Title

A randomized, open-label, pre-surgical, window of opportunity study comparing the pharmacodynamic effects of the novel oral SERD AZD9496 with fulvestrant in patients with newly diagnosed ER+ HER2- primary breast cancer.

Authors and affiliations

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Running title

Pre-surgical study of the novel oral SERD AZD9496 in ER+ BC

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Notes about the manuscript

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Translational relevance statement

Endocrine therapy is highly effective and the mainstay treatment for estrogen receptor positive (ER+) breast cancer; unfortunately, resistance inevitably occurs. Fulvestrant is the first-in-class selective estrogen receptor degrader (SERD), and is clinically effective in both endocrine naïve- and resistant- ER+ breast cancers. At present, fulvestrant is injected intramuscularly with an approved dose of 500 mg. Orally bioavailable SERDs may achieve greater exposure and anti-ER degrading activity than fulvestrant, which may translate into improved clinical outcomes. In this pre-surgical, window of opportunity study, the novel oral SERD AZD9496 reduced ER, progesterone receptor and Ki-67 expression, and is the first to show that an oral SERD is able to impact its key biological targets in this setting. While AZD9496 250 mg twice daily was not superior to fulvestrant 500 mg, pre-surgical studies represent an important assessment of the proof of mechanism of novel SERDs in early clinical development.

Word count: 150/150

Abstract

Purpose: Fulvestrant, the first-in-class selective estrogen receptor (ER) degrader (SERD), is clinically effective in patients with ER+ breast cancer, but it has administration and pharmacokinetic (PK) limitations. Pharmacodynamic (PD) data suggests complete ER degradation is not achieved at fulvestrant's clinically feasible dose. This pre-surgical study (NCT03236974) compared the PD effects of fulvestrant with AZD9496, a novel, orally bioavailable, non-steroidal, potent SERD, in treatment-naive patients with ER+ human epidermal growth factor receptor 2 negative primary breast cancer awaiting curative intent surgery.

Methods: Patients were randomized 1:1 to receive AZD9496 250 mg twice daily from Day (D) 1 for 5–14 days, or fulvestrant 500 mg on D 1. On-treatment imaging-guided core tumor biopsies were taken between D 5–14 and compared with pre-treatment diagnostic biopsies. The primary objective was to compare the effects of AZD9496 and fulvestrant on ER expression. Secondary objectives included changes in progesterone receptor (PR) and Ki-67 PK/PD relationships and safety.

Results: Forty-six women received treatment (AZD9496 n=22; fulvestrant n=24); 35 paired biopsies were evaluable (AZD9496 n=15; fulvestrant n=20). The least square mean estimate for ER H-score reduction was 24% after AZD9496 *versus* 36% after fulvestrant treatment (p=0.86). AZD9496 also reduced PR H-scores (-33.3%) and Ki-67 levels (-39.9%) from baseline, but was also not superior to fulvestrant (PR: -68.7%, p=0.97; Ki-67: -75.4%, p=0.98). No new safety findings were identified.

Conclusion: This was the first pre-surgical study to demonstrate that an oral SERD impacts its key biological targets. However, AZD9496 was not superior to fulvestrant at the dose tested.

Word count: 250/250

Introduction

Approximately 75% of breast cancers are estrogen receptor positive (ER+), 60% of which are also progesterone receptor (PR+).[1,2] Endocrine therapy is highly effective and represents the mainstay treatment of ER+ breast cancers.[3] Primary and secondary resistance occur in a high proportion of patients, which ultimately limits the use of these agents.[4] Despite resistance to one or more endocrine therapies, tumors continue to depend on ER activity for growth.[5,6] Therefore, ER remains an important target in the endocrine-resistant setting, emphasizing the need for more effective endocrine treatments.

Fulvestrant is the first-in-class SERD and was the first ER targeting agent to be described as a pure anti-estrogen, referring to its lack of agonism in all ER+ tissues.[7] Fulvestrant is clinically effective in patients with ER+ breast cancer, both naive and resistant to endocrine therapy;[8-13] it has been shown to be effective in patients who have disease progression after receiving tamoxifen (a selective estrogen receptor modulator [SERM]) therapy in Phase 3 trials;[8,9] other SERMs were cross-resistant to tamoxifen despite promising Phase 2 results.[14,15] Fulvestrant is also effective after third-generation aromatase inhibitors (AIs),[10,12,16] and is more efficacious than these agents in the first-line setting in patients naive to endocrine treatment, both in terms of progression-free survival (PFS; FIRST[17] and FALCON[18] studies) and overall survival (OS; FIRST[19]). SERDs may also represent a more efficacious therapeutic option in the adjuvant setting as compared with tamoxifen and third-generation AIs; however, this remains to be proven in prospective randomized trials.

Fulvestrant has low oral bioavailability and is administered via intramuscular (IM) injection. The clinically approved dose of fulvestrant is 500 mg administered monthly as two 250 mg 5 mL IM injections, with a loading dose on D 15 of the first cycle. Observations from fulvestrant studies suggest that its maximum clinical efficacy and biomarker impact may not have been achieved even at this dose. In a pre-surgical, window of opportunity study comparing three doses of fulvestrant (50 mg, 125 mg, and 250 mg), tamoxifen 20 mg, and placebo, all doses of fulvestrant were associated with dose-dependent reductions in ER and Ki-67 expression compared with placebo. ER reduction was significantly greater than tamoxifen at the fulvestrant 250 mg dose, and numerically but not statistically greater with fulvestrant dosed at 250 mg compared with 125 mg (59% *vs* 50%).[13] Higher doses of fulvestrant have been explored; fulvestrant 750 mg IM monthly was effective at reducing proliferation in pre-menopausal women with ER+ breast cancer,[20] and fulvestrant 1,000 mg monthly (500 mg IM dosed on D 1, 8, and 15 of a 28-day cycle, and on D 1 and 15 thereafter) is currently being investigated in the plasmaMATCH trial (NCT03182634).

In the neoadjuvant NEWEST trial, the degree of ER degradation in tumors was greater in early breast cancer patients receiving 4 weeks of fulvestrant 500 mg treatment (dosed on D 0, 14, and 28, and every 28 days thereafter) compared with 250 mg (dosed on D 0 and 28, and every 28 days thereafter; -50.3% *vs* -13.7%; $p<0.0001$).[21] The dose-dependent pharmacodynamic (PD) effects of fulvestrant shown in this study were echoed in the larger clinical efficacy Phase 3 study (CONFIRM) in the advanced disease setting, where fulvestrant 500 mg (dosed on D 0, 14, and 28, and every 28 days thereafter) was superior to fulvestrant 250 mg (dosed every 28 days) with respect to PFS and OS.[16,22] In addition to ER, reductions in Ki-67 levels in the neoadjuvant setting have also shown to be predictive of long-term clinical efficacy of endocrine therapies in early breast cancer (IMPACT[23] and ATAC[24] trials, among others).

AZD9496 is an orally bioavailable, non-steroidal, selective and potent ERα degrader and ER antagonist[25] that has shown anti-tumor activity in both endocrine-sensitive and -resistant models.[26] In an HCC1428 long-term estrogen-deprived breast model, which is independent of estrogen for growth and as such represents a model of AI resistance, AZD9496 caused tumor regression and significant ERα degradation.[27]

In a Phase 1 dose-escalation, dose-expansion study of 45 patients (NCT02248090), AZD9496, dosed up to 600 mg twice daily (BID) in heavily pre-treated patients with ER+/human epidermal growth factor receptor 2 negative (HER2-) advanced breast cancer, was well tolerated. Six patients (three of whom had an *ESR1* mutation) experienced prolonged disease stabilization (defined as progression-free survival of more than 52 weeks), and one patient, who received AZD9496 250 mg BID, had a confirmed partial response.

The current study was designed to assess and compare the effects of AZD9496 and fulvestrant after short-term administration on PD biomarkers ERα, PR, and Ki-67 in treatment-naive patients with ER+ HER2- primary breast cancer awaiting surgery of curative intent. The study also assessed the pharmacokinetics (PK) of AZD9496 and fulvestrant on the day of biopsy, associated PK/PD relationships, and the safety and tolerability of AZD9496 compared with fulvestrant.

Patients and methods

Study design and patients

In this open-label, randomized, multicenter pre-surgical trial (NCT03236974), patients were randomized 1:1 to receive either AZD9496 250 mg (BID orally for 5–14 days commencing on D 1 and continuing up to and including the day of the on-treatment biopsy) or fulvestrant 500 mg (administered as two 5 mL IM injections on D 1). The residual core-cut biopsy sample taken as a standard hospital diagnostic procedure was used as the pre-treatment tumor tissue comparator for each patient if taken up to 6 weeks prior to starting study treatment (D 1). A new pre-treatment biopsy was taken if diagnostic biopsies were of insufficient quality or taken more than 6 weeks prior to starting study treatment. After 5–14 days of study treatment (and approximately 2 hours after the last AZD9496 dose), up to three core-cut, on-treatment, imaging-guided biopsy samples were taken, either at the time of definitive surgery or at a separate visit prior to surgery. The 5- to 14-day window for surgery was considered adequate to allow fulvestrant plasma concentrations after the single 500 mg dose to be within the C_{max} and C_{min} observed when fulvestrant 500 mg is at steady state when administered using the standard therapeutic regimen in patients with advanced breast cancer.[28,29] Steady state exposure of AZD9496 is reached after 5 days of treatment.[30]

The study included post-menopausal women with newly diagnosed, resectable primary invasive breast cancer, histologically confirmed as ER+ (in this context defined by ER staining of ≥10% of tumor cell nuclei), HER2- (defined as negative by *in situ* hybridization or an immunohistochemistry status of 0 or 1+), and with a palpable tumor of any size, or a tumor with an ultrasound-assessed diameter of at least 1 cm. Patients were excluded from the trial if they had evidence of metastatic disease; had received prior systemic or local treatment for the new primary breast cancer currently under investigation; were receiving medication or herbal supplements known to be strong inhibitors/inducers of CYP3A4/5, strong inhibitors of CYP2C8, or sensitive substrates of CYP2C8

inhibition; had received hormone replacement therapy anytime between 4 weeks before the pre-treatment biopsy and the start of study treatment; had inflammatory breast cancer; or had any evidence of severe or uncontrolled systemic diseases, including uncontrolled hypertension, uncontrolled diabetes, or active infection, including hepatitis B, hepatitis C, and human immunodeficiency virus, as judged by the investigator.

Assessments

Tumor samples were sectioned and scored manually for ER, PR, and Ki-67 protein biomarkers by central pathology review. The percentage of ER+ or PR+ tumor epithelial cell nuclei in each staining category (negative; weak +; moderate ++; strong +++) were recorded for each sample. Results were expressed as an H-score, where H-score = (1 × % of +) + (2 × % of ++) + (3 × % of +++), with a range of 0–300. Ki-67 index was assessed and expressed as the percentage of positively stained tumor nuclei, following the International Ki-67 in the Breast Cancer Working Group recommendations.[31]

Blood samples for determining AZD9496 plasma concentrations were taken at the time of on-treatment biopsy (approximately 2 hours after last AZD9496 dose) and 1–2 hours afterwards. An additional sample was taken 8–12 hours after the last dose, or at discharge from patients undergoing surgery on the day of biopsy. Patients who were undergoing surgery on a separate day to the biopsy had an optional sample taken 3–4 h after on-treatment biopsy. Only one blood sample for fulvestrant PK analysis was taken on the day of biopsy, any time before on-treatment biopsy.

Safety was assessed in terms of adverse events (AEs; graded according to Common Terminology Criteria for Adverse Events [CTCAE] 4.0), laboratory data, vital signs, and electrocardiogram changes. AEs were monitored from screening through to the follow-up visit 28 ± 3 days after the last AZD9496 study dose or after fulvestrant administration.

This study was carried out in accordance with the principles of the International Conference on Harmonisation Guideline for Good Clinical Practice, the Declaration of Helsinki, and all applicable national and local laws. All patients gave their written consent to participate before enrolling in the study. The protocol was approved by the respective regulatory authorities and the research ethics committee of each participating site, and was subject to Ethics Committee and Institutional Review Board approvals.

Statistical methods

The primary endpoint was the treatment effect on ER expression in tumor biopsy samples obtained before and during treatment. Secondary endpoints were the treatment effects on PR and Ki-67 expression in tumor biopsy samples obtained before and during treatment, plasma concentrations of AZD9496 or fulvestrant during treatment, and safety and tolerability. An analysis of covariance (ANCOVA) model, adjusted for baseline expression and day of on-treatment biopsy, was used to estimate the treatment effects on ER, PR, and Ki-67 expression. As this study was designed to assess the superiority of AZD9496 over fulvestrant, one-sided testing was performed; a p-value <0.1 was needed to declare AZD9496 superior to fulvestrant. The least square (LS) mean, along with 80% confidence intervals (CIs) for each treatment group was expressed as estimated percentage change from baseline. One sided p-values were presented. PR and Ki-67 were log transformed before being analyzed, and then back-transformed to the original scale. Analysis for all PK/PD and PD/PD relationships in individual patients was limited to exploratory correlation plots using linear

regression. In addition, for PD/PD relationships, R and p-values were calculated by Spearman's rank correlation. The treatment effect (difference in LS means, or geometric mean ratios [GMRs] for log transformed data) was calculated, together with CIs. The sample size was determined based on the fulvestrant data reported previously;[32] sample size calculations indicated that 20 evaluable patients per treatment group would detect an absolute mean percent change difference in ER expression of 20% with a power of 80%, and a one-sided significance level of 10%. Assuming a drop-out rate of approximately 15%, the recruitment target was set at 24 patients per treatment group. Plasma concentrations of AZD9496 and fulvestrant were compared with PK models that were developed using historical data obtained from patients with metastatic ER+ breast cancer.

The safety analysis set was defined as all patients who received at least one dose of treatment. The PD analysis set included all evaluable patients, defined as those who received at least 80% of the AZD9496 predicted dose; received the last dose of AZD9496 on the day of on-treatment biopsy; had on-treatment biopsy within 5–14 days of AZD9496 therapy or fulvestrant administration; had evaluable paired tumor samples by central pathology assessment, and had no major protocol deviations that could have impacted biomarker analysis. The PK analysis set was defined as all patients who received at least one dose of study treatment, and had at least one measured AZD9496 or fulvestrant concentration at a scheduled PK timepoint post dose.

Results

The study commenced on October 5, 2017; the last patient's last visit was on February 12, 2019, and final data cut off was April 1, 2019. Patients were recruited from 12 sites in Germany and the UK; 49 were enrolled and randomized. Three patients were excluded from the study before receiving study treatment: one withdrew consent, and two were ruled ineligible. Of the 46 patients who completed the trial, 22 received AZD9496 and 24 received fulvestrant (Figure 1). Paired biopsy samples from 35 patients were evaluable for biomarker analysis (AZD9496 n=15; fulvestrant n=20) and were included in the PD analysis set. Eleven paired biopsy samples were not evaluable: nine on-treatment tumor biopsies were surgical resections, one patient did not receive the last dose of AZD9496 on the day of on-treatment biopsy, and one patient did not have the on-treatment biopsy. Patients' characteristics were well balanced between the two groups and as expected per the inclusion exclusion criteria (Table 1).

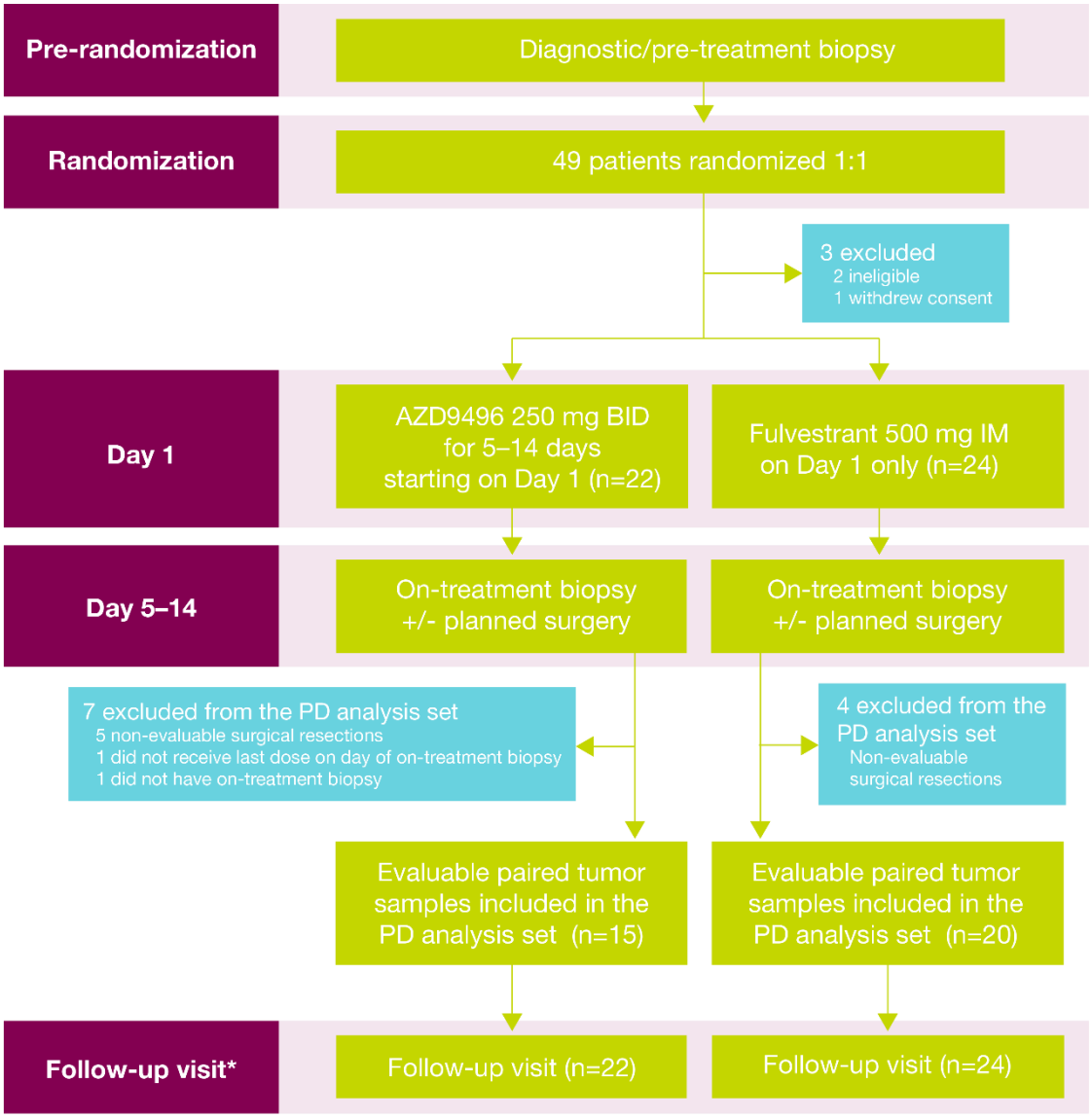

Figure 1. Trial profile.

*AZD9496 follow-up visit: 28±3 days after last dose; fulvestrant follow-up visit: 28±3 days after treatment.
BID: twice daily; IM: intramuscular; PD: pharmacodynamic.

Table 1. Baseline characteristics of study population.

	AZD9496 (n=22)	Fulvestrant (n=24)	Total (N=46)
Median age, years (range)	61.5 (52–83)	66.5 (52–87)	63.5 (52–87)
ECOG PS, n (%)			
0	21 (95)	18 (75)	39 (85)
1	1 (5)	6 (25)	7 (15)
Primary tumor, n (%)			
T1	10 (45)	9 (38)	19 (41)
T2	11 (50)	11 (46)	22 (48)
T3	1 (5)	4 (17)	5 (11)
Regional lymph nodes, n (%)			
N0	20 (91)	21 (88)	41 (89)
N1	0	3 (13)	3 (7)
Missing	2 (9)	0	2 (4)
Tumor grade, n (%)			
G1	3 (14)	5 (21)	8 (17)
G2	13 (59)	17 (71)	30 (65)
G3	5 (23)	2 (8)	7 (15)
GX	1 (5)	0	1 (2)
PR, n (%)			
Positive	16 (73)	19 (79)	35 (76)
Negative	6 (27)	4 (17)	10 (22)
Missing	0	1 (4)	1 (2)
HER2, n (%)			
IHC-borderline and FISH-negative	2 (9)	2 (8)	4 (9)
IHC-borderline and CISH-negative	0	1 (4)	1 (2)
Negative*	20	21	41
ER positive, n (%)	22 (100)	24 (100)	46 (100)

*Both IHC and FISH/CISH negative.

CISH: chromogenic *in situ* hybridization; ECOG PS: Eastern Cooperative Oncology Group performance status; FISH: fluorescence *in situ* hybridization IHC: immunohistochemistry; PR: progesterone receptor.

Pharmacodynamic analysis

The median biopsy day was similar between the two treatment groups (D 8 in the AZD9496 group and D 8.5 in the fulvestrant group), and in the AZD9496 group most biopsies (67%) took place 2–4 hours (range: 1–6) after the patient's last dose of AZD9496. The LS mean reduction in ER H-scores after adjusting for baseline and day of biopsy was 24.3% (80% CI: 14.3, 34.4) in the AZD9496 group, and 36.3% (80% CI: 27.7, 44.9) in the fulvestrant group. One-sided testing for AZD9496 superiority over fulvestrant was not significant (12%, p=0.86; Figure 2a).

Figure 2

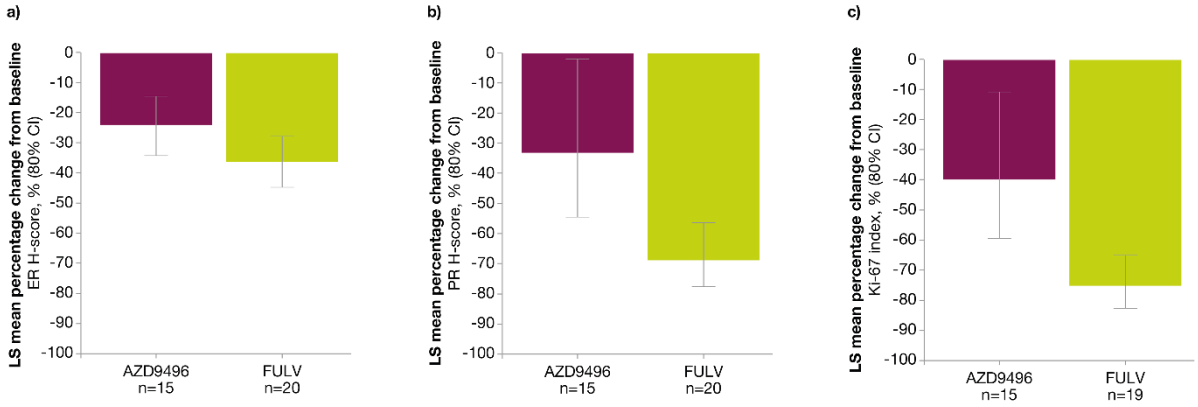

Figure 2. LS mean percentage change in PD markers from baseline to on-treatment biopsy.

LS mean percentage change from baseline in a) ER H-score, b) PR H-score, and c) Ki-67 index levels. One-sided ANCOVA was used to assess treatment effects. PR H-score and Ki-67 index data were log transformed prior to analysis, with results back-transformed to represent percentage change. Error bars represent 80% CIs.
ANCOVA: analysis of covariance; CI: confidence interval; ER: estrogen receptor; LS; least square; PD: pharmacodynamic; PR: progesterone receptor.

PR H-scores were reduced from baseline in both the AZD9496 group (LS mean reduction: 33.3% [80% CI: 2.2, 54.5]) and the fulvestrant group (LS mean reduction: 68.7% [80% CI: 56.4, 77.5]). The treatment effect between fulvestrant and AZD9496 was not significant, with a GMR of fulvestrant to AZD9496 of 2.13 (p=0.97 [one-sided testing AZD9496 superior to fulvestrant]; Figure 2b). Ki-67 levels were reduced from baseline by a mean of 39.9% (80% CI: 10.8, 59.5) in the AZD9496 group, and 75.4% (80% CI: 65.1, 82.7) in the fulvestrant group. Using one-sided testing, AZD9496 was determined to be not superior to fulvestrant, with a GMR of fulvestrant to AZD9496 of 2.4 (p=0.98; Figure 2c).

In the AZD9496 group, all correlations between individual percent changes in ER, PR, and Ki-67 were not statistically significant (ER and Ki-67 [R=0.31, p=0.26]; PR and Ki-67 [R=0.059, p=0.83]), with the exception of the correlation between individual ER and PR percent changes (R=0.59, p=0.022). However, because of the number of tests conducted, this correlation may likely due to chance (Supplementary Figures 1a and 2). For patients in the fulvestrant group, the correlation was not statistically significant between ER and PR (R=0.37, p=0.11), ER and Ki-67 (R=0.21, p=0.38), and PR and Ki-67 (R=0.16, p=0.51; Supplementary Figure 1b). However, ER reduction was accompanied by concurrent reductions in PR and Ki-67 in most of the patients in both the AZD9496 and fulvestrant groups (Supplementary Figure 2).

Pharmacokinetic analysis

In the 44 patients included in the PK analysis set, plasma exposure of AZD9496 was lower than predicted based on modeled PK data from the Phase 1 trial in patients with advanced breast cancer.[30] Compared with the steady-state plasma concentration observed in the Phase 1 AZD9496 250 mg BID treatment group, the area under the concentration–time curve (AUC) for the current study was 31% lower, and the C_{max} was 25% lower (Supplementary Figure 3). Fulvestrant plasma exposure was consistent with historical data (AstraZeneca data on file).[32] No clear PK/PD relationship between plasma concentration at biopsy and change in PD markers relative to baseline was observed for AZD9496 (Supplementary Figure 4a,b,c) or fulvestrant (Supplementary Figure 4d).

Safety and tolerability

The safety analysis set included 46 patients. The median treatment duration of AZD9496 was 9.5 days (range: 6–15). Despite one patient missing their last dose of AZD9496 on the day of on-treatment biopsy, compliance to study treatment was high, with a relative dose intensity of 100% (range: 90–125; upper end of range due to one patient not returning leftover study treatment). One dose interruption was reported, where a patient forgot to take a second dose of AZD9496 on D 6. She resumed normal dosing on D 7 and was deemed eligible to continue the study. AZD9496 and fulvestrant were both well tolerated, and no new safety findings were identified. Twenty-five (54.3%) patients experienced at least one AE, irrespective of causality: 11 (50.0%) in the AZD9496 group and 14 (58.3%) in the fulvestrant group. Most AEs were CTCAE Grade 1 (21/25, 90.9%); no Grade 3 or higher toxicities were reported. Nausea was the most common AE observed in the AZD9496 group (n=4, 18.2%), while hot flush was the most common AE observed in the fulvestrant group (n=3, 12.5%; Table 2). Thirteen (28.3%) patients experienced AEs that were considered by the investigator to be related to the study drug: 6 (27.3%) in the AZD9496 group and 7 (29.2%) in the fulvestrant group. No drug discontinuations occurred, and no serious AEs were reported during the treatment and follow-up periods.

Table 2. Most common adverse events (>5% of patients), irrespective of causality, occurring during the study.

AE, by preferred term	Number of patients (%)*		
	AZD9496 250 mg BID (n=22)	Fulvestrant 500 mg single dose (n=24)	Total (N=46)
Any AE	11 (50.0)	14 (58.3)	25 (54.3)
Nausea	4 (18.2)	2 (8.3)	6 (13.0)
Fatigue	2 (9.1)	2 (8.3)	4 (8.7)
Hot flush	1 (4.5)	3 (12.5)	4 (8.7)
Back pain	1 (4.5)	2 (8.3)	3 (6.5)
Pain in extremity	2 (9.1)	1 (4.2)	3 (6.5)

*Safety analysis set.
AE: adverse event; BID: twice daily.

Discussion

This was the first pre-surgical window of opportunity study to demonstrate that an oral SERD can impact its key biological targets, and the first randomized study to compare two SERDs (AZD9496 and fulvestrant). The treatment groups were well balanced in age, disease stage, and other tumor characteristics.

AZD9496 250 mg BID reduced ER expression in primary untreated breast tumors. However, the magnitude of ER reduction was not statistically superior from the effect of the clinically approved dose of fulvestrant. AZD9496 reduced PR and Ki-67 expression compared with baseline, but was not superior to fulvestrant. Preclinically, AZD9496 produced statistically significant ER degradation in the HCC1428 long-term estrogen-deprived breast model and the patient-derived xenograft CTC174.[27] In the MCF-7 xenograft model, AZD9496 demonstrated greater tumor growth inhibition than

fulvestrant.[27] In other endocrine-sensitive and -resistant breast cancer models, the effects of AZD9496 and fulvestrant were comparable.[26]

AZD9496 plasma exposure was lower than expected based on data from the previous Phase 1 study. This could have contributed to the lower than anticipated ER degradation.[30] Reasons for the lower exposure are unclear; however, interstudy variability, the sparse PK sampling schedule and the use of population PK analysis required to make comparisons in this study may have contributed to the variability of results. Similarly, differences in concomitant medications used by the newly diagnosed, treatment-naive breast cancer patients in this study and the advanced, heavily treated patients in the Phase 1 study may also have impacted the results. Additionally, it has been reported that patients with advanced cancer may have altered drug PK. This is due to the inflammatory state induced by their disease and changes in cytochrome P450 expression in the liver, most notably CYP3A, leading to reduced metabolic clearance of certain drugs.[33-35] As AZD9496 is a substrate and inducer of CYP3A clinically[30], in contrast to fulvestrant, this may have contributed to the interstudy variability in exposure seen with AZD9496 but not with fulvestrant.

AZD9496 250 mg BID was the selected dose for the present study, based on evidence of tolerability and biological activity at this dose in the previous Phase 1 study. In that study, dose-limiting toxicities (DLTs) were observed in three patients: one patient (150 mg BID) experienced abnormal hepatic functions, another (400 mg BID) developed Grade 3 diarrhea and elevated liver function tests, and another (600 mg BID) developed Grade 3 diarrhea; the maximum tolerated dose (MTD) was not reached and therefore, 600 mg BID was the maximum dose explored and declared the maximum feasible dose (MFD).[30] The use of a higher dose of AZD9496 in the present study may have resulted in greater PD activity but this remains to be demonstrated.

Fulvestrant performed as expected, based on historical data, in terms of PD biomarker modulation (ER, PR, and Ki-67),[21,32] achieving a mean 36% reduction in ER H-score. In previous studies, fulvestrant reduced ER H-score from baseline by 41%.[32]

Fulvestrant reduced PR levels by 69% and Ki-67 levels by 75% from baseline. These reductions are in line with two previous studies, where fulvestrant 500 mg reduced PR H-score by 34% and 81%, and Ki-67 levels by 75% and 79%.[21,32]

AZD9496 and fulvestrant were well tolerated and no new safety signals were identified. No Grade 3 or higher toxicities, or serious AEs developed, and no patient discontinued study treatment. Hot flush was the most commonly reported AE in patients in the fulvestrant group, consistent with the safety profiles of fulvestrant 500 mg in previous studies.[16,32] Nausea was the most common AE in the AZD9496 group, and judged as causally related in all four patients. Dose limiting toxicities in the Phase 1 study included Grade 3 liver toxicities and diarrhea. However, in the current trial, no liver toxicities were reported and only one patient experienced an AZD9496 causally related Grade 1 diarrhoea.[30]

The median day of biopsy was similar for both groups. Based on Phase I data, the PK steady state of AZD9496 was expected to be 5 days.[30] Within 5–14 days of exposure, fulvestrant concentration is anticipated to be within the C_{max} and C_{min} observed at steady state in patients with advanced breast cancer receiving fulvestrant 500 mg as a standard therapeutic regimen.[28,29] In the context of the present study, a 5- to 14-day window was considered sufficient to observe the biomarker changes

associated with fulvestrant, and appropriate to provide a comparison to assess the PD activity of AZD9496.

The rate of non-evaluable patient samples was higher than predicted because biopsy samples were taken from surgical resections instead of core needle biopsies in some patients. Surgical samples were not included in the analysis of this study, as the POETIC trial (NCT02338310) showed evidence that surgery *per se* can affect Ki-67 expression in tumor tissue.[36]

One limitation of this study is the lack of a placebo group to compare the consistency in ER, PR, and Ki-67 expression. In addition, examining a range of AZD9496 doses would have been useful to determine any dose–response relationship more robustly.

In conclusion, this is the first window of opportunity study where an oral SERD has shown relevant biomarker modulations in patients with ER+ breast cancer. However, AZD9496 was not superior to fulvestrant at the dose tested. Window of opportunity studies represent an important means to test the proof of mechanism and degree of PD activity of novel SERDs early in clinical development. They can also inform dosing decisions for future Phase 2 and 3 trials, thus reducing the reliance on pre-clinical PK modeling.

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Data sharing statement

Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure.

Role of the study sponsor

AstraZeneca funded this study, and participated in the study design, data collection, data analysis, data interpretation, and the writing of the study report. AstraZeneca reviewed the publication, without influencing the opinions of the authors, to ensure medical and scientific accuracy, and the protection of intellectual property. The corresponding author had access to all data in the study, and had the final responsibility for the decision to submit the manuscript for publication.

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Tables and figures

Tables

Figures

Supplementary material