Written informed consent was obtained according to institutional guidelines and the declaration of Helsinki. Inclusion criteria were as follows: (1) Diagnosis of ISM, ASM or SM with associated hematologic non-mast cell disease (SM-AHNMD); (2) Age ≥ 18 years; (3) Minimum of 2 weeks since any major surgery or completion of radiation; (4) Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2; (5) Adequate liver function as shown by serum bilirubin ≤ 1.5 × upper limit of normal (ULN), and serum alanine aminotransferase ≤ 3 × ULN; (6) Prothrombin time and activated partial thromboplastin time within normal limits. Patients with ISM were required to have uncontrolled symptoms related to the disease, despite optimal supportive care, to participate.

All patients were required to continue birth control for the duration of the trial and at least 3 months after the last dose of everolimus. Exclusion criteria were as follows: (1) Treatment with any conventional (specifically interferon or cladribine) investigational therapy for SM within the preceding 4 weeks; (2) Concurrent severe medical diseases (such as severely impaired lung function, uncontrolled diabetes, unstable angina or New York Heart Association Class III or IV congestive heart failure, ventricular arrhythmias, active ischemic heart disease, myocardial infarction in the past 6 months, chronic liver or renal disease, active upper gastrointestinal ulcer disease); (3) Chronic treatment with systemic steroids or other immunosuppressive agents; (4) Other malignancies within the past 3 years except for adequately treated carcinoma of cervix or basal or squamous cell carcinomas of the skin; (5) Known history of HIV seropositivity; (6) Patients with a bleeding diathesis or those on oral vitamin K antagonists; (7) Impairment of gastrointestinal function or gastrointestinal disease that may significantly alter the absorption of everolimus (e.g. history of inflammatory bowel disease, malabsorption, or small bowel resection); (8) Patients who had previously received an mTOR inhibitor (e.g. sirolimus, temsirolimus); (9) Patients unwilling or unable to comply with the study protocol.

Treatment

Everolimus was administered orally at a dose of 10 mg daily. Thirty days of therapy was considered as one cycle. Dose adjustments were permitted if patients were unable to tolerate the protocol-specified dosing schedule. Treatment was interrupted for clinically significant grade 2 hematologic and non-hematologic toxicity, and held until toxicity had resolved to grade 1, then resumed at the same dose. If grade 2 toxicity recurred, everolimus was again held until resolution of the toxicity to grade 1, and then resumed at a lower dose. For grade 3 hematologic and non-hematologic toxicity, everolimus was held until the toxicity resolved to grade 1 and then resumed at a lower dose. If Grade 3 toxicity recurred after re-introduction of everolimus at a reduced dose, therapy was discontinued. For both hematologic and non-hematologic Grade 4 toxicities, treatment with everolimus was discontinued. Patients were to receive everolimus therapy for at least 3–6 months, or longer if beneficial to the patient. Patients were taken off the study protocol if they had disease progression or developed unacceptable toxicity. Patients were allowed to continue usage of medications for the relief of SM-associated symptoms (e.g. H₁ and H₂ blockers, cromolyn sodium).

Follow-up and response assessment

Upon enrollment into the trial, all patients underwent a complete physical examination, complete blood count and chemistries, serum tryptase level, and bone marrow aspiration and biopsy (for morphology and immunohistochemistry, cytogenetics, cell co-expression of CD2 and CD25 by flow cytometry, and polymerase-chain-reaction (PCR) test for D816V KIT mutation status). Patients were followed monthly for the initial 3 months and then every 3 months. A complete blood count with chemistries and serum tryptase levels were obtained every month and a bone marrow examination with biopsy was done every 3 months while the patients were enrolled in the study.

The response criteria used in this study for patients with ASM have been previously published [12]. Response criteria for ISM and SM-AHNMD have not been established. Therefore, to assess objective response in patients with ISM, we measured serum tryptase levels and bone marrow mast cell percentage while patients were on therapy. Subjective improvements in symptoms were also ascertained during each follow-up visit. We required that 'a response' be durable (evident on subsequent evaluation) to be accounted as such.

Statistical methods

This was an open-label, non-comparative Phase II study of everolimus in patients with SM. Bayesian phase II study design was used and the goal was to target an improvement in objective response of greater than 30%. A minimum of 10 and maximum of 25 patients were planned to be enrolled in the trial. The trial would be stopped if the probability that an objective response rate of greater than 30% will be achieved is less than 12.5%. In other words, if there are no objective responses among first 10 patients on the study, or if there are only two responders among 15 patients on the study, the trial will be terminated. Time to progression (duration of time elapsed from the date of initial treatment to the date of relapse or disease progression) and duration of response (from beginning of response to the time of relapse) for responding patients will be analyzed by the Kaplan–Meier method.

