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CLINICAL TRIAL

Sirolimus and trastuzumab combination therapy for HER2-positive metastatic breast cancer after progression on prior trastuzumab therapy

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Abstract Constitutive activation of the PI3K/Akt/mTOR pathway has been suggested as a mechanism of resistance to trastuzumab therapy. This phase II trial was designed to evaluate the safety and clinical activity of daily oral sirolimus, a mammalian target of rapamycin (mTOR) inhibitor, in combination with trastuzumab in HER2-positive metastatic breast cancer following disease progression on prior trastuzumab therapy. Sirolimus 6 mg oral daily dose was administered with a standard dose and schedule of trastuzumab weekly or every 3 weeks. Pharmacodynamic studies included Western blot analysis of S6K1, phospho-S6K1, and mTOR in peripheral mononuclear cells, circulating tumor cells (CTC), and endothelial cells (CEC). Eleven patients were evaluable for safety; and nine were evaluable for response assessment. Subsequent enrollment was stopped due to slow accrual. Study treatment-related grade 3 toxicity included pneumonitis, myelosuppression (leukopenia/anemia), and dermatologic reactions (mucositis, nail changes and rash), with no grade 4 events. One patient received eight cycles (58 weeks) and achieved a partial response. Five patients treated for a total of 101 weeks (median 12 weeks, range 8–47 weeks) achieved stable disease as best response. Overall response rate was 1/9 (11 %) and clinical benefit rate was 4/9 (44 %). There was no statistically significant correlation between response and post-treatment change in levels of the mTOR pathway biomarkers, CTCs, HER2 CTCs, or CECs. Sirolimus 6 mg administered daily with trastuzumab appears to be well tolerated in patients with metastatic HER2-

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positive breast cancer following disease progression on prior trastuzumab therapy, with evidence of disease activity. mTOR inhibition may overcome resistance to trastuzumab in some HER2-positive tumors.

Keywords mTOR · Trastuzumab · Sirolimus · Breast cancer · Circulating tumor cells

Introduction

Human epidermal growth factor receptor 2 (HER2) overexpression or gene amplification occurs in approximately 25 % of all breast cancers, and is associated with decreased disease-free survival (DFS) and overall survival (OS) [1]. Trastuzumab, a humanized monoclonal antibody directed against the extracellular domain of HER2, is a critical component of treatment of HER2-positive breast cancer [2]. Some patients with HER2-positive breast cancer may benefit from the combination of trastuzumab with other novel therapies, without the need for cytotoxic chemotherapy [3–8].

The mammalian target of rapamycin (mTOR) is a serine threonine kinase member of the cellular phosphatidylinositol 3-kinase (PI3K) pathway, which is involved in multiple biologic functions, such as transcriptional and translational control. It is a downstream mediator in the PI3K/Akt signaling pathway and plays a critical role in cell survival. Inhibitors of mTOR have been shown to be promising agents in reducing tumor growth in several solid cancers, both in vitro and in vivo [5, 9].

Intrinsic and acquired mechanisms of resistance to trastuzumab have been reported, and include activation of the PI3K/Akt/mTOR pathway through various mechanisms. [10, 11] Preclinical data show that activation of the PI3K/Akt/mTOR pathway in trastuzumab-resistant cell lines can be overcome by either a PI3K inhibitor or by sirolimus. Preclinical studies in mice bearing HER2 overexpressing and PTEN deficient breast tumor xenografts studies demonstrated that mTOR inhibition reduces tumor formation and growth, and sensitizes response to trastuzumab [12, 13]. In clinical studies, the mTOR inhibitor everolimus has been evaluated in combination with trastuzumab [14], with paclitaxel and trastuzumab [15] and with vinorelbine and trastuzumab [16], exhibiting promising results with an overall response rate (ORR) ranging from 14 to 44 % and a median progression free survival (PFS) ranging from 4.1 to 8.5 months.

