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Phase I study of panobinostat (LBH589) and letrozole in post-menopausal metastatic breast cancer patients

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Abstract

Introduction/Background—Histone deacetylase inhibitors have been found to restore sensitivity to the estrogen receptor in endocrine resistant and triple negative breast cancer cell lines. We decided to test panobinostat, a pan histone deacetylase inhibitor, based on preclinical data in combination with letrozole in a Phase I study.

Patients and Methods—We enrolled patients with metastatic breast cancer with the aim to determine the safety and tumor response by RECIST criteria. Dose Level 1 was panobinostat 20 mg per-oral three times weekly with letrozole 2.5 mg per-oral daily. Dose level 2 was panobinostat 30 mg per-oral three times a week with same dose of letrozole.

Results—Twelve patients (six each dose level) were enrolled. Forty-three cycles of treatment were given. There was 1 dose-limiting toxicity (out of 6 at the 20 mg dose level, increased in creatinine). At the 30 mg dose level, 3/6 patients had dose-limiting toxicity, one grade 3 thrombocytopenia with bleeding, one grade 4 thrombocytopenia, and one grade 3 diarrhea. The maximum tolerated dose was the 20 mg dose level. Among the 12 patients, two had a partial response and five had stable disease. The most common severe adverse event was thrombocytopenia in 4/12 patients.

Conclusion—The recommended Phase II starting dose is panobinostat 20 mg per-oral three times weekly (MWF) and letrozole 2.5 mg per-oral daily. This dose should be escalated to 30 mg per-oral three times weekly if there is no grade 3 toxicity since the partial responses were found in patients with this dose.

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Keywords

Phase I; Panobinostat; HDAC inhibitors; metastatic breast cancer; aromatase refractory

Introduction

Changes in chromosome structure by epigenetic alterations play critical roles in the control of gene transcription. These epigenetic alterations include modification of histones and other proteins by acetylation and/or phosphorylation^{1, 2}. Normally, these modifications are finely balanced and are highly reversible in normal tissues, but they may be imbalanced and heritable in tumor cells. Histone deacetylase inhibitors (HDACi) increase histone acetylation, thereby modulating the expression of a subset of genes in a coordinated fashion. Tumor suppressor genes are noted to be repressed by epigenetic mechanisms in several cancers^{3, 4}. Thus, therapy with HDACi may alter tumor phenotype and inhibit tumor growth⁵. Additionally, breast cancer cell lines that are estrogen resistant or triple negative, were made to respond to an aromatase inhibitor or tamoxifen after pretreatment with HDACi^{6, 7}. Panobinostat had been found to be a powerful pan HDACi in preclinical studies in combination with letrozole. So we embarked on a phase I clinical trial that evaluated the safety and tolerability of panobinostat, and letrozole in patients with metastatic breast cancer (MBC).

Patients and Methods

The study was approved by the Mayo Clinic IRB and trial was registered in clinicaltrials.gov, NCT01105312. Patients were eligible to participate in this study if they had histologic proof of breast cancer that was metastatic at registration; evidence of MBC confirmed by radiological imaging; measurable or non-measurable (evaluable) disease; post-menopausal, any receptor status, estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2 (HER2) level acceptable; with or without prior treatment and Eastern Cooperative Oncology Group Performance Status of 0–2. Reasons for excluding study participation included uncontrolled intercurrent illness; chemotherapy or radiation therapy four weeks prior to registration; radiation to >25% of bone marrow; reversible effects of prior therapy, known central nervous system metastases, or seizure disorder; and receiving concurrent CYP3A4 inhibitors. Dose Level 1 was panobinostat 20 mg per-oral three times weekly (MWF) with letrozole 2.5 mg per-oral daily. Dose level 2 was panobinostat 30 mg per-oral three times a week with letrozole 2.5 mg per-oral daily.

