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Phase Ib Study of the Oral Proteasome Inhibitor Ixazomib (MLN9708) and Fulvestrant in Advanced ER+ Breast Cancer Progressing on Fulvestrant

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Key Words. Breast cancer • Anti-estrogen • Endocrine therapy • Proteasome inhibitor

Trial Information

- **ClinicalTrials.gov Identifier**: NCT02384746
- **Sponsor**: Dartmouth-Hitchcock Medical Center
- **Principal Investigators**: Todd Miller, Gary Schwartz
- **IRB Approved**: Yes

Lessons Learned

- Fulvestrant is a selective estrogen receptor (ER)-downregulating antiestrogen that blocks ER transcriptional activity and is approved for ER-positive breast cancer.
- Fulvestrant also induces accumulation of insoluble ER and activates an unfolded protein response; proteasome inhibitors have been shown to enhance these effects in preclinical models.

Abstract

Background. Fulvestrant is a selective estrogen receptor (ER)-downregulating antiestrogen that blocks ER transcriptional activity and is approved for ER-positive (+) breast cancer. Fulvestrant also induces accumulation of insoluble ER and activates an unfolded protein response; proteasome inhibitors have been shown to enhance these effects in preclinical models.

Methods. This is a single-center phase Ib study with a 3+3 design of fulvestrant and the proteasome inhibitor ixazomib (MLN9708) in patients with advanced ER+ breast cancer that was progressing on fulvestrant. A dose-escalation design allowed establishment of the ixazomib maximum tolerated dose (MTD). Secondary objectives included progression-free survival, pharmacokinetics, and tumor molecular analyses.

Results. Among nine evaluable subjects, treatment was well-tolerated without dose-limiting toxicities The MTD of ixazomib was 4 mg in combination with fulvestrant. Plasma concentrations of the active form of ixazomib (MLN2238) in the 4-mg dose cohort had a median (range) maximal concentration (C_{max}) of 155 (122–171) ng/mL, time of maximal concentration (T_{max}) of 1 (1–1.5) hour, terminal elimination half-life of 66.6 (57.3–102.6) hour after initial dose, and area under the curve (AUC) of 5,025 (4,160–5,345) ng*h/mL. One partial response was observed, and median progression-free survival was 51 days (range, 47–137).

Conclusion. This drug combination has a favorable safety profile and antitumor activity in patients with fulvestrant-resistant advanced ER+ breast cancer that justifies future testing. *The Oncologist* 2021;26:467–e924