Sirolimus is a specific mTOR antagonist that targets the PI3K/Akt/mTOR pathway and blocks the downstream signaling elements. It first binds to FKBP-12, and binding of this complex to mTOR then leads to repression of specific protein phosphatases, resulting in the dephosphorylation of downstream effector molecules which ultimately lead to cell cycle arrest in the G1 phase [9]. This agent has been found to have antiproliferative and angiogenic actions in a diverse range of experimental human tumors, including breast cancer [17]. Sirolimus is currently approved as an immunosuppressive agent for organ transplantation and more recently, as a component of cardiac arterial stents because of its potent antiproliferative effects on fibroblasts responsible for restenosis after such a procedure [18, 19]. Sirolimus is commonly administered orally on a daily basis, in doses ranging from 2 to 40 mg/day. S6K1 is one of the principal kinases downstream of mTOR. Inhibition of the S6K1 is one of the best-characterized effects of sirolimus and has been related to the antiproliferative activity of the agent. Furthermore, the phosphorylation of the S6K1 in peripheral mononuclear cells (PBMC) has been demonstrated to decrease in a concentration-dependent fashion after exposure to sirolimus and has been used to monitor this drugs effect in transplant patients. Sirolimus has been also evaluated in a phase I clinical trial in advanced cancer patients by Cohen et al. with a similar toxicity profile and pharmacokinetics compared with other mTOR inhibitors [20]. In addition, there are several clinical trials evaluating daily sirolimus in combination with other cancer therapies in advanced solid tumors.

Detection of circulating tumor cells (CTCs) in blood and lymph systems has the potential to aid clinical decision making in the treatment of cancer [21–23]. The presence of CTCs is a prognostic marker in patients with metastatic breast cancer and has the potential to indicate relapse or disease progression. Circulating endothelial cells (CECs) have been proposed as a surrogate marker of antiangiogenic activity, as it is believed that these cells are active agents for neoangiogenesis [21]. The observed association between HER2 overexpression and higher VEGF expression indicates that HER2 may be involved in the regulation of angiogenesis in breast cancer [24–26].

Targeting the PI3K/Akt/mTOR pathway has the potential to reverse resistance to trastuzumab. Here we report the results of a safety cohort from a phase II trial designed to evaluate the safety and clinical activity of daily oral sirolimus in combination with trastuzumab in patients with HER2-positive metastatic breast cancer after progression on prior trastuzumab therapy.

Patients and methods

Patients

Patients with measurable metastatic HER2-positive breast cancer were eligible. HER2 positivity was defined as 3+ positive for HER2 overexpression by immunohistochemistry (IHC) (Dako Herceptest) or gene amplification [ratio of

HER2 to chromosome 17 centromere (CEP17) of >2.0] by fluorescence in situ hybridation (FISH) by local review. Patients were considered eligible for clinical trial participation if they had documented disease progression following at least 8 weeks of standard doses of trastuzumab or a trastuzumab-containing regimen in the metastatic setting. All patients must have been off trastuzumab or HER2-targeted therapy for a minimum of 3 weeks prior to initiation of study treatment. Patients must have had measurable disease by response evaluation criteria in solid tumors (RECIST) 1.0. Bone only disease was allowed if a lesion measured 10 mm or greater on MRI or if a lytic bone lesion measured 20 mm or larger on plain film or conventional computed tomography (CT) scan, or 10 mm or larger on a spiral CT scan.

Eligible patients had a life expectancy >3 months; age >18 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤2; adequate bone marrow function [absolute neutrophil count (ANC) >1500/μL, platelets ≥ 100,000/μL,hemoglobin >9 g/dL]; fasting serum cholesterol <350 mg/dL and triglycerides <400 mg/dL; bilirubin <1.5 upper limit of normal (ULN), aspartate aminotransferase and alanine aminotransferase <2.5 × ULN; creatinine <1.5 × ULN; and lack of cardiac dysfunction [defined as left ventricular ejection fraction (LVEF) <50 %]. Patients with a history of brain metastases were eligible, provided that they had completed the treatment of their brain metastases at least 30 days before enrollment and had no evidence of progression on a post-treatment MRI.

Patients with active infections, uncontrolled intercurrent illness, and human immunodeficiency virus infection were excluded. Patients were excluded if they received chemotherapy within the 4 weeks of study initiation. Prior treatment with sirolimus, sirolimus analogs, or experimental agents targeting mTOR was not allowed. Radiation therapy within the last 4 weeks, prior radiation therapy to indicator lesion, and prior investigational agents within 30 days before enrollment were also exclusion criteria. In addition, patients with concomitant malignancies or previous malignancies within the last 5 years, with the exception of adequately treated basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix, were excluded.

The protocol was approved by the institutional review board of the participating institution, and all patients provided written informed consent.

Study design

This study was a phase II clinical trial designed to evaluate the safety and clinical activity of daily oral sirolimus in combination with trastuzumab in patients with HER2-positive metastatic breast cancer with evidence of disease progression following prior trastuzumab therapy. The primary endpoint was to determine the proportion of patients who are progression free at 16 weeks, defined by complete response (CR), partial response (PR), or stable disease (SD). Secondary objectives were to assess the objective response rate (ORR) by response evaluation criteria (RECIST) 1.0, duration of response, safety, and pharmacodynamic endpoints. An early stopping rule was included for excess toxicity with plan to terminate trial enrollment if three or more cases of grades 3 or 4 drug-related toxicities were observed within the first 4 weeks of treatment among the first nine patients enrolled.