A patient was deemed to have had a dose-limiting toxicity (DLT) if they had any of the following adverse events (AE) in the first cycle of treatment that was deemed at least possibly related to study treatment: grade 3 electrocardiogram QT corrected interval prolonged; grade 4 thrombocytopenia (or grade 3 thrombocytopenia with bleeding); grade 4 neutropenia (for at least 5 days); grade 4 anemia; febrile neutropenia; grade 3 nausea, vomiting, or diarrhea that did not responded to conventional therapy; any other grade 3+ non-hematologic event; creatinine increased at least two times from baseline; and any cardiac arrhythmia requiring new cardiac medication and/or hospitalization.

The primary endpoint of this study was to determine the maximum tolerated dose (MTD) of panobinostat in combination with letrozole. The MTD was defined as the highest dose that led to fewer than 2 DLTs (out of 6). The single secondary endpoint for this Phase I study was tumor response determined by RECIST version 1.1.

Results

Twelve patients (six at dose level 1 and six at dose level 2) were enrolled.

Ten patients were ER positive and two patients had triple negative breast cancer. Six had endocrine resistant disease, five progressed on an aromatase inhibitor, and four had prior treatment. Among the 12 patients, six had at least one prior chemotherapy treatment and one hormonal therapy treatment, four had 1–10 chemotherapy treatments, and two had no prior chemotherapy or hormonal therapy treatments. None of the patients had hormonal treatment alone with letrozole and one had tamoxifen, aromatase inhibitor, and fulvestrant. Baseline patient characteristics can be found in Table 1.

A total of 43 cycles of treatment were given. The initial cohort of three patients at the 20 mg dose level had no DLT. At the subsequent 30 mg dose level, three patients had DLT (two patients with thrombocytopenia, one grade 3 with bleeding, one grade 4, and one patient with grade 3 diarrhea). On the subsequent cohort of three patients at the 20 mg dose level, one had DLT manifested as an increase in creatinine. The most common severe AE for the study were thrombocytopenia (4 out of 12) and neutropenia and hyponatremia (both in 3 out of 12) patients. There were eight patients on-study with measurable disease and four with evaluable disease only. Of the patients with measurable disease, two patients had a partial response (endocrine resistant and chemotherapy resistant and treated at 30 mg dose level) and one patient had stable disease (triple negative and chemotherapy resistant). Among them one patient had a confirmed partial response and remained on study for six cycles of treatment at the 30 mg dose (time to progression was 5.1 months). This patient experienced a DLT (grade 3 diarrhea) and also had grade 3 anorexia. The remaining five patients with measurable disease experienced progression at their first evaluation. One patient with partial response remained on study after six cycles of treatment at the 30 mg dose, the other patient had PR was also at 30 mg. Of the four patients that had stable disease, three were at 20 mg and 1 was on the 30 mg dose level. A patient by patient listing of disease characteristics versus outcomes can be found in Table 2.

Of the four patients that had evaluable disease, all were ER positive and endocrine and chemotherapy resistant and experienced stable disease for a median duration of 5.6 months (range 3.9–9.2months). All of these patients went off-study without progression due to refusal, AE, or decision to start new treatment with another agent. None of the evaluable disease patients experienced a DLT; however, one experienced grade 3 thrombocytopenia and grade 3 neutropenia, another experienced grade 3 hypocalcemia, grade 3 hyponatremia, and grade 3 bladder infection, and one experienced grade 3 hyponatremia. Additional AE data, by dose level, may be seen in Tables 3 and 4 for dose levels 20 and 30 mg, respectively.

Discussion

Loss of ER alpha (ER α) gene expression has been associated with insensitivity to endocrine therapy in human breast cancer patients after treatment with antiestrogen⁴. HDACi have been found to restore sensitivity to the ER by modulation of the ER and PR. Several preclinical studies have supported the importance of HDAC inhibition as a potential target for treatment of endocrine insensitive breast cancer and potentially triple negative breast cancer cells lines. Keen et al showed that inhibition of DNA methylation with HDACi resulted in functional ER mRNA and protein expression⁸. Using ER-negative cell lines, MDA-231, MDA-MB-435, and Hs578t, they demonstrated restoration of ER function. Furthermore, there was significant inhibition of tumor and increased acetylation of H3 and H4 histones. They also demonstrated dose-dependent re-expression of the PR, which indicated that induced ER is functional. The xenograft mouse model MDA-MD-231 showed decreased tumor growth following treatment with the HDACi Trichostatin A (TSA) and the hypomethylating agent or methyltransferase inhibitor azacytidine.

