A first in human study of the new oral selective estrogen receptor degrader AZD9496 for ER+/HER2− advanced breast cancer

Authors and affiliations

Erika P. Hamilton[1], Manish R. Patel[2], Anne C. Armstrong[3], Richard D. Baird[4], Komal Jhaveri[5], Matthias Hoch[6], Teresa Klinowska[6], Justin P.O. Lindemann[6], Shethah R. Morgan[6], Gaia Schiavon[6], Hazel M. Weir[7], Seock-Ah Im[8]

[1]Sarah Cannon Research Institute/Tennessee Oncology, PLLC, Nashville, TN, USA.

[2]Sarah Cannon Research Institute/Florida Cancer Specialists, Sarasota, FL, USA.

[3]Department of Medical Oncology, The Christie NHS Foundation Trust and the Faculty of Biology, Medicine and Health, The University of Manchester, UK.

[4]Breast Cancer and Early Phase Clinical Trials Teams, Cancer Research UK Cambridge Centre, UK.

[5]Memorial Sloan Kettering Cancer Center, New York, NY, USA.

[6]IMED Biotech Unit, AstraZeneca, Cambridge, UK.

[7]IMED Biotech Unit, AstraZeneca, Macclesfield, UK.

[8]Department of Internal Medicine, Seoul National University Hospital, Cancer Research Institute, Seoul National University College of Medicine, Seoul, South Korea.

Running title

Phase 1 study of oral SERD AZD9496

Keywords

ER+, metastatic breast cancer, oral SERD, pharmacokinetic, Phase 1

Additional information

Financial support

This study was sponsored by AstraZeneca. The authors thank InterComm International Ltd, Cambridge, UK, for providing medical writing support, which was funded by AstraZeneca, Cambridge, UK, in accordance with Good Publication Practice (GPP3) guidelines (http://www.ismpp.org/gpp3).

Corresponding author

Full name: Dr Erika Hamilton

Mailing address: 250 25th Ave. N Suite 200, Nashville, TN, 37203, USA

Phone: +1 615-320-5090

Fax: +1 615-524-4961

Email: ehamilton@tnonc.com

Conflicts of interest disclosure

MH, TK, JPOL, SRM and GS are employees of AstraZeneca UK. HMW was an employee of AstraZeneca when the study was conducted. Data from this work were partially reported at the San Antonio Breast Cancer Symposium meeting, December 5–9 2016, San Antonio, TX, USA (poster number P6–12–03).

Notes about the manuscript

Word count (body text only, excluding tables, figures, and citations): 3,875

Number of figures/tables: Six

Statement of translational relevance

Endocrine resistance is a challenge for patients with estrogen receptor (ER) positive breast cancer. Fulvestrant, a selective estrogen receptor degrader (SERD), is a standard of care medication for advanced ER+/HER2− metastatic breast cancer, but its intramuscular administration restricts the maximum feasible dose. Orally bioavailable SERDs may achieve greater clinical anti-ER activity than fulvestrant, which may translate into improved clinical outcomes.

This Phase 1 study reports safety, tolerability, pharmacokinetics, and preliminary antitumor activity of the oral SERD AZD9496, which shows prolonged disease stabilization in some heavily pre-treated patients with ER+/HER2− metastatic breast cancer, including those previously treated with fulvestrant. These results support the further clinical development of AZD9496.

Oral SERDs could be the next generation of endocrine therapy and are a priority for clinical investigation.

Abstract

Purpose: AZD9496 is an oral non-steroidal, small-molecule inhibitor of estrogen receptor alpha (ERα), and a potent and selective antagonist and degrader of ERα. This first in human Phase 1 study determined the safety and tolerability of ascending doses of oral AZD9496 in women with estrogen receptor (ER)+/HER2– advanced breast cancer, characterized its pharmacokinetic (PK) profile, and made preliminary assessment of antitumor activity.

Experimental design: Forty-five patients received AZD9496 (20 mg once daily to 600 mg twice daily) in a dose-escalation, dose- expansion 'rolling 6' design. Safety, tolerability, and PK activity in each cohort was reviewed before escalating to the next dose. PK was determined by mass spectrometry. Adverse events (AEs) were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0. Objective tumor response was evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1.

Results: Most common causally related AEs were diarrhea (35.6%), fatigue (31.1%), and nausea (22.2%), and seven patients had grade ≥3 AEs. Three patients experienced a dose-limiting toxicity (DLT): one each at 150 mg BID (abnormal hepatic function), 400 mg BID (diarrhea and elevated liver function tests) and 600 mg BID (diarrhea), and all were reversible. The maximum tolerated dose was not reached. Partial response was confirmed in one patient, who also had decreased tumor marker Ca15.3. Four patients had stable disease at 12 months' follow up.

Conclusions: AZD9496 is well tolerated with an acceptable safety profile, showing evidence of prolonged disease stabilization in heavily pre-treated patients with ER+/HER2– advanced breast cancer.

74 # [Body text]

75 ## Introduction

76 Approximately 70% of breast cancers are estrogen receptor (ER) positive, and inhibiting estrogen
77 receptor (ER) signaling is a mainstay of treatment (1). Three classes of endocrine agents are used:
78 aromatase inhibitors, selective estrogen receptor modulators (SERMs), and selective estrogen
79 receptor degraders (SERDs); each with unique modes of action. Aromatase inhibitors prevent the
80 conversion of androgens to estrogens (2), SERMs bind to the ER and act as mixed
81 antagonists/agonists (3), and SERDs bind to, antagonize, and degrade the ER (4).

82 Current endocrine therapies can be effective, but many patients develop primary or secondary
83 resistance, ultimately leading to disease progression and death. Therefore, drug resistance is a major
84 clinical challenge (1). Only around 30% of patients with metastatic breast cancer achieve objective
85 tumor regression with initial endocrine treatment, and another 20% experience prolonged stable
86 disease (5). Resistance mechanisms include deregulation of the ER pathway itself, alterations in cell
87 cycle and cell survival signaling molecules, development of escape pathways, and acquisition of
88 activating mutations in the ER gene (*ESR1*) that allow tumors to survive and proliferate without
89 depending on estrogen (5). Although the benefit of SERMs and aromatase inhibitors declines after
90 resistance develops, it is well known that the ER itself remains involved in the pathogenesis and
91 progression of advanced disease, and therefore remains an important therapeutic target (6-9).