Treatment

Patients received oral sirolimus 6 mg daily in combination with weekly trastuzumab administered intravenously with a loading dose of 4 mg/kg followed by 2 mg/kg weekly in a 28-day cycle. A subsequent amendment allowed trastuzumab to be administered every 3 weeks for patient convenience, with a loading dose of 8 mg/kg followed by a 6 mg/kg in a 21-day cycle. Sirolimus was administered at a 6 mg oral daily dose. Cycles were repeated on an every 21 or 28-day schedule until disease progression, unacceptable toxicity, or the development of any of the criteria for study removal. Doses were reduced or discontinued based on tolerability. Events necessitating sirolimus dose reduction (from 6 to 4 to 2 mg) included ANC <1000 cells/mm^3, grade 3 or 4 thrombocytopenia, grade 3 non-hematologic event (excluding alopecia and non-premedicated nausea/vomiting), or grade 3 or 4 hyperlipemia. On the 3rd occurrence of any of these events, sirolimus was discontinued. Grade 2 or 3 non-infectious pneumonitis required sirolimus interruption and/or dose reduction until recovery to grade ≤1, or withdrawal from study if not within 3 weeks. Any grade 4 non-hematologic event (except hyperlipemia) resulted in study discontinuation. Dose modifications of trastuzumab therapy were not permitted. There were no dose or schedule adjustments for trastuzumab based on specific trastuzumab-related toxicity criteria, except for cardiac toxicity. Patients underwent cardiac evaluation with an echocardiogram or multi-gated acquisition scan at baseline, before study entry, and every three or four cycles (12 weeks) during treatment.

Assessments

At screening, patients were assessed by history, physical examination, documentation of performance status, complete blood count, chemistry panel, baseline electrocardiogram, serum pregnancy test (when indicated), lipid panel, and baseline tumor measurements. Toxicity was evaluated according to the National Cancer Institute common toxicity criteria for adverse events (CTCAE v3.0). Tumor measurements were assessed by conventional

imaging at baseline, at the end of cycle 3, and every 8–9 weeks thereafter, measured by RECIST 1.0.

Biomarkers

Circulating tumor and endothelial cells

Blood samples were collected for CTCs and CEC analysis at baseline, 2-week post-treatment, and monthly thereafter. CTCs were isolated, fluorescently labeled and analyzed by the CellSearch System (Veridex, LLC, Warren, New Jersey) in our pathology Core laboratory, per manufacturer's instructions. Analysis of HER2 labeling on malignant cells (HER2 CTC's) was performed after isolating CTCs utilizing CellSearch™Epithelial Cell Kit and CellSearch™-Tumor Phenotyping Reagent. CECs were isolated, fluorescently labeled, and analyzed by the CellTracks® Analyzer II (Veridex LLC, Warren, New Jersey). CTC and CEC results were reported as number of cells per 7.5 ml.

mTOR pathway evaluation in PBMCs

The effects of sirolimus treatment on S6K1 and mTOR activity were assessed by Western blot analysis in PBMCs. Blood samples were collected at baseline and at day 15 (±2 days) of cycle 1. Cells were lysed with CelLytic M Cell Lysis Reagent (Sigma) with protease inhibitor (Complete, Roche). Samples were sonicated on ice for 10 s. Protein concentrations were determined for gel loading with the Bio-Rad QuickStart Bradford Assay. A total of 8 ug protein were loaded in each lane. Protein was electrophoresed on a 4–20 % gradient gel (Bio-Rad) and transferred onto PVDF Mini transfer membranes (Bio-Rad) by standard protocols. Membranes were incubated overnight with gentle agitation at 4° in 30 ml of blocking buffer with a mixture containing primary antibodies for one of the following: mTOR, S6K1, and pS6K1 (CellSignaling Technology, Danvers, MA) or Actin (Sigma, St Louis, MO).ImageLab software version 4.1 (Bio-Rad) was used for image acquisition and densitometric analysis of the blots. The software interprets the raw data in three dimensions with the length and width of the band defined by the ''Lanes and Bands'' tool in concert with the ''Lane Profile'' tool. Quantitation of the bands was done using the "Relative Quantity Tab" in the "Quantity Tools". Normalization was done by comparing the test antibody against the actin which was normalized to 1.0

Statistical methods

The primary outcome of the planned phase II clinical trial was to determine the proportion of patients who are progression-free (CR, PR, or SD) at 16 weeks. Sample size calculations were based on the assumption that progression-free from baseline has an exponential distribution; therefore, we estimated that a median progression-free duration of 12 weeks will exhibit 44 % progression-free at 16 weeks. If 17 or more of the 30 patients are progression-free at 16 weeks then we will reject this null hypothesis in favor of an increased progression-free alternative hypothesis. This criterion has significance level 0.094. If the median progression-free survival can be increased to 25.7 weeks (5.9 months), then the power of this study is 0.87. This alternative hypothesis also corresponds to 65 % progression-free at 16 weeks, again assuming exponential survival distributions.

An early stopping rule was included for excess toxicity with plans to terminate trial enrollment if three or more cases of grades 3 or 4 drug-related toxicities were observed within the first 4 weeks of treatment among the first nine patients enrolled. However, subsequent enrollment on the clinical trial was stopped due to slow accrual.

For the exploratory mTOR pathway biomarker analysis, an association with response was assessed through logistic regression adjusted for baseline levels, and association between relative biomarker changes through linear regression analysis. For trend analysis of CTC and CEC profiles, a linear mixed effects (LME) regression mode was used to assess significance of time trend and effect of response group accounting for the longitudinal aspect of the data.

Results

Patient population

Eleven patients were enrolled from April 2007 to February 2010. The study was closed before completion of planned sample size enrollment due to slow patient accrual, partly due to patient concerns about omitting cytotoxic chemotherapy. A total of 11 patients were accrued to the study. Patient characteristics are described in Table 1. The median age was 57 (range 38–62). ECOG performance status was 0 ($n = 7$) and one ($n = 4$). Race was white ($n = 8$), black ($n = 2$), and other ($n = 1$). Prior lines of chemotherapy regimens were one ($n = 2$), two ($n = 3$), three ($n = 3$), and more than three ($n = 3$). All 11 patients had prior trastuzumab and four also received prior lapatinib therapy.

Anti tumor activity

A total of 49 cycles (range 1–12, median three) were administered. One patient had one cycle of trastuzumab held

due to decreased LVEF. The first seven patients enrolled on clinical trial received weekly trastuzumab, and the remaining four patients received trastuzumab every 21 days. Nine patients were evaluable for response assessment (Table 2). Two patients withdrew from study before first response assessment (one for noncompliance and the other for toxicity). One patient achieved a PR as best response, and was treated for a duration of 58 weeks. An additional five patients achieved SD and were treated for a median of 12 weeks, with a range of 8–47 weeks (patient #3 for 47 weeks, #5 for 12 weeks, #7 for 8 weeks, #8 for 26 weeks and #11 for 20 weeks). The overall ORR was 1/9 (11 %), and clinical benefit rate was 6/9 (66.6 %).

Table 1 Patients characteristics

Characteristic	$n = 11$
Age (years)	
Median	57
Range	38–62
ECOG PS [*n*(%)]	
0	7
1	4
Race	
White	8
Black	2
Unknown	1
ER/PR status [*n*(%)]	
ER+ or PR+	5 (45.4)
Prior number of chemotherapy regimens for MBC	
1	2
2	3
3	3
>3	3
Prior lapatinib therapy	4
Prior trastuzumab containing regimen	11

Table 3 Adverse events

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic				
Leukopenia	1	2	2	
Neutropenia	1		2	
Anemia	3	4	1	
Thrombocytopenia	4			
Non hematologic				
Nail changes	1		1	
Nausea	3			
Fatigue	1	1		
Dyspepsia		1		
Mucositis	2	1	2	
Xerostomia	1			
Dysgeusia		1		
Hemorrhage, pulmonary/ upper resp	1			
Pneumonitis			1	
Rash			2	
Anorexia		1		
Hypertryglyceredemia	1	1		
Hyperglycemia	2			1*
AST/ALT	3	1		

* Grade 4 hyperglycemia was non-fasting and therefore did not meet prespecified definition of grade 4 toxicity

Table 2 Prior treatment and best response

Pt #	Prior trastuzumab	Prior lapatinib	Treatment duration (weeks)	Best response	Reason off study
1	Y	N	58	PR	PD
2	Y	N	3	PD	Tox
3	Y	N	47	SD	PD
4	Y	Y	5	U	W
5	Y	N	12	SD	PD
6	Y	N	7	U	Tox
7	Y	Y	8	SD	Tox
8	Y	Y	26	SD	PD
9	Y	N	8	PD	PD
10	Y	Y	8	PD	PD
11	Y	N	8	SD	PD

Y yes, *N* no, *PR* partial response, *PD* progression of disease, *SD* stable disease, *U* unknown, *Tox* toxicities, *W* withdrawal

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