Yang et al described re-expression of the silenced ER α induced by TSA in MDA-231 ER-negative cells and implicated histone deacetylation as a critical component of ER gene silencing in human breast cancer cells⁹. The use of HDACi to reactivate the silenced ER α gene demonstrated increased sensitivity of ER-negative cells to tamoxifen. It was also recognized that the ER α gene has a methylated promoter region in hormone-resistant disease and the re-expression of the ER gene is enhanced by treatment with an HDACi and a methyltransferase inhibitor, 5-aza-2'-deoxycytidine. Studies with panobinostat in MDA-231 and MDA-435 ER-negative breast cancer lines demonstrated that ER α was reactivated without demethylation of the ER promoter.

Zhou et al demonstrated that treatment with panobinostat restores ER mRNA and protein without demethylation of the CpG island at the ER promoter. The expression was sustained for at least 96 hours after withdrawal of panobinostat⁶. Panobinostat treatment led to release of DNMT1, HDAC1, and the H3 lysine 9 methyltransferase SUV39H1 from the ER promoter. Such changes caused an active chromatin formation manifested as accumulation of acetylated histone H3 and H4, a decrease in methylated H3-K9, and an impaired binding of heterochromatin protein1 at the promoter.

Kubo et al studied panobinostat in acquired aromatase inhibitor resistant breast cancer cell lines and tumors. He found that panobinostat abrogated growth of AI resistant cells in vitro and in vivo, causing cell cycle G2 arrest and induced apoptosis. This is due to the effects of panobinostat in decreasing levels of NF-kB1 in tumors^{6,7}.

Luu et al, in a phase II study of vorinostat in metastatic breast cancer, treated 14 patients with 200mg vorinostat orally twice daily for 14 days during each 21-day cycle. She observed the following side effects: fatigue, nausea, diarrhea, and lymphopenia. There were no complete or partial response by RECIST criteria; however, 4 had stable disease between 4–14 months¹⁰.

Munster in a phase II study of a combination of vorinostat and tamoxifen in 43 patients with metastatic ER positive breast cancer revealed a response rate of 19% and clinical benefit

(response and stable disease) of 40%. The median response duration was 10.3 months. Histone hyperacetylation and higher baseline HDAC2 levels correlated with response ¹¹.

In a Phase II, double blind, placebo-controlled study of exemestane with or without entinostat in postmenopausal women with metastatic, ER positive breast cancer progressing on aromatase inhibitors, Yardley found that combination entinostat and exemestane improved median progression survival from 2.3 months with exemestane to 4.3 with the entinostat combination ($P=.11$). An exploratory endpoint showed an improved median overall survival from 19 months to 28.1 months for the combination ($P=.036$) ¹². Based on this trial, a Phase III trial is ongoing to validate this data. All these studies show the clinical potential of HDAC inhibitors in patients with metastatic breast cancer.

Our study demonstrated that combination therapy of an aromatase inhibitor (letrozole) and the HDACi panobinostat is a potentially effective therapy in patients with MBC, especially on those considered to have endocrine and chemotherapy resistant disease. Seven of the 12 patients (six with ER-positive, but endocrine resistant, disease and one with ER/PR/HER2 negative disease) that participated in this trial had either a partial response (2/12 were ER-positive and had endocrine and chemotherapy resistant disease) or stabilization of disease upon therapy (5/12 [four with ER-positive and endocrine resistant disease and one with triple negative and chemotherapy resistant disease]). The duration of response in these pre-treated patients was encouraging, being 5.6 months for the patients with evaluable disease achieving stable disease, 5.1 months for the patient achieving a partial response, and 3.7 months for the patients with measurable disease also achieving stable disease.