Discussion

Approved therapeutics for the treatment of ER-positive breast cancer include antiestrogens that target ER, such as the selective ER downregulator fulvestrant. Unfortunately, treatment responses to antiestrogen therapy for metastatic disease are unpredictable, and approximately half of these patients experience disease progression within 6 months [1–6]. The mechanism of fulvestrant action involves antagonizing ER transcriptional activity, inducing degradation of nuclear ER via the proteasome, and inducing the formation of cytoplasmic aggregates containing ER [7–9]. The accumulation of these aggregates invokes a generalized unfolded protein response
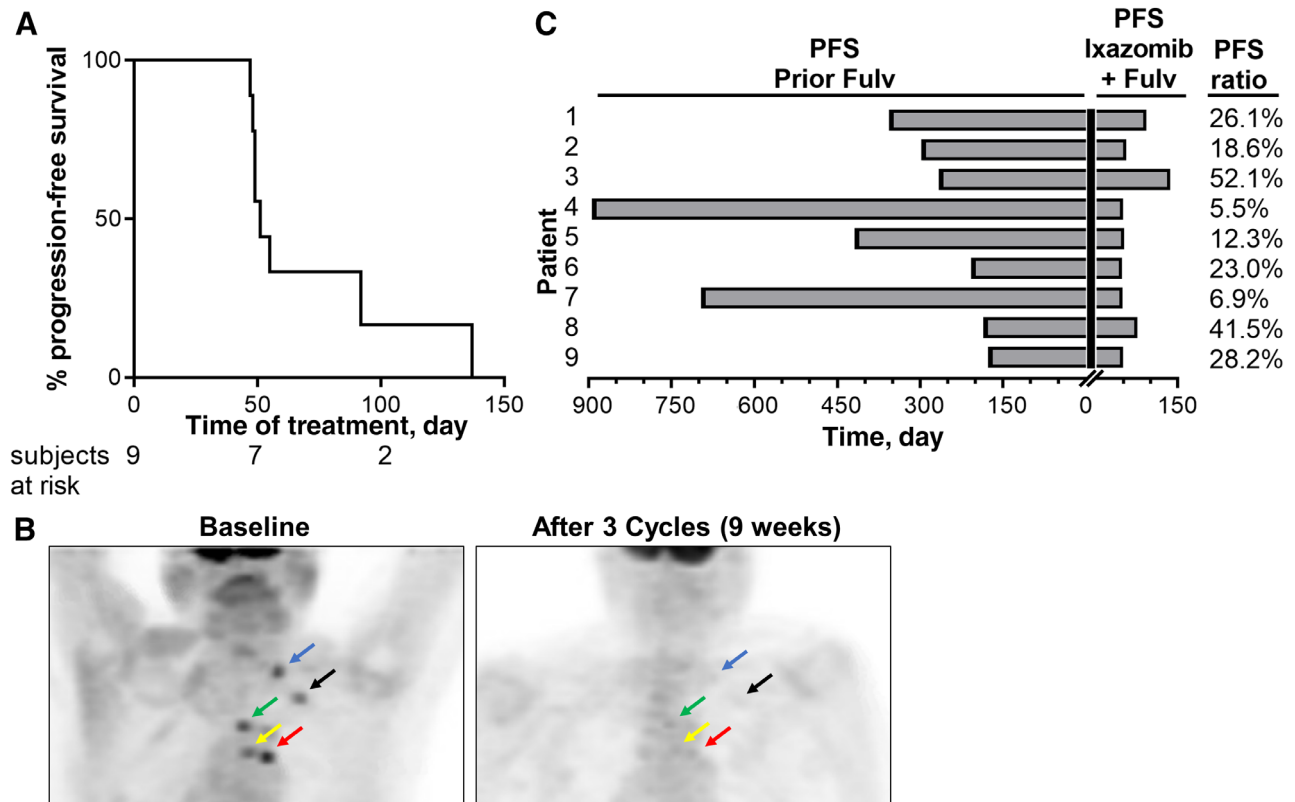
Figure 1. Progression-free survival and response to ixazomib plus fulvestrant. **(A):** Distribution of PFS for patients treated with fulvestrant plus ixazomib. **(B):** The addition of ixazomib induced a partial tumor response in a subject with fulvestrant-resistant breast cancer. Positron emission tomography imaging results are shown from baseline and after 3 cycles of ixazomib (4 mg) plus fulvestrant. Arrows indicate corresponding tumor locations. **(C):** Comparison of PFS on prior fulvestrant regimen to fulvestrant/ixazomib regimen.
Abbreviations: Fulv, fulvestrant; PFS, progression-free survival.

that can lead to DNA fragmentation and apoptosis [10]. However, proteasome activity may contribute to resistance to fulvestrant by clearing ER aggregates, thereby protecting cancer cells from death. Therefore, inhibition of proteasomal protein degradation represented a logical therapeutic opportunity to test in combination with fulvestrant [11, 12].