Results

Patients and disease characteristics

Between April 2007 and October 2008, ten patients were enrolled into the study (Table I). Seven patients were diagnosed with ISM and three with ASM (two had cytogenetic abnormality: del17q and del20q, respectively). Six patients had previously received other targeted therapies for SM, including a varying combination of imatinib mesylate, dasatinib, cladribine, and daclizumab (anti CD-25 antibody).

Efficacy

The median time on everolimus therapy for all patients was 4 months (range, 0.25–18 months) (Table II). Of the 7 patients with ISM, four had subjective improvement in symptoms, including decreased flushing, itching, shortness of breath and frequency of diarrhea, and improvement in fatigue. None had an objective response in serum tryptase levels or percent bone marrow mast cells. Median duration of symptomatic improvement was 3 months (range, 3–15 months). Two out of these four patients were negative for the D816V mutation. However, all patients have stopped the study medication due to loss in response. None of the three patients with ASM achieved subjective improvement in symptoms or an objective response (i.e. change in serum tryptase level or bone marrow mast cell percentage).

Safety and tolerability

All patients received an initial dose of 10 mg of everolimus daily. This dose was not modified in four patients who received the drug at a dose of 10 mg daily for a median of 6 months (range 3–18 months). The dose was decreased to 5 mg daily, after interruption in therapy for a brief period of time, in five patients due to varying toxicities such as Grade 1–3

diarrhea, neutropenia, and mucositis (Table III). No Grade 4 toxicities were noted. Since there were no responses noted in the first 10 patients enrolled in the study, further accrual was stopped as described in the methods section above.

Discussion

In a recent retrospective analysis of more than 300 patients from Mayo Clinic, Lim *et al.* have shown that the survival of patients with ISM is identical to sex and age matched cohort of normal US population [13]. However, the median survival of patients with ASM and SM-ANHMD is only 3.5 and 2 years, respectively. Clearly, these subsets of patients with SM would benefit the most from new targeted therapy. D816V *KIT* mutation has been observed in more than 90% of patients with SM, resulting in constitutive activation of *kit* tyrosine kinase, thought to play an important role in the pathophysiology of the disease. Efforts are therefore underway to develop an inhibitor of the mutated *kit* enzyme that would provide significant clinical benefit to patients with SM. Although initial clinical data about the efficacy of imatinib mesylate in patients with SM were mixed [14–16], it is now very well established that imatinib does not inhibit D816V mutated *kit*, and does not have clinical activity in patients with D816V *KIT*-positive SM [17]. Surprisingly, however, other agents proven in preclinical testing to inhibit D816V *KIT* mutation carrying cells (e.g. dasatinib) [18], have also not shown significant clinical activity [19,20]. PKC412 (midostaurin), a multikinase inhibitor, was reported to induce remission in a patient with mast cell leukemia positive for D816V mutation [21], and is currently being tested in a Phase II multicenter trial. Since the mTOR pathway was described as the downstream regulatory mechanism constitutively activated by mutated D816V *kit*; we chose to study a novel mTOR inhibitor, everolimus (RAD001), in patients with SM.

Our study shows that everolimus does not result in appreciable clinical activity in patients with SM. No patient achieved an objective response to therapy, and subjective improvement in symptoms seen in four patients with ISM was short-lasting. A limitation of the present study is the difficulty in evaluating symptomatic improvement reported by patients. Since this study was not randomized or placebo controlled and did not employ established quality-of-life assessment, it is quite likely that ‘placebo effect’ significantly contributed to the observed improvements. Another limitation of our study is lack of correlative studies to monitor mTOR-dependent phosphorylation events in patient samples, to assess if everolimus indeed affected this pathway. However, there are several clinical studies published in the literature linking the exposure of patients to everolimus at a dose of 10 mg daily and the inhibition of the mTOR pathway leading to clinical improvement [22–24].

Given the unfavorable toxicity profile and lack of objective efficacy of everolimus in our patients, this study was closed after the enrollment of 10 patients. Other mTOR inhibitors have been developed and are used in solid tumors. Temsirolimus is approved for the treatment of metastatic renal cell cancer. Since its mechanism of action, pharmacokinetics and toxicity profile is comparable to everolimus, it is questionable whether it would be effective in treating SM. Other targeted therapy agents such as bcl-2 antagonists (obatoclax) and novel *kit* inhibitors (masatinib) are being currently tested in clinical trials for patients with SM.