Several observations and conclusions were made that will help future trials with panobinostat. The 20 mg three times weekly dose was tolerable without significant grade three toxicity. At this dose there were no CR or PR. Five patients had stable disease. However, the patients that had PR were treated at the 30 mg dose level. We propose that in future trials that patients be started on the 20 mg dose level if no significant toxicities are seen, the dose should be escalated to the 30 mg level. This combination is effective even in pretreated patients with aromatase inhibitors. Six patients had AI resistant disease. The PR were found in 2 patients with AI and chemotherapy resistant disease, one of them had 4 prior chemotherapy regimens. Four of the patients with stable disease had AI resistant and 1 had triple negative disease. This combination should be further tested in AI and chemotherapy resistant patients. Additionally, this should be tested in the triple negative patients since chemotherapy is the only option for these patients. A break from chemotherapy would always be welcomed since after multiple chemotherapy treatments significant toxicities are often noted. Ten of the patients had been treated with chemotherapy prior to entering the study.

Although tissue samples were planned to be collected it was optional. This was due to the fact that community physicians do not mandate follow-up biopsies on their patients on progression of disease.

Conclusion

Our Phase I study determined that the recommended starting dose for subsequent Phase II studies with this combination is panobinostat 20 mg per-oral three times weekly (MWF) and letrozole 2.5 mg per-oral daily. This dose should be escalated to 30 mg per-oral three times weekly if there is no grade 3 toxicity since the PR were found in patients with this dose. The encouraging responses observed in this study support further evaluation of this combination in MBC, in the endocrine resistant and triple negative populations.

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List of Abbreviations

AE	adverse events
DLT	dose-limiting toxicity
ER	estrogen receptor
ERα	ER alpha
HDACi	histone deacetylase inhibitors
HER2	human epidermal growth factor receptor 2
MBC	metastatic breast cancer
MTD	maximum tolerated dose
PR	progesterone receptor
TSA	Trichostatin A

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Clinical Practice Points

Histone deacetylase inhibitors are important in cancer pathogenesis. Panobinostat, a histone acetylase inhibitor, was recently approved as treatment for multiple myeloma that have tumor progression after immunomodulating agents and bortezomib. We tested the safety of the oral combination of panobinostat and letrozole and found a safe and tolerable dose in patients with metastatic breast cancer (MBC). The combination led to partial response in patients with prior aromatase inhibitor treatment. This drug regimen could be a potential, chemotherapy sparing, oral option for patients with recurrent MBC. Currently, there are multiple studies evaluating these agents in aromatase refractory and triple negative MBC patients. Additionally, targeting this novel pathway could potentially open the door for further testing of these drugs in other solid tumors.

Table 1

Baseline Patient Characteristics

	N=12
Age	
Median	63.5
Range	43–75
Female	12 (100%)
Performance Score	
0	10 (83%)
1	2 (17%)
Histologic Type	
Infiltrating Ductal	8 (67%)
Infiltrating Lobular	4 (33%)
Number of Mets *	
Median	2.5
Range	1–5
Prior Therapies	
No prior	2
Chemo and hormone	6
Chemotherapy	4
Hormone alone	0

* Most common were: bone (10), nodalaxillary (5), non-axillary nodal (4), lung (4), liver (3), chest wall (3)

Table 2

Outcomes Versus ER/PR/HER2 Status

Pt	Disease	Dose	ER	PR	HER2	DLT	Best Response
1	Non measurable	20 mg	+	+	Unknown	No	Stable
2	Non measurable	20 mg	+	–	+	No	Stable
3	Non measurable	20 mg	+	+	–	No	Stable
4	Measurable	30 mg	–	–	+	No	PD
5	Non measurable	30 mg	+	+	Unknown	No	Stable
6	Measurable	30 mg	–	–	–	Grade 3 Thrombocytopenia with bleeding	PD
7	Measurable	30 mg	+	+	+	Grade 4 Thrombocytopenia	PR
8	Measurable	30 mg	+	+	+	Grade 3 Diarrhea	PR
9	Measurable	30 mg	+	Unknown	+	No	PD
10	Measurable	20 mg	–	–	+	No	PD
11	Measurable	20 mg	–	–	–	No	Stable
12	Measurable	20 mg	–	–	+	Doubled Creatinine	PD