At the time this study was initiated, the oral proteasome inhibitor ixazomib (MLN9708) was in phase III testing in combination with lenalidomide and dexamethasone for multiple myeloma; this combination was subsequently U.S. Food and Drug Administration approved. We conducted a phase Ib trial to test the safety and efficacy of the novel combination of fulvestrant and ixazomib in patients with advanced ER+ breast cancer progressing on fulvestrant. No dose-limiting toxicities were observed during cycle 1. This study fulfilled its primary objective of establishing a safety profile for ixazomib and identifying a maximum tolerated dose of 4 mg on days 1, 4, 8, and 11 in a 21-day cycle when combined with fulvestrant. The ixazomib plasma pharmacokinetic profile was generally consistent with prior findings [13]. The addition of ixazomib in patients with disease progression on fulvestrant resulted in a delay of disease progression (progression-free survival; PFS) in all nine patients by a median of 51 days (maximum 137 days; Fig. 1A). One patient had a partial response by RECIST version 1.1 (Fig. 1B); this patient had previously received four lines of therapy. Comparison of PFS on study treatment with ixazomib plus fulvestrant to that on single-agent fulvestrant as the prior line of therapy revealed PFS ratios ranging from 0.05 to 0.52 (Fig. 1C), indicating that PFS on study treatment was shorter than PFS on the prior line of (fulvestrant) therapy in all cases.

TRIAL INFORMATION	
Disease	Breast cancer
Stage of Disease/Treatment	Metastatic/advanced
Prior Therapy	No designated number of regimens
Type of Study	Phase I, 3+3
Primary Endpoint	Maximum tolerated dose
Secondary Endpoints	Safety, tolerability, pharmacodynamics, correlative endpoint, efficacy

Additional Details of Endpoints or Study Design

Eligibility criteria: Patients were required to be postmenopausal with histologically confirmed ER-positive, HER2-negative metastatic or locally advanced breast cancer. Patients were required to have a favorable performance status (ECOG 0–1) without significant hematologic, hepatic, and renal impairment as measured by laboratory measurements, as well as cancer that was progressing by RECIST 1.1 criteria while taking fulvestrant for ≥56 days as the most recent line of treatment.

Adverse event severity was graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Dose-limiting toxicity was defined as any grade 3 or higher nonhematologic toxicity or any grade 4 or higher hematologic toxicity during cycle 1 that was considered related to ixazomib.

Safety (adverse events and hematological/chemistry laboratory parameters) and physical status (including ECOG performance status) were assessed at baseline and at the end of each 21-day cycle. Adverse event severity was graded using the NCI CTCAE version 4.03. Dose-limiting toxicity was defined as any grade 3 or higher nonhematologic toxicity or any grade 4 or higher hematologic toxicity during cycle 1 that was considered related to ixazomib. All patients underwent computed tomography (CT) scanning of the chest, abdomen, and pelvis and a bone scan, or a positron emission tomography-CT scan within 21 days of study entry. Disease reassessments were repeated with the same imaging modality approximately every 6 weeks on study. All lesions measurable by radiologic study or physical exam, up to a maximum of five, were identified as "target lesions" and measured at baseline and after each imaging session. Tumor response was assessed per RECIST criteria v1.1. Clinical benefit was defined as complete response, partial response, or stable disease for ≥24 weeks. PFS was calculated. PFS ratio was then calculated as (PFS on ixazomib + fulvestrant)/(PFS on prior line of fulvestrant).

Investigator's Analysis	Active and should be pursued further

DRUG INFORMATION

Ixazomib	
Generic/Working Name	Ixazomib
Trade Name	Ninlaro
Drug Type	Small molecule
Drug Class	Proteasome
Dose	2.3, 3, or 4 mg per flat dose
Route	oral (po)
Schedule of Administration	Days 1, 4, 8, and 11 of a 21-day cycle [19, 20]
Fulvestrant	
Generic/Working Name	Fulvestrant
Trade Name	Faslodex
Drug Type	Small molecule
Drug Class	Estrogen receptor
Dose	500 mg per flat dose
Route	Intramuscular
Schedule of Administration	Day 1 of a 28-day cycle

PATIENT CHARACTERISTICS

Number of Patients, Male	0
Number of Patients, Female	9
Stage	IV
Age	Median (range): 60 (37–67) years
Number of Prior Systemic Therapies	Median (range): 5 (2–6)
Performance Status: ECOG	0 — 6 1 — 3 2 — 0 3 — 0 Unknown — 0
Other	In addition to fulvestrant, all patients had received other prior endocrine therapy, and four of nine subjects had received prior chemotherapy. Although bone was the most common site of metastatic disease (8/9 subjects), all had accompanying soft-tissue disease.