92 Fulvestrant is the only SERD approved for treating advanced ER+/HER2− metastatic breast cancer,
93 and is effective in both endocrine treatment-naïve patients, and in those whose disease has
94 progressed whilst on other endocrine therapies (10-12). Indeed, although *ESR1* mutations appear to
95 predict resistance to aromatase inhibitor therapy, such mutations do not appear to influence
96 outcomes in patients treated with fulvestrant (13). Fulvestrant is a standard of care medication for
97 advanced ER+/HER2− metastatic breast cancer, but has some limitations: intramuscular injection

restricts the maximum feasible dose (MFD) to 500 mg once a month, and steady state plasma concentrations are not reached until 3 to 6 months after first administration (14,15). Furthermore, a recent study indicated that the MFD of fulvestrant may be insufficient to fully reduce ER in some patients, and this can be associated with earlier disease progression (16). These limitations suggest that an agent inducing even greater combined ER targeting and degradation than fulvestrant would be highly desirable (15).

An orally bioavailable SERD may overcome some of the limitations associated with intramuscular fulvestrant, help patients avoid painful injections, and ease delivery in pressured healthcare systems. An oral SERD may reach steady state more quickly, and might be given at higher relative doses; enhancing target engagement, and potentially deliver superior clinical benefits to patients with ER+ breast cancer.

AZD9496 is a new oral, non-steroidal, small-molecule inhibitor of ERα, and is a potent and selective antagonist and degrader of ERα in ER+ breast cancer models (IC_{50}s from different assays are ≤1 nM) (17). Data show that AZD9496 significantly inhibits tumor growth and decreases expression of progesterone receptor (PR) protein in estrogen-dependent MCF-7 xenograft models and in patient-derived *ESR1* mutant *in vivo* models (17). AZD9496 also caused tumor regression and downregulated ERα expression in the HCC1428 cell long-term estrogen-deprived breast cancer model of resistance to aromatase inhibitor treatment (17).

This first in human study investigated the safety and tolerability of ascending doses of AZD9496 when given orally to women with advanced ER+/HER2− metastatic breast cancer, and characterized its pharmacokinetic (PK) profile.

Patients and methods

Study design and objectives

This study (NCT02248090) was a multicenter, global, Phase 1, open-label, first in human study that comprised two parts: dose escalation and dose expansion. This study was carried out in accordance with the principles of the International Conference on Harmonization guidelines for Good Clinical Practice, the Declaration of Helsinki, and all applicable laws.

The primary objective was to investigate the safety and tolerability of ascending doses of oral AZD9496 in patients with metastatic or locoregionally recurrent ER+/HER2− advanced breast cancer. Secondary objectives were to characterize the PK of AZD9496 and its metabolites after a single oral dose and at steady state after multiple doses, and to obtain a preliminary assessment of anti-tumor efficacy. Exploratory analyses included investigating potential determinants of response or resistance to AZD9496 in plasma (such as *ESR1* mutation status in circulating tumor DNA), and pharmacodynamic biomarker changes in tumor tissue and circulating tumor cells will be reported separately (manuscript in preparation).

Patient selection and screening

Patients were recruited from hospitals in the US, UK, and Korea. 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. All patients provided their written informed consent at study enrollment. Patients were screened within 28 days prior to study admission to gather demographic data and standard medical and surgical history.

Patient eligibility

Key inclusion criteria included: female patients of any menopausal status, aged at least 18 years, and with a diagnosis of ER+/HER2− adenocarcinoma of the breast, metastatic or locoregionally recurrent, and not amenable to treatment with curative intent. Pre- or peri-menopausal women must have

started luteinizing hormone-releasing hormone (LHRH) agonist treatment at least 4 weeks before study treatment, and must have continued this treatment throughout the study. Disease must have progressed after at least 6 months of endocrine therapy for ER+ breast cancer. (Before protocol amendment 21 August 2015, patients must have spent ≥6 months on a line of endocrine therapy in the advanced setting). Radiological or objective evidence of progression on or after the last systemic therapy was needed before starting study treatment.

Key exclusion criteria included receipt of more than two lines of chemotherapy for advanced disease, or systemic anti-cancer therapy within 14 days of the first dose of study treatment. Radiotherapy for palliation was permitted if received more than 1 week before the first dose of study treatment. Patients were excluded if they were receiving any medications known to induce or inhibit CYP3A4/5 or CYP2C8, or had life-threatening visceral, central nervous system or pulmonary lymphangitic metastases, inadequate bone marrow reserve or organ function, unexplained symptomatic endometrial disorders, uncontrolled symptomatic thyroid dysfunction, or an Eastern Cooperative Oncology Group (ECOG) performance status of ≥2.

Dose escalation and dose expansion

A 'rolling 6' design was employed, in which each cohort of at least three and up to six patients received AZD9496 at an escalating dose (18). Dosing began at 20 mg once-daily (QD). Patients were dosed in cycles: Cycles 1 to 6 each were 4 weeks long, and further cycles each were 6 weeks long. Dose-limiting toxicities (DLTs) were assessed for the first 28 days of treatment (Cycle 1), and the dose was escalated in the next cohort if no DLTs were observed in the previous cohort. If two or more patients in any cohort experienced a DLT, the dose was considered non-tolerated. If only one patient experienced a DLT, the cohort was expanded to include six evaluable patients, and if no further DLTs occurred, dose escalation could continue. Dose interruptions and reductions were permitted if patients experienced adverse events (AEs). Dose escalations were planned to continue until the maximum tolerated dose (MTD; the last dose below the non-tolerated dose) or MFD (a

reasonable number of acceptably sized tablets given, or evidence of saturation of absorption observed) was reached. Patients were dosed until confirmed disease progression, or unacceptable toxicity.