In summary, our study shows that everolimus, an oral mTOR inhibitor, does not result in appreciable clinical activity in patients with SM and given its toxicity profile should not be considered a therapeutic option in these patients.

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Table 1
Baseline characteristics and objective response parameters of all patients enrolled in the study ($n=10$).

Patient number	Age (years)	Sex	Type of SM	Previous therapy	Karyotype	D816V <i>KIT</i> mutation	CD25 positivity	Serum tryptase (ng/mL)				Bone marrow MC percent			
								Base	1 mo.	2 mo.	3 mo.	6 mo.	Base	3 mo.	6 mo.
1	47	M	ISM	Imatinib	Diploid	Positive	Positive	69	81	70	58	–	10	–	–
2	50	F	ASM	Cladribine, Imatinib	Del 7q	Positive	Positive	116	–	152	159	158	35	40	–
3	61	F	ISM	Dasatinib	Diploid	Positive	Positive	144	77	100	95	–	15	20	–
4	49	F	ISM	None	Diploid	Negative	Positive	44	51	50	45	39	10	10	15
5	56	F	ISM	None	Diploid	Negative	Positive	12	13	9	8	8	<5	<5	<5
6	74	M	ASM	Cladribine	Diploid	Positive	Positive	191	147	171	153	125	10	–	–
7	43	F	ISM	Imatinib, Dasatinib	Diploid	Positive	Positive	22	25	20	–	–	10	–	–
8	54	F	ISM	None	Diploid	Positive	Positive	144	140	138	–	134	40	30	–
9	57	M	ISM	None	Diploid	Positive	Positive	55	–	60	69	–	15	–	15
10	73	M	ASM	Daclizumab	Del 20q	Positive	Positive	162	–	–	–	–	45	–	–

SM, systemic mastocytosis; ISM, indolent systemic mastocytosis; ASM, aggressive systemic mastocytosis; Base, Baseline; MC, mast cell; mo., months; –, values not obtained.

Table II

Subjective response assessment in all patients enrolled in the study ($n = 10$).

Patient number	Type of SM	Baseline	Symptoms					Duration on trial (mo.)
			1 mo.	2 mo.	3 mo.	6 mo.	12–18 mo.	
1	ISM	Skin rash, pruritus, diarrhea, abdominal pain	No response	No response	No response	–	–	3
2	ASM	Skin rash, pruritus, diarrhea, anaphylaxis	No response	No response	–	No response	–	6
3	ISM	Skin rash, flushing, pruritus, anaphylaxis	No response	No response	No response	–	–	3
4	ISM	Flushing, shortness of breath, anaphylaxis	Improved shortness of breath, flushing	Improved shortness of breath, flushing	Improved shortness of breath, flushing	Loss of response	–	6
5	ISM	Skin rash, diarrhea, fatigue, flushing, abdominal pain	Improvement in all symptoms	Improvement in all symptoms	Improvement in all symptoms	Improvement in all symptoms	Loss of response at 15 months	18
6	ASM	Skin rash, fatigue, flushing, pruritus	No response	No response	No response	–	–	3
7	ISM	Skin rash, diarrhea, anaphylaxis	No response	No response	No response	–	–	3
8	ISM	Skin rash, flushing, fatigue, diarrhea	Improvement in all symptoms	Improvement in all symptoms	Improvement in all symptoms	Loss of response	–	6
9	ISM	Skin rash, headache, fatigue, pruritus	Improvement in all symptoms	Improvement in all symptoms	Improvement in all symptoms	Loss of response	–	6
10	ASM	Skin rash, fatigue, diarrhea, flushing, abdominal pain	–	–	–	–	–	0.2

SM, systemic mastocytosis; ISM, indolent systemic mastocytosis; ASM, aggressive systemic mastocytosis; mo., months; –, assessment not obtained.

Table III

Number and grade of adverse events during treatment with everolimus.

Adverse event	Grade 1	Grade 2	Grade 3
Anemia	1	–	–
Thrombocytopenia	–	1	–
Neutropenia	1	1	1
Diarrhea	1	1	2
Mucositis	2	3	2
Fatigue	4	–	1
Fever	–	1	–
Headache	3	1	–
Nausea/vomiting	2	–	1
Hyperglycemia	–	1	–
Hyperlipidemia	1	2	–
Cough	1	1	–