Baseline characteristic	Subjects, n (%)
Median age (range), yr	60 (37–67)
ECOG performance status	
0	6 (67)
1	3 (33)
Receptor status (%)	
ER-positive	9 (100)
PR-positive	9 (100)
HER2-positive	0 (0)
Sites of metastatic disease	
Bone	8 (89)
Liver	6 (67)
Lung or pleura	3 (33)
Lymph nodes	2 (22)
Omentum	1 (11)
Lines of endocrine therapy in the metastatic setting	
0–1	0 (0)
2	3 (33)
3	3 (33)
4	1 (11)
5	2 (22)
Prior endocrine therapies in the metastatic setting	
Aromatase inhibitor(s)	9 (100)
Tamoxifen	4 (44)
Fulvestrant	9 (100)
Everolimus	1 (11)
Palbociclib	4 (44)
Abemaciclib	2 (22)
Lines of chemotherapy in metastatic setting	
0	5 (56)
1	2 (22)
2	1 (11)
>2	1 (11)

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.

PRIMARY ASSESSMENT METHOD

Title	Maximum tolerated dose
Number of Patients Screened	9
Number of Patients Enrolled	9
Number of Patients Evaluable for Toxicity	9
Number of Patients Evaluated for Efficacy	9
Evaluation Method	Adverse events
Response Assessment PR	*n* = 1 (11%)
Response Assessment PD	*n* = 8 (88%)
(Median) Duration Assessments PFS	51 Days, CI: 20

Outcome Notes

We performed a 3+3 dose-escalation trial to assess the safety and efficacy of fulvestrant with three dose cohorts of oral ixazomib: 2.3 mg (cohort A), 3 mg (cohort B), and 4 mg (cohort C). Subjects were treated with fulvestrant (500 mg) by intramuscular injection once every 28 days starting on day 1. Subjects were treated with ixazomib orally on days 1, 4, 8, and 11 of a 21-day cycle based on prior clinical studies [19, 20]. The dose of ixazomib started at 2.3 mg, slightly greater than 50% of the phase III dose in another

ongoing study at the time (NCT01850524). MLN2238 is the biologically active, boronic acid form of ixazomib citrate; in aqueous systems, the equilibrium shifts from ixazomib citrate to MLN2238. Plasma MLN2238 (ixazomib) concentration versus time profiles were determined [21] during cycle 1 day 1 through cycle 2 day 1. Subjects with visceral or soft-tissue disease had a tumor biopsy prior to treatment with ixazomib to confirm ER, progesterone receptor (PR), and HER2 status and to provide a baseline specimen for molecular analysis. When safely accessible, a biopsy of the same tumor was obtained on cycle 1 day 11 after dosing with ixazomib. Treatments continued without interruption until disease progression, severe or intolerable toxicity, or participant withdrawal of consent.