DLT: dose-limiting toxicity; ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; PR: progesterone receptor; Pt: patient

Table 3

Maximum Grade AE – 20 mg Patients (no of patients/percent of patients)

Toxicity	Grade 3	Grade 4	Grade 5	Total
Platelet count decreased	2 (33%)	0 (0%)	0 (0%)	5 (83%)
White blood cell decreased	0 (0%)	0 (0%)	0 (0%)	5 (83%)
Anemia	0 (0%)	0 (0%)	0 (0%)	4 (67%)
Neutrophil count decreased	2 (33%)	0 (0%)	0 (0%)	4 (67%)
Hypermagnesemia	0 (0%)	0 (0%)	0 (0%)	3 (50%)
Creatinine increased	0 (0%)	0 (0%)	0 (0%)	3 (50%)
Hypocalcemia	1 (17%)	0 (0%)	0 (0%)	2 (33%)
Aspartate aminotransferase increased	0 (0%)	0 (0%)	0 (0%)	1 (17%)
Bladder infection	1 (17%)	0 (0%)	0 (0%)	1 (17%)
Hyperkalemia	0 (0%)	0 (0%)	0 (0%)	1 (17%)
Hypokalemia	0 (0%)	0 (0%)	0 (0%)	1 (17%)
Hypomagnesemia	0 (0%)	0 (0%)	0 (0%)	1 (17%)
Hyponatremia	1 (17%)	0 (0%)	0 (0%)	1 (17%)
Peripheral sensory neuropathy	0 (0%)	0 (0%)	0 (0%)	1 (17%)
Abdominal pain	0 (0%)	0 (0%)	0 (0%)	1 (17%)
Fatigue	0 (0%)	0 (0%)	0 (0%)	1 (17%)

Table 4

Maximum Grade Adverse Events – 30 mg Patients (no of patients/percent of patients)

Toxicity	Grade 3	Grade 4	Grade 5	Grade 1–5 Total
Neutrophil count decreased	0 (0%)	1 (17%)	0 (0%)	6 (100%)
Platelet count decreased	1 (17%)	1 (17%)	0 (0%)	6 (100%)
White blood cell decreased	1 (17%)	0 (0%)	0 (0%)	4 (67%)
Hypocalcemia	0 (0%)	0 (0%)	0 (0%)	4 (67%)
Creatinine increased	0 (0%)	0 (0%)	0 (0%)	4 (67%)
Anemia	0 (0%)	0 (0%)	0 (0%)	2 (33%)
Blood bilirubin increased	0 (0%)	0 (0%)	0 (0%)	2 (33%)
Hypercalcemia	0 (0%)	0 (0%)	0 (0%)	2 (33%)
Hyperkalemia	0 (0%)	0 (0%)	0 (0%)	2 (33%)
Hypermagnesemia	0 (0%)	0 (0%)	0 (0%)	2 (33%)
Hypokalemia	0 (0%)	0 (0%)	0 (0%)	2 (33%)
Hypomagnesemia	0 (0%)	0 (0%)	0 (0%)	2 (33%)
Hyponatremia	1 (17%)	1 (17%)	0 (0%)	2 (33%)
Fatigue	0 (0%)	0 (0%)	0 (0%)	2 (33%)
Nausea	0 (0%)	0 (0%)	0 (0%)	2 (33%)
Alopecia	0 (0%)	0 (0%)	0 (0%)	1 (17%)
Hypothyroidism	0 (0%)	0 (0%)	0 (0%)	1 (17%)
Anorexia	1 (17%)	0 (0%)	0 (0%)	1 (17%)
Diarrhea	1 (17%)	0 (0%)	0 (0%)	1 (17%)
Dysgeusia	0 (0%)	0 (0%)	0 (0%)	1 (17%)
Vomiting	0 (0%)	0 (0%)	0 (0%)	1 (17%)