At selected doses, escalation cohorts were expanded to include six evaluable patients in order to further investigate safety, tolerability, PK, and biological activity of AZD9496.Safety and tolerability assessments

Safety was assessed in terms of AEs (including treatment emergent adverse events [TEAEs; any event not present prior to receipt of first dose of study drug, or a worsening of an existing event], serious adverse events [SAEs], causally related AEs [any event deemed related to the study drug in the investigator's opinion], AEs leading to discontinuation, and AEs leading to death), laboratory data, vital signs, electrocardiogram changes, and ECOG assessment. AE severity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE), Version 4. An independent Safety Review Committee reviewed the safety, tolerability, and preliminary PK data (if available) from patients in each escalation cohort before escalating to the next dose.

Pharmacokinetic assessments

Plasma PK parameters (including AUC, maximum plasma concentration [C_{max}], and time to maximum plasma concentration [t_{max}]) were determined for AZD9496 and its metabolites M3 and M5 (30- and 3-fold lower potency than parent, respectively, and both formed by oxidation of the parent) after a single dose, and at steady state after multiple dosing (i.e. 13 days of dosing in the dose escalation cohorts and 11 days in the dose expansion cohort). AZD9496 concentration was also determined in urine for patients in the dose escalation cohorts only. 4β-hydroxy-cholesterol:cholesterol ratios were determined as a marker of hepatic CYP3A4 induction potential by AZD9496.

AZD9496 and metabolites were determined in plasma using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The validated range was 1.00 to 5,000 ng/mL for

AZD9496, 1.00 to 2,000 ng/mL for M3 and 0.1 to 200 ng/mL for M5. AZD9496 concentrations were also determined in urine using a validated LC-MS/MS method with a validated range of 50.0 to 50,000 ng/mL. For patients in the dose escalation cohorts, in Cycle 1 venous blood samples were taken pre-dose and at regular intervals on Day 1 (over 24 h) and Day 15 (over 10 h), and pre-dose on Days 2 and 16. In Cycles 2–4, samples were taken pre-dose on Day 1. For patients in the dose expansion cohorts, in Cycle 1 blood samples were taken on Day 1 (over 72 h) and Day 15 (over 10 h), and pre-dose on Day 8. In Cycles 2–4, samples were taken pre-dose on Day 1. For patients participating in PK profiling (those in the dose expansion cohort), two additional blood samples were taken pre-dose on Days 1 and 15 of Cycle 1, and Day 1 of Cycles 2–4, to determine 4β-hydroxy-cholesterol:cholesterol ratios. Urine samples were collected pre-dose, and 0 to 4, 4 to 8, 8 to 10, and 10 to 24 hours post-dose on Days 1 and 15 (Cycle 1 only) from patients in the dose escalation cohorts.

Anti-tumor efficacy assessment

Objective tumor response assessment was based on the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 guidelines for response (19). Computed tomography/magnetic resonance imaging (CT/MRI) was performed of the chest, abdomen, and pelvis (and any other sites at which new disease was suspected) of all patients at baseline (within 28 days of study start), at 8, 16, and 24 weeks after the start of treatment, and every 12 weeks thereafter until objective disease progression was confirmed. Patients underwent a bone scan or skeletal survey at baseline, and at follow-up visits if clinically indicated.

Data derivation and analysis

The number of patients was chosen based on the desire to obtain adequate data while exposing as few patients as possible to the investigational product and procedures. The safety analysis set was all patients who received at least one dose of AZD9496. The PK analysis set was all patients who

received at least one dose of AZD9496, and who have at least one measured concentration of AZD9496 at a scheduled post-dose PK time point.

PK parameters were derived by standard non-compartmental methods using Phoenix™ WinNonlin® (Certara), Version 6.4. No formal statistical analysis was done for this study; data were summarized using standard summary statistics (SAS Version 9.2).

Results

The study commenced in October 2014, and recruitment was completed on 26 February 2016, ahead of the final data cut-off on 31 January 2017. Forty-five patients were enrolled: all met the inclusion criteria and received AZD9496 at various doses (from 20 mg QD to 600 mg twice daily [BID]; Figure S1). Patients were allocated to cohorts containing between four and six patients, and each cohort received AZD9496 at an escalating dose. Six further patients were selected for an expansion cohort after the 400 mg BID dose escalation, and received AZD9496 at 250 mg BID at the same time as the 600 mg BID cohort.

Baseline characteristics

Baseline patient demographics are shown in Table 1. Patients were mostly white (*n* = 31; 68.9%) with a median age of 62 years (range 41 to 83 years). All patients had metastatic disease on study entry. Most patients had measurable disease (*n* = 39; 86.7%) and many had visceral disease (*n* = 36; 80.0%). Twenty-five patients (55.6%) had received prior treatment with fulvestrant before enrolling in the study. Of these, ten received fulvestrant as the immediate therapy prior to enrollment; five as a monotherapy, and five as part of combination treatment.

Safety and tolerability

Forty-four patients (97.8%) experienced at least one AE, and most were CTCAE grade 1 or 2. The most common AEs of any grade were fatigue (*n* = 19; 42.2%), nausea (*n* = 18; 40.0%), and diarrhea (*n* = 17; 37.8%).

Forty patients (88.9%) experienced AEs that were considered by the investigator, using his/her clinical judgment, to be related to the study drug. The most common causally related AEs of any grade were diarrhea (*n* = 16; 35.6%), fatigue (*n* = 14; 31.1%), nausea (*n* = 10; 22.2%), and upper abdominal pain (*n* = 6; 13.3%), grading of these AEs are shown in Table 2. Causally related SAEs occurred in two patients (4.4%; diarrhea, abnormal hepatic function), and causally related AEs of CTCAE grade ≥3 or higher occurred in seven patients (15.6%). These were diarrhea (*n* = 3; 6.7%), increased ALT (*n* = 2; 4.4%), and fatigue, vomiting, and increased AST (each *n* = 1; 2.2%).

247 Table 1. Baseline characteristics of study population

	20 mg QD (*n* = 4)	40 mg BID (*n* = 6)	80 mg BID (*n* = 5)	150 mg BID (*n* = 6)	250 mg BID[a] (*n* = 12)	400 mg BID (*n* = 6)	600 mg BID (*n* = 6)	Total (*n* = 45)
Median age, years (range)	70.0 (63–82)	57.0 (44–75)	50.0 (43–83)	60.0 (44–75)	58.0 (41–75)	57.5 (46–67)	64.0 (48–69)	62.0 (41–83)
Race, n (%)								
White	3 (75.0)	4 (66.7)	3 (60.0)	5 (83.3)	9 (75.0)	4 (66.7)	3 (50.0)	31 (68.9)
Black or African American	1 (25.0)	0	0	0	1 (8.3)	0	0	2 (4.4)
Asian	0	2 (33.3)	2 (40.0)	1 (16.7)	2 (16.7)	2 (33.3)	3 (50.0)	12 (26.7)
Post-menopausal, n (%)	4 (100.0)	6 (100.0)	3 (60.0)	5 (83.3)	11 (91.7)	4 (66.7)	5 (83.3)	38 (84.4)
ECOG category 0, n (%)	2 (50.0)	4 (66.7)	3 (60.0)	2 (33.3)	3 (25.0)	2 (33.3)	3 (50.0)	19 (42.2)
Measurable disease, n (%)	4 (100.0)	6 (100.0)	5 (100.0)	6 (100.0)	8 (66.7)	5 (83.3)	5 (83.3)	39 (86.7)
Visceral disease[b], n (%)	4 (100.0)	5 (83.3)	4 (80.0)	6 (100.0)	8 (66.7)	4 (66.7)	5 (83.3)	36 (80.0)
Number of prior chemotherapy regimens, median (range)								
(Neo) adjuvant setting	0 (0–1)	0 (0–1)	0 (0–1)	1 (0–1)	1 (0–1)	1 (0–1)	1 (0–1)	1 (0-1)
Advanced setting	0.5 (0–1)	1.5 (0–2)	0 (0–2)	0 (0–2)	1 (0–2)	0.5 (0–1)	1 (0–2)	1 (0–2)
Number of prior endocrine regimens, median (range)								
Any setting	3.5 (2–4)	3 (2–6)	2 (2–4)	3.5 (1–5)	3 (1–5)	2.5 (1–4)	3 (1–4)	3 (1–6)
Prior treatment with an AI (total), n (%)								
Adjuvant setting	1 (25.0)	1 (16.7)	0 (0.0)	1 (16.7)	3 (25.0)	3 (50.0)	0 (0.0)	9 (20.0)
Metastatic setting	4 (100.0)	6 (100.0)	5 (100.0)	5 (83.3)	12 (100.0)	3 (50.0)	6 (100.0)	41 (91.0)
Prior treatment with fulvestrant, n (%)	3 (75.0)	4 (66.7)	2 (40.0)	2 (33.3)	7 (58.3)	4 (66.7)	3 (50.0)	25 (55.6)
Prior treatment with CDK4/6 inhibitors, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	2 (33.3)	4 (33.3)	1 (16.7)	0 (0.0)	7 (15.6)
Prior treatment with mTOR inhibitors, n (%)	2 (50.0)	3 (50.0)	0 (0.0)	3 (50.0)	6 (50.0)	2 (33.3)	2 (33.3)	18 (40.0)

248 [a]Pooled data from dose escalation and expansion groups.
249 [b]Visceral disease includes patients with disease site at baseline of lung, liver (including biliary tract), hepatic, brain, pleural,
250 and/or peritoneal involvement.
251 AI=aromatase inhibitor; BID=twice daily; CDK=cyclin-dependent kinase; ECOG=Eastern Cooperative Oncology Group;
252 mTOR=mechanistic target of rapamycin; QD=once daily.

253 Table 2. Causally related[a] AEs occurring in more than three patients (≥5%) treated with AZD9496

Causally related AEs by preferred term, n (%)	20 mg QD *n* = 4	40 mg BID *n* = 6	80 mg BID *n* = 5	150 mg BID *n* = 6	250 mg BID[b] *n* = 12	400 mg BID *n* = 6	600 mg BID *n* = 6	Total *n* = 45			
								Grade 1	Grade 2	Grade 3	All grades
Diarrhea	0	2 (33.3)	2 (40.0)	0	5 (41.7)	4 (66.7)	3 (50.0)	**10 (22.2)**	**3 (6.7)**	**3 (6.7)**	**16 (35.6)**
Fatigue	3 (75.0)	2 (33.3)	1 (20.0)	1 (16.7)	4 (33.3)	2 (33.3)	1 (16.7)	**8 (17.8)**	**5 (11.1)**	**1 (2.2)**	**14 (31.1)**
Nausea	0	0	0	3 (50.0)	2 (16.7)	3 (50.0)	2 (33.3)	**9 (20.0)**	**1 (2.2)**	**0**	**10 (22.2)**
Abdominal pain (upper)	0	1 (16.7)	1 (20.0)	1 (16.7)	1 (8.3)	0	2 (33.3)	**6 (13.3)**	**0**	**0**	**6 (13.3)**
Hot flush	1 (25.0)	0	0	0	1 (8.3)	3 (50.0)	0	**5 (11.1)**	**0**	**0**	**5 (11.1)**
ALT increased	0	0	0	0	1 (8.3)	2 (33.3)	1 (16.7)	**1 (2.2)**	**1 (2.2)**	**2 (4.4)**	**4 (8.9)**
Vomiting	0	1 (16.7)	0	1 (16.7)	0	2 (33.3)	0	**3 (6.7)**	**0**	**1 (2.2)**	**4 (8.9)**
AST increased	0	0	0	0	1 (8.3)	2 (33.3)	0	**2 (4.4)**	**0**	**1 (2.2)**	**3 (6.7)**
Asthenia	0	0	0	0	2 (16.7)	0	1 (16.7)	**3 (6.7)**	**0**	**0**	**3 (6.7)**
Flatulence	0	0	0	0	1 (8.3)	0	2 (33.3)	**2 (4.4)**	**1 (2.2)**	**0**	**3 (6.7)**
Flushing	0	2 (33.3)	0	0	1 (8.3)	0	0	**3 (6.7)**	**0**	**0**	**3 (6.7)**
Myalgia	0	1 (16.7)	1 (20.0)	1 (16.7)	0	0	0	**3 (6.7)**	**0**	**0**	**3 (6.7)**
Vaginal discharge	0	0	0	1 (16.7)	1 (8.3)	1 (16.7)	0	**3 (6.7)**	**0**	**0**	**3 (6.7)**

254 [a]Causally related to the study drug in the investigator's opinion.

255 [b]Pooled data from dose escalation and expansion groups.

256 AEs=adverse events; ALT=alanine aminotransferase; AST=aspartate aminotransferase; BID=twice daily; CTCAE=Common Terminology Criteria for AEs; QD=once daily.

Three patients experienced DLTs, which were reversible in all patients. One patient in the 150 mg BID cohort experienced abnormal hepatic functions (elevated aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma-glutamyl transferase [GGT] [grade 3], bilirubin, and alkaline phosphatase [ALP] [grade 2]). AZD9496 was withdrawn, and the abnormal hepatic functions returned to baseline. One patient in the 400 mg BID cohort experienced grade 3 diarrhea and grade 3 elevated AST, ALT, and GGT, and was managed with dose interruption and reduction. A further patient, in the 600 mg BID cohort, experienced grade 3 diarrhea, which was managed with dose interruption. Dose escalation was stopped at 600 mg BID. All other causally related grade ≥3 events resolved, and no AEs leading to death were reported.

Pharmacokinetics

Single dose pharmacokinetics of AZD9496

Following a single dose on Day 1, AZD9496 was rapidly absorbed at all dose levels, with median T_{max} 1.55–3.0 hours (Figure 1 Panel A; Table 3). Plasma concentrations underwent a rapid and biphasic decline following the peak, with a mean alpha half-life of 0.99–1.99 hours and a mean terminal half-life of 1.4–5.7 hours (Table 3).

272 Table 3. PK parameters for AZD9496 following single doses (Day 1) and multiple doses (Day 15)

	20 mg QD (N = 4)	40 mg BID (N = 6)	80 mg BID (N = 5)	150 mg BID (N = 6)	250 mg BID[a] (N = 12)	400 mg BID (N = 6)	600 mg BID (N = 6)
Day 1							
C_{max}, ng/mL (gCV%) (*n*)	260 (52) (*n* = 4)	338 (73) (*n* = 4)	536 (40) (*n* = 5)	1,163 (95) (*n* = 5)	2,779 (26) (*n* = 9)	2,577 (53) (*n* = 6)	7,313 (60) (*n* = 3)
AUC_{inf}, h·ng/mL (gCV%) (*n*)	546 (56) (*n* = 4)	1,046 (98) (*n* = 3)	1,368 (22) (*n* = 3)	4,550 (99) (*n* = 4)	11,040 (19) (*n* = 5)	11,580 (77) (*n* = 3)	36,390 (69) (*n* = 3)
t_{max}, h (min, max)	1.75 (1.50, 2.00) (*n* = 4)	1.50 (1.00, 4.05) (n = 4)	2.00 (1.50, 3.00) (*n* = 5)	2.95 (1.50, 3.00) (*n* = 5)	2.03 (1.55, 3.00) (*n* = 9)	2.10 (1.12, 3.00) (*n* = 6)	3.00 (2.00, 4.05) (*n* = 6)
α-$t_{½}$, h (SD) (*n*)	0.92 (0.15) (*n* = 4)	1.1 (0.17) (*n* = 4)	1.2 (0.23) (*n* = 5)	1.2 (0.25) (*n* = 5)	1.3 (0.10) (*n* = 9)	1.5 (0.36) (*n* = 6)	1.9 (0.30) (*n* = 3)
$t_{½}$, h (SD) (*n*)	1.37 (0.42) (*n* = 4)	2.33 (1.94) (*n* = 3)	1.79 (0.95) (*n* = 3)	4.23 (1.28) (*n* = 4)	5.72 (2.68) (*n* = 5)	3.95 (0.74) (*n* = 3)	2.30 (0.52) (*n* = 3)
Day 15							
C_{max}, ng/mL (gCV%) (*n*)	200 (53) (*n* = 4)	215 (62) (*n* = 6)	385 (26) (*n* = 5)	591 (44) (*n* = 6)	1,478 (57) (*n* = 9)	1,195 (65) (*n* = 5)	2,758 (61) (*n* = 6)
AUC_{tau}, h·ng/mL (gCV%) (*n*)	585 (156) (*n* = 4)	637 (58) (*n* = 6)	1,025 (31) (*n* = 5)	1,664 (51) (*n* = 6)	3,841 (67) (*n* = 9)	3,642 (53) (*n* = 5)	8,676 (50) (*n* = 6)
t_{max}, h (min, max)	1.50 (1.42, 2.20) (*n* = 4)	1.49 (0.95, 2.00) (*n* = 6)	1.98 (1.17, 3.00) (*n* = 5)	1.50 (1.00, 3.00) (*n* = 6)	1.50 (1.00, 2.00) (*n* = 9)	2.00 (1.00, 3.00) (*n* = 5)	1.99 (1.50, 3.00) (*n* = 6)
α-$t_{½}$, h (SD) (*n*)	1.1 (0.65) (*n* = 4)	1.2 (0.55) (*n* = 6)	3.3 (3.24) (*n* = 5)	1.2 (0.31) (*n* = 6)	1.0 (0.23) (*n* = 9)	1.1 (0.23) (*n* = 5)	1.1 (0.59) (*n* = 5)
Temporal change for AUC (SD)	1.35 (1.17) (*n* = 4)	0.76 (0.21) (*n* = 3)	0.65 (1.16) (*n* = 3)	0.40 (0.16) (*n* = 4)	0.43 (0.09) (*n* = 3)	NC	0.23 (0.07) (*n* = 3)

273 [a]Pooled data from dose escalation and expansion groups.
274 Temporal change for AUC calculated as follows: AUC_{tau}/AUC_{inf}.
275 Data are geometric mean (gCV%) for C_{max} and the AUC variables, arithmetic mean (SD) for α-$t_{½}$, $t_{½}$ and temporal change for AUC, and median (min, max) for t_{max}.
276 α-$t_{½}$=effective (alpha) half-life; $t_{½}$=terminal elimination half-life; AUC_{inf}=area under the concentration–time curve from zero to infinity; AUC_{tau}=area under the concentration–time curve at
277 steady-state over the dosing interval; BID=twice daily; C_{max}=maximum plasma concentration; gCV=geometric coefficient of variation; EXP=expansion cohort; NC=not calculated (since n<3);
278 QD=once daily; SD=standard deviation; t_{max}=time to observed C_{max}.

Following a single AZD9496 dose of 20 mg up to 400 mg (Day 1), the area under the concentration–time curve (AUC) increased in reasonable proportion to the increasing dose. At 600 mg, a more than dose-proportional increase in AUC and maximum concentration (C_{max}) was observed.

Multiple dose pharmacokinetics of AZD9496

Multiple dose AUC and C_{max} were consistently and dose-dependently lower than those for single dose for 40 mg up to 600mg. Based on the temporal change parameter (TCP) which compares AUC_{tau} on Day 15 to AUC_{inf} on Day 1, a time-dependent reduction in AZD9496 exposure was observed across the BID dose range, with more marked reductions at higher doses (mean reduction of 24% and 77% for the 40 mg BID and 600 mg BID dose level, respectively). No reduction in exposure was observed for the 20 mg QD dose group (Figure 1 Panel B, Table 3). These data correlated with the dose-dependent increase in the marker for hepatic cytochrome P450 (CYP) induction (4β-hydroxy-cholesterol:cholesterol ratio). The median (min, max) percentage change from baseline (Day 1) in 4β-hydrox-ycholesterol:cholesterol ratio to Day 15 was between –5.7% (–16.4, 8.00) for the 20 mg QD dose and 247% (106, 298) for the 600 mg BID dose.

Pharmacokinetics of metabolites

Following single doses and at steady state, the plasma concentration–time profiles of metabolites M3 (around 30-fold lower in potency on ERα degradation than AZD9496) (20) and M5 (around 3-fold lower potency on ERα degradation than AZD9496) (20) closely followed that of AZD9496 but at lower concentrations (Figure S2; Table S1 and S2): around 9 to 20% was detected for M3 and around 2% was detected for M5, relative to AZD9496 exposure. AZD9496 was not detected in urine.

Preliminary anti-tumor efficacy

Duration on treatment

The median duration on treatment with AZD9496 was 2.1 months (range 0.7 to 21.1 months, across the range of doses examined). Twelve patients (26.6%) received AZD9496 for 6 months or longer,

and 10 patients (22.2%) and four patients (8.9%) exhibited stable disease at 6 and 12 months' follow-up, respectively. Treatment was ongoing in six patients (13.3%) up to the data cut-off of 31 January 2017 (Figure 2).

Tumor responses

One patient in the 250 mg BID cohort was observed to have had a partial response at Cycle 9 (Day 251), which was confirmed by a subsequent scan 4 weeks later (Figure 3). This patient had metastatic breast cancer at study entry and had received eight prior chemotherapy regimens' she was fulvestrant naïve, and had not received prior CDK 4/6 or mTOR inhibitor therapy (Figure 2). In this patient, the serum tumor marker Ca15.3 (raised at baseline: 60 U/mL) started to decrease early (2 months after starting AZD9496) and steadily, to reach normal levels after Cycle 8 (23 U/mL). This biochemical response was maintained at the time of RECIST partial response (15 U/mL) and at the last assessment before data cut-off, 2 months later (10 U/mL).

Panels C and D: CT confirming RECIST partial response at Cycle 9. No change compared with previous scan performed 4 weeks prior (05 October 2016).

Discussion

Resistance to endocrine therapies is an important clinical challenge, and continues to drive the search for more effective agents (1). Fulvestrant is effective in patients with metastatic breast cancer, including those who experience progression after endocrine treatment, but is associated with administration and PK limitations at its approved 500 mg once-monthly intramuscular dose. An orally bioavailable SERD, without the bioavailability and PK limitations of fulvestrant, is clearly an unmet medical need.

This first in human study investigated the safety and tolerability of AZD9496: a new, non-steroidal small-molecule inhibitor of ERα, which has shown promise in preclinical models of ER+ advanced breast cancer (17). To our knowledge, this is the first published manuscript reporting results of a completed first in human study with an oral SERD.

AZD9496 was shown to have a tolerable safety profile, with most AEs of CTCAE grade 1 or 2. The most common causally related AEs were diarrhea, fatigue, nausea, and upper abdominal pain, but these were largely mild (grade 1 or 2) and manageable without dose reduction or interruption. Two patients (4.4%) experienced a causally related SAE (diarrhea and abnormal hepatic function tests), and seven patients (15.6%) experienced a causally related grade 3 AE. DLTs were observed in three patients, and all were reversible. One patient (150 mg BID) experienced abnormal hepatic functions, another (400 mg BID) developed grade 3 diarrhea and abnormal hepatic functions, and another (600 mg BID) developed grade 3 diarrhea. However, only one of these patients (receiving 150 mg BID) permanently discontinued AZD9496, following which the abnormal hepatic functions returned to baseline. The other two DLTs (in patients receiving 400 and 600 mg BID) were resolved with dose reduction and/or interruption, and the patients remained on-study. Because no two patients in any cohort experienced a DLT, the MTD was not reached, and 600 mg BID was the maximum dose explored. 600 mg BID was regarded as the MFD on the basis of the number of tablets required for each dose. These findings suggest that AZD9496 is well tolerated and has an acceptable safety profile in this population.

The PK of AZD9496 was characterized by a rapid absorption and fast biphasic decline with a short alpha phase half-life. Based on the interim PK analysis of the first 20 mg QD dose group, the dosing regimen was switched from QD to BID to prolong target coverage. The single-dose AUC increased in reasonable proportion to the increasing dose, up to 400 mg. At 600 mg, a more than dose-proportional increase in AUC and C_{max} was observed. Following multiple dosing, AUC and C_{max} were consistently and dose-dependently lower than for a single AZD9496 dose for 40 mg up to 600 mg. This was presumed to result from auto-induction of cytochrome P450 (CYP) isoenzymes (e.g. CYP3A), as suggested by *in vitro* studies and supported by the dose-dependent increase in the marker for hepatic CYP3A induction (4β-hydroxy-cholesterol:cholesterol ratio). It was assumed that steady-state conditions were reached at Day 15. The clinical relevance of this CYP induction with regards to co-

353 medications and combinability with other cancer drugs is currently unknown and needs further
354 investigation in future clinical studies.

355 We obtained evidence of therapeutic activity in one patient at the 250 mg BID dose who had a
356 confirmed partial response, and experienced a steady fall in levels of tumor marker Ca15.3. The
357 steady-state exposure observed in patients at a dose of 250 mg BID was comparable to that in the
358 pre-clinical patient-derived MCF-7 xenograft model in mice observed at a dose of 5 mg/kg AZD9496,
359 which was the minimal dose required to see significant tumor growth inhibition in this model (21).
360 Furthermore, ten patients (22.2%) were deemed to have stable disease at 6 months or longer
361 follow-up. Based on the clinical activity and the safety and tolerability profile of AZD9496 at the 250
362 mg BID dose, this was selected as the recommended dose for the subsequent AZD9496 study. Paired
363 evaluable tumor biopsies were obtained from five of the 45 patients in the study highlighting the
364 challenges in conclusively assessing proof-of-mechanism in tumor tissue in Phase 1 studies. The
365 pharmacodynamic biopsy data will be presented separately (manuscript in preparation).

366 We note some limitations to this study. Firstly, cohorts were small, containing between four and six
367 patients only, and this may have been insufficient to detect the less frequent effects of AZD9496
368 treatment. Secondly, the minimum washout period between previous anti-cancer regimens and
369 starting AZD9496 treatment was 14 days. Since fulvestrant has a half-life of 50 days, the possibility
370 that these results include synergistic effects of AZD9496 and fulvestrant cannot be ruled out. Thirdly,
371 this study was a non-randomized, non-comparator trial, so assessment of both efficacy and safety
372 may be difficult in this heavily pre-treated, heterogeneous population.

373 This Phase 1 study suggests that AZD9496 has an acceptable safety and tolerability profile, and
374 shows preliminary evidence of prolonged stabilization of disease in some women with heavily
375 pre-treated, advanced breast cancer, including in those previously treated with fulvestrant.
376 A pre-surgical window of opportunity study (NCT03236974) will now compare the pharmacodynamic

effects of AZD9496 (expression of ER, PR, and Ki67 in tumor tissue) with those of fulvestrant in women with hormone receptor positive early breast cancer awaiting surgery with curative intent.

Acknowledgements

We thank all the investigators and site staff, with special thanks to the patients and families. We also wish to thank the following contributors to this study: Razak Abdalla, Aisling Barton-Twomey, Nicola Bateman, Sarah Bujac, Howard A Burris III, Clive Cannon, Karen Clegg, Brian Dayton, Nicola Dearden, Angela Dymond, Wendy Burke, Leigh Ferris, Stuart Findlay, Graham Fisher, Amandine Gojon, Joanne Herbert, Victoria Holmes, Audrey Lawrence, Gayle Marshall, Tony Nash, Sabina Patel, Rebecca Peagram, Terri Peterson, Graham Richmond, Nitharsan Sathiyayogan, Atif Saeed, Mythili Shastry, Edith Simeon, Terry Ulatowski, Ewa Warwick, Denise Yardley, the NIHR/Cancer Research UK Christie Clinical Research Facility and Cancer Research UK, ECMC and NIHR/BRC infrastructure funding for the Cambridge Cancer Centre.

References

1. Jeselsohn R, Buchwalter G, De Angelis C, Brown M, Schiff R. ESR1 mutations-a mechanism for acquired endocrine resistance in breast cancer. Nat Rev Clin Oncol. 2015;12:573–83.

2. Ma CX, Reinert T, Chmielewska I, Ellis MJ. Mechanisms of aromatase inhibitor resistance. Nat Rev Cancer 2015;15:261–75.

3. Dutertre M, Smith CL. Molecular mechanisms of selective estrogen receptor modulator (SERM) action. J Pharmacol Exp Ther 2000;295:431–7.

4. Carlson RW. The history and mechanism of action of fulvestrant. Clin Breast Cancer 2005;6 Suppl 1:S5–8.

5. Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. Annu Rev Med 2011;62:233–47.

6. McDonnell DP, Wardell SE, Norris JD. Oral Selective Estrogen Receptor Downregulators (SERDs), a Breakthrough Endocrine Therapy for Breast Cancer. J Med Chem 2015;58:4883–7.

7. Nardone A, De Angelis C, Trivedi MV, Osborne CK, Schiff R. The changing role of ER in endocrine resistance. Breast 2015;24 Suppl 2:S60–6.

8. Dodwell D, Wardley A, Johnston S. Postmenopausal advanced breast cancer: options for therapy after tamoxifen and aromatase inhibitors. Breast 2006;15(5):584–94.

9. Masri S, Phung S, Wang X, Wu X, Yuan YC, Wagman L*, et al.* Genome-wide analysis of aromatase inhibitor-resistant, tamoxifen-resistant, and long-term estrogen-deprived cells reveals a role for estrogen receptor. Cancer Res 2008;68:4910–8.

10. Di Leo A, Jerusalem G, Petruzelka L, Torres R, Bondarenko IN, Khasanov R*, et al.* Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer. J Clin Oncol 2010;28:4594–600.

11. Vergote I, Robertson JF. Fulvestrant is an effective and well-tolerated endocrine therapy for postmenopausal women with advanced breast cancer: results from clinical trials. Br J Cancer 2004;90 Suppl 1:S11–4.

12. Robertson JF, Bondarenko IM, Trishkina E, Dvorkin M, Panasci L, Manikhas A*, et al.* Fulvestrant 500 mg versus anastrozole 1 mg for hormone receptor-positive advanced breast cancer (FALCON): an international, randomised, double-blind, phase 3 trial. Lancet 2016;388(10063):2997–3005.

13. Spoerke JM, Gendreau S, Walter K, Qiu J, Wilson TR, Savage H*, et al.* Heterogeneity and clinical significance of ESR1 mutations in ER-positive metastatic breast cancer patients receiving fulvestrant. Nat Commun 2016;7:11579.

14. Robertson JF, Harrison M. Fulvestrant: pharmacokinetics and pharmacology. Br J Cancer 2004;90 Suppl 1:S7-10 doi 10.1038/sj.bjc.6601630.

15. Robertson JF, Harrison M. Fulvestrant: pharmacokinetics and pharmacology. Br J Cancer on JF. Fulvestrant (Faslodex) – how to make a good drug better. Oncologist 2007;12:774–84.

16. van Kruchten M, de Vries EG, Glaudemans AW, van Lanschot MC, van Faassen M, Kema IP*, et al.* Measuring residual estrogen receptor availability during fulvestrant therapy in patients with metastatic breast cancer. Cancer Discov 2015;5:72–81.

17. Weir HM, Bradbury RH, Lawson M, Rabow AA, Buttar D, Callis RJ*, et al.* AZD9496: An Oral Estrogen Receptor Inhibitor That Blocks the Growth of ER-Positive and ESR1-Mutant Breast Tumors in Preclinical Models. Cancer Res 2016;76:3307–18.

18. Skolnik JM, Barrett JS, Jayaraman B, Patel D, Adamson PC. Shortening the timeline of pediatric phase I trials: the rolling six design. J Clin Oncol 2008;26:190–5.

19. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R*, et al.* New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.

20. AstraZeneca. Data on File. 2017.

21. Weir HM, Bradbury RH, Lawson M, Rabow AA, Buttar D, Callis RJ, et al. AZD9496: An Oral Estrogen Receptor Inhibitor That Blocks the Growth of ER-Positive and ESR1-Mutant Breast Tumors in Preclinical Models. Cancer Res. 2016;76(11):3307-18.

CCR-17-3102R1: Figure titles and legends

Figure 1 Title:

AZD9496 geometric mean plasma concentration–time profiles following a single dose or multiple doses (n = 3–5 subjects)

Figure 1 Legend:

Panel A: AZD9496 geometric mean plasma concentration following a single dose of AZD9496 on Day 1 (semi-log scale). Panel B: Geometric mean plasma concentration following multiple doses of AZD9496 on Day 15 (semi-log scale). On Day 15 at all doses except 20 mg, the 24 h time point is the 12 h trough concentration following the evening dose of AZD9496 and therefore not shown.

[a]Pooled data from dose escalation and expansion groups.

BID=twice daily; QD=once daily; EXP=expansion cohort.

Figure 2 Title:

Duration on AZD9496 treatment by dose (cohort) and prior fulvestrant

Figure 2 Legend:

Data cut off: 31 January 2017. Patients are ordered on the y-axis by cohort. When a patient received fulvestrant in several lines, the duration of most the most recently received is shown.

BID=twice daily; EXP=dose expansion group; PR=partial response; QD=once daily.

Figure 3 Title:

CT scans showing confirmed partial response in one patient (250 mg BID).

Figure 3 Legend:

Panels A and B: Baseline staging CT. Right pleural nodules with further nodules extending in the right pericardiophrenic fat. Multiple nodules extending along the right oblique and horizontal fissures. Multiple pulmonary nodules.

Figure 2

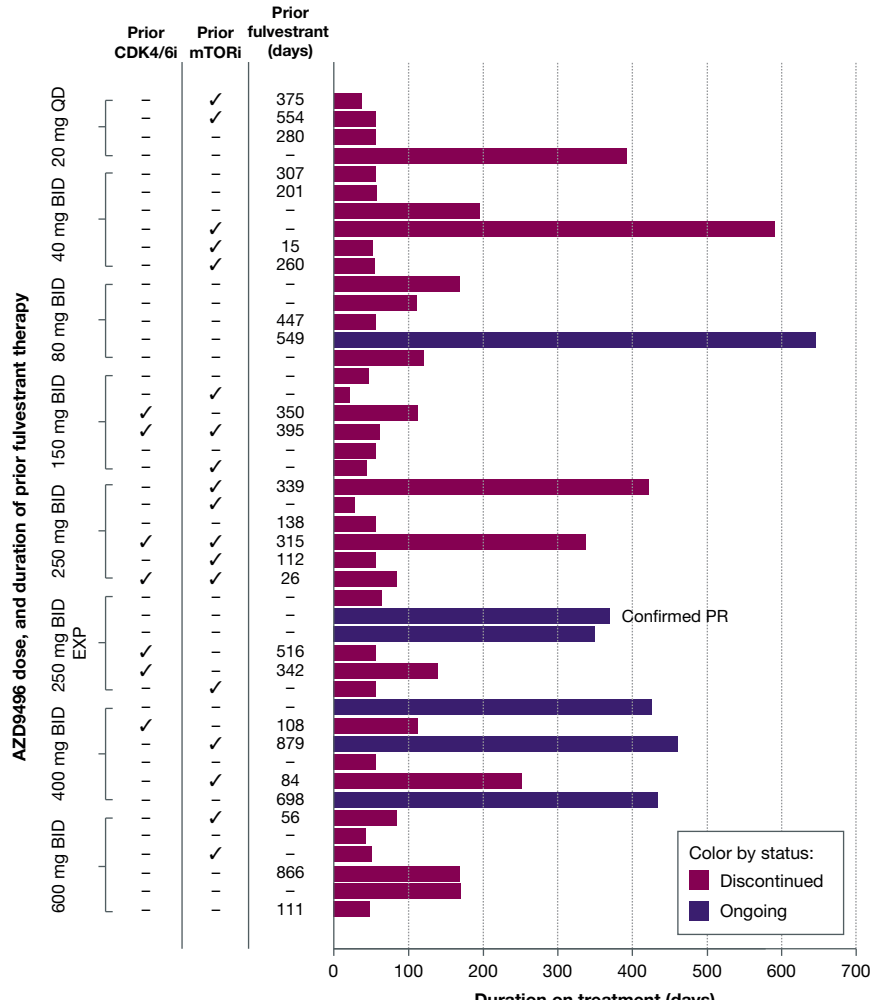