ADVERSE EVENTS

Name	NC/NA	1	2	3	4	5	All grades
Anemia	67%	0%	11%	22%	0%	0%	33%
Leukocytes (total WBC)	78%	0%	22%	0%	0%	0%	22%
Lymphopenia	89%	0%	11%	0%	0%	0%	11%
Neutrophil count decreased	78%	0%	22%	0%	0%	0%	22%
Platelets	56%	0%	0%	22%	22%	0%	44%
Anorexia	89%	0%	11%	0%	0%	0%	11%
Arthralgia	78%	0%	22%	0%	0%	0%	22%
Cough	78%	0%	22%	0%	0%	0%	22%
Dizziness	89%	0%	11%	0%	0%	0%	11%
Dysgeusia	89%	0%	11%	0%	0%	0%	11%
Dyspepsia	89%	0%	11%	0%	0%	0%	11%
Dyspnea (shortness of breath)	67%	0%	22%	11%	0%	0%	33%
Alanine aminotransferase increased	89%	0%	11%	0%	0%	0%	11%
Aspartate aminotransferase increased	89%	0%	0%	11%	0%	0%	11%
Blood bilirubin increased	89%	0%	11%	0%	0%	0%	11%
Fatigue (asthenia, lethargy, malaise)	78%	0%	22%	0%	0%	0%	22%
Hot flashes/flushes	89%	0%	11%	0%	0%	0%	11%
Hypertension	33%	0%	67%	0%	0%	0%	67%
Hypotension	89%	0%	11%	0%	0%	0%	11%
Hypoxia	89%	0%	0%	11%	0%	0%	11%
Heart failure	89%	0%	11%	0%	0%	0%	11%
Lip infection	89%	0%	11%	0%	0%	0%	11%
Nausea	78%	0%	11%	11%	0%	0%	22%
Pulmonary hypertension	89%	0%	0%	11%	0%	0%	11%
Rash	78%	0%	11%	11%	0%	0%	22%
Right ventricular dysfunction	89%	0%	11%	0%	0%	0%	11%
Insomnia	89%	0%	11%	0%	0%	0%	11%

Cycle 1 toxicities in any dose level.
Abbreviations: NC/NA, no change from baseline/no adverse events; WBC, white blood cell.

PHARMACOKINETICS/PHARMACODYNAMICS

Dose level	Dose of drug: Ixazomib	Dose of drug: fulvestrant	Number enrolled	C_{max} (ng/mL) mean ± SD	T_{max}(h) (min - max)	AUC_{0-12} (ng*h/mL) mean ± SD	$T \frac{1}{2}$(h) mean ± SD
A	2.3 mg	500 mg	3	50.5 ± 25.8	0.5–2	1978.8 ± 461.6	160.2 ± 48.0
B	3 mg	500 mg	3	61.5 ± 34.3	1–2	2,507.1 ± 890.4	101.3 ± 26.2
C	4 mg	500 mg	3	149.3 ± 25.0	1–1.5	5,459.4 ± 20.6	75.5 ± 23.9

Abbreviations: AUC, area under the curve; C_{max}, maximal concentration; T_{max}, time of maximal concentration.

ASSESSMENT, ANALYSIS, AND DISCUSSION

Completion	Did not fully accrue
Investigator's Assessment	Active and should be pursued further

This phase Ib study investigated the safety and efficacy of ixazomib in combination with fulvestrant and revealed no dose-limiting toxicities (DLTs) during cycle 1. This study fulfilled its primary objective of establishing a safety profile for ixazomib and identifying a maximum tolerated dose of 4 mg p. o. on days 1, 4, 8, and 11 in a 21-day cycle when combined with fulvestrant in patients with fulvestrant-resistant metastatic breast cancer. The most common grade 3/4 treatment-related adverse event was thrombocytopenia, which occurred in four subjects (lasting one cycle each) in a dose-dependent manner and is consistent with reports of ixazomib use [20, 22, 23]. Other observed grade 2–4 adverse events included anemia (n = 3 subjects), nausea (n = 2), hypertension (n = 6 subjects), dyspnea (n = 3), cough (n = 2), pulmonary hypertension (n = 1), and pneumonia (n = 1). Pulmonary adverse events may also be related to treatment with fulvestrant. For example, the CONFIRM trial found that fulvestrant (500 mg) was associated with cough and dyspnea in 19 of 361 (5.3%) and 16 of 361 (4.4%) of patients, respectively [24].

Three patients were enrolled into each of the 2.3-mg, 3-mg, and 4-mg ixazomib dose cohorts. No DLTs were observed; thus, the maximum tolerated dose of ixazomib is 4 mg on the schedule used herein in combination with fulvestrant. The adverse event profile among all subjects (grade $\geq$ 2) is shown in Table 2. Grade 3 or 4 hematologic adverse events included anemia (in 2 subjects) and thrombocytopenia (in 4 subjects). Grade 3 or 4 nonhematologic adverse events included dyspnea, hypoxia, pulmonary hypertension, rash, nausea, and elevated aspartate aminotransferase. Notably, six of nine subjects developed grade 2 hypertension, suggesting that ixazomib plus fulvestrant may elicit cardiovascular complications with longer-term treatment. Pulmonary hypertension was diagnosed in one subject in the 2.3-mg ixazomib dose cohort with metastatic breast cancer in lung and axillary lymph nodes. She had a history of bilateral pulmonary emboli diagnosed 10 months prior to study enrollment, and she was prescribed the anticoagulant enoxaparin. At the time of enrollment, she had stable exertional dyspnea and a chronic cough. She was diagnosed with secondary pulmonary hypertension while on study. Imaging showed segmental and subsegmental pulmonary emboli in the lung; the pulmonary emboli and resultant pulmonary hypertension were ascribed to her underlying thromboembolic disease and metastatic breast cancer, and thought to be unrelated to fulvestrant or ixazomib. Pleural effusions and pneumonia were diagnosed in one subject in the 4-mg cohort with metastatic breast cancer in the brain and lymph nodes. She was hospitalized after 7 weeks of study treatment for hypoxemia secondary to bilateral pleural effusions and pneumonia. Her hypoxemia resolved with diuresis and antibiotics, and she came off study 3 weeks later. The development of pleural effusions and pneumonia in this patient were thought to possibly be due to treatment with fulvestrant and ixazomib. Grade 4 thrombocytopenia was seen in 2 subjects in the 4-mg cohort. The first subject experienced a drop in platelet counts to 7,000/uL, which required platelet transfusion on cycle 2 day 10. The second subjected experienced a drop in platelet counts to 17,000/uL, which required platelet transfusion on cycle 2 day 14

The ixazomib pharmacokinetic disposition profile (Fig. 2) showed a median T_{max} of 1 h, which indicated rapid absorption and is consistent with prior findings [13]. The ixazomib median elimination half-lives in the 2.3-mg, 3-mg, and 4-mg dose cohorts were 148, 97, and 67 h, respectively. The overall ixazomib exposure, as determined by the area under the curve (AUC)$_{(last)}$, was found to increase with dose, with median values of 1,236, 1,957, and 5,025 ng/mL per hour in the 2.3-mg, 3-mg, and 4-mg cohorts, respectively. The elimination half-life and the AUC$_{(last)}$ values were similar to those previously reported for ixazomib monotherapy administered twice weekly when pharmacokinetic data were analyzed using noncompartmental analysis [25]. However, the elimination half-life values in this study were shorter than the cited mean elimination half-life of 9.5 days (228 hours) [13, 26]. This difference in observed terminal elimination half-life was most likely due to our inability to fit a multicompartment model to the plasma ixazomib concentration vs. time data in our study, and our limited blood sampling time points. Pharmacodynamic studies showed potent inhibition of proteasome activity in blood following 11 days of combination treatment at all dose levels studied (Fig. 3). Sufficient tumor tissue in biopsy specimens from both baseline and Day 11 time points was available from six study subjects for Ki67 IHC, and from four subjects for ER IHC and TUNEL studies. Significant changes between baseline and Day 11 data were not observed, but TUNEL studies showed a trend of decreasing proportion of apoptotic cells following ixazomib treatment (p=.094; Fig. 4).

Despite encouraging preclinical data, clinical studies of proteasome inhibitors in solid tumors including breast cancer have thus far failed to demonstrate efficacy [27]. Engel et al. reported the results of a phase II trial of single-agent bortezomib in subjects with metastatic breast cancer, and they saw no objective responses among 12 evaluable subjects [28]. Trinh et al. reported on a phase I study in which subjects with hormone receptor-positive metastatic breast cancer received the combination of bortezomib and an aromatase inhibitor (8 subjects), or bortezomib and tamoxifen (1 subject). There were no objective responses, but two of the nine subjects experienced stable disease for $\geq$15 weeks [29]. Based on these results, the activity of single-agent ixazomib or the combination of ixazomib with an aromatase inhibitor or tamoxifen in metastatic breast cancer was hypothesized to be very low. Alternatively, the addition of proteasome inhibitors to fulvestrant has been shown to increase apoptosis in breast cancer cell lines and mouse xenografts [11, 12], leading to the initiation of clinical testing of this combination in breast cancer. Adelson et al. reported the results of a randomized phase II trial comparing bortezomib plus fulvestrant to fulvestrant alone, and showed an improved 12-month progression-free survival (PFS; 28.1% vs. 13.6%; p = .03) [18].

Although efficacy was not a primary endpoint of our study, we found that the addition of ixazomib in patients with disease progressing on fulvestrant resulted in a delay of disease progression in all patients by a median of 51 days, up to as many as 137 days. Furthermore, one patient had a partial response by RECIST version 1.1. Of note, this patient had previously received 3 lines of endocrine therapy including letrozole plus palbociclib, single-agent anastrozole, and single-agent fulvestrant, as well as

chemotherapy with docetaxel and cyclophosphamide. Although the overall rate of clinical benefit (11%) of ixazomib in combination with fulvestrant observed in this study is modest, it is worth noting that this patient population has disease resistant to multiple agents with recent progression on fulvestrant. This is unlike the majority of clinical trials for fulvestrant-containing regimens in advanced breast cancer, which typically involve patients who have progressed on a single endocrine agent such as an aromatase inhibitor and have not had previous exposure to fulvestrant. Additionally, four of nine (44%) subjects in our study population had disease that progressed on combination therapy with palbociclib and an endocrine agent, which is now considered a standard regimen for advanced endocrine-resistant hormone receptor-positive disease. Based on our observation of a response in one subject in our study population, and a PFS in one subject of 137 days in the lowest dose cohort studied, ixazomib with fulvestrant in fulvestrant-naive patients with advanced endocrine-resistant disease may be considered for testing by other investigators.

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A

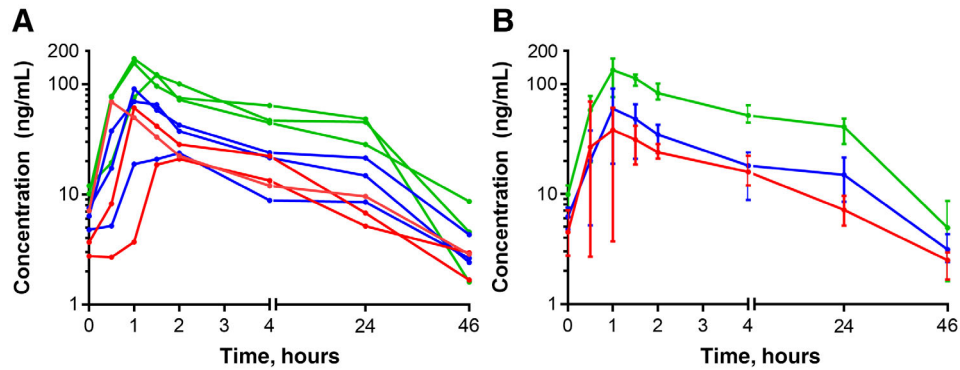**B**

Figure 2. Log plasma ixazomib (MLN2238) concentration versus time profiles. Individual **(A)** and cohort-averaged **(B)** pharmacokinetic data for 9 subjects. In **(B)**, data are shown as mean $\pm$ range. Pre-time point was sampled prior to start of study treatment. Other time points were sampled after the cycle 1 day 11 dose of ixazomib.
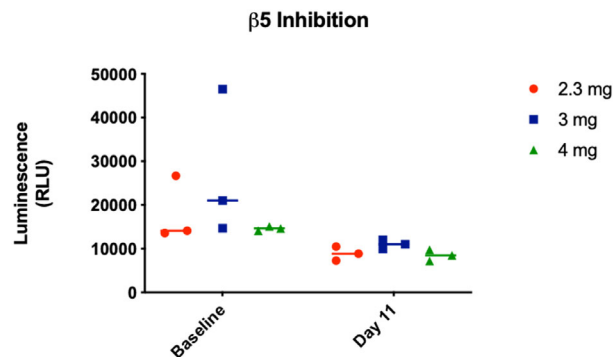
β5 Inhibition

Figure 3. Ixazomib suppresses blood 20S proteasome activity. Whole-blood samples acquired at baseline and on cycle 1 day 11 at 2 hours postdosing were analyzed by β5 chymotrypsin-like proteasome activity assay. Although proteasome activity was widely variable at baseline, postixazomib samples showed a significant decrease of proteasome activity compared with baseline (Fig. 2; two-way analysis of variance [ANOVA] time effect p = .026). The postixazomib samples showed residual proteasome activity, which was not significantly different between dose cohorts, suggesting that maximal proteasome inhibition in blood was achieved with the lowest dose (2.3 mg) of ixazomib in this study. Luminescence per sample is shown according to dose cohort. Data were analyzed by two-way ANOVA followed by Tukey-adjusted comparisons between dose cohorts, which revealed only a statistically significant effect of time on proteasome activity.
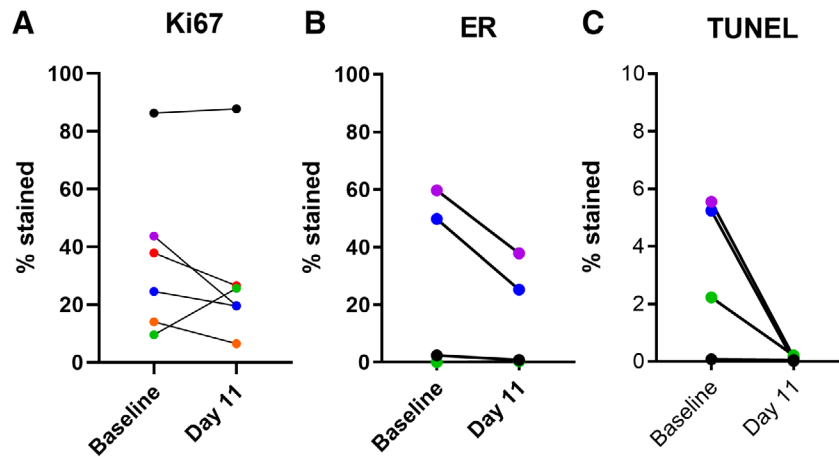Abbreviation: RLU, relative luminescence unit.

Figure 4. Immunohistochemical (IHC) analysis of paired baseline and cycle 1 day 11 tumor tissue specimens. Sufficient tumor tissue in biopsy specimens from both baseline and day 11 time points was available from six study subjects for Ki67 IHC and from four subjects for ER IHC and TUNEL studies. IHC scoring of Ki67 **(A)** and ER **(B)**, and TUNEL scoring **(C)** from tumor specimens obtained at baseline or on cycle 1 day 11. Tissue specimens are color-coded by subject across panels. Significant changes between baseline and day 11 data were not observed, but TUNEL studies showed a trend of decreasing proportion of apoptotic cells following ixazomib treatment (paired t test p = .094). Tumor specimens from one subject were ER-negative despite this subject having a history of ER+ metastatic disease.
Abbreviations: ER, estrogen receptor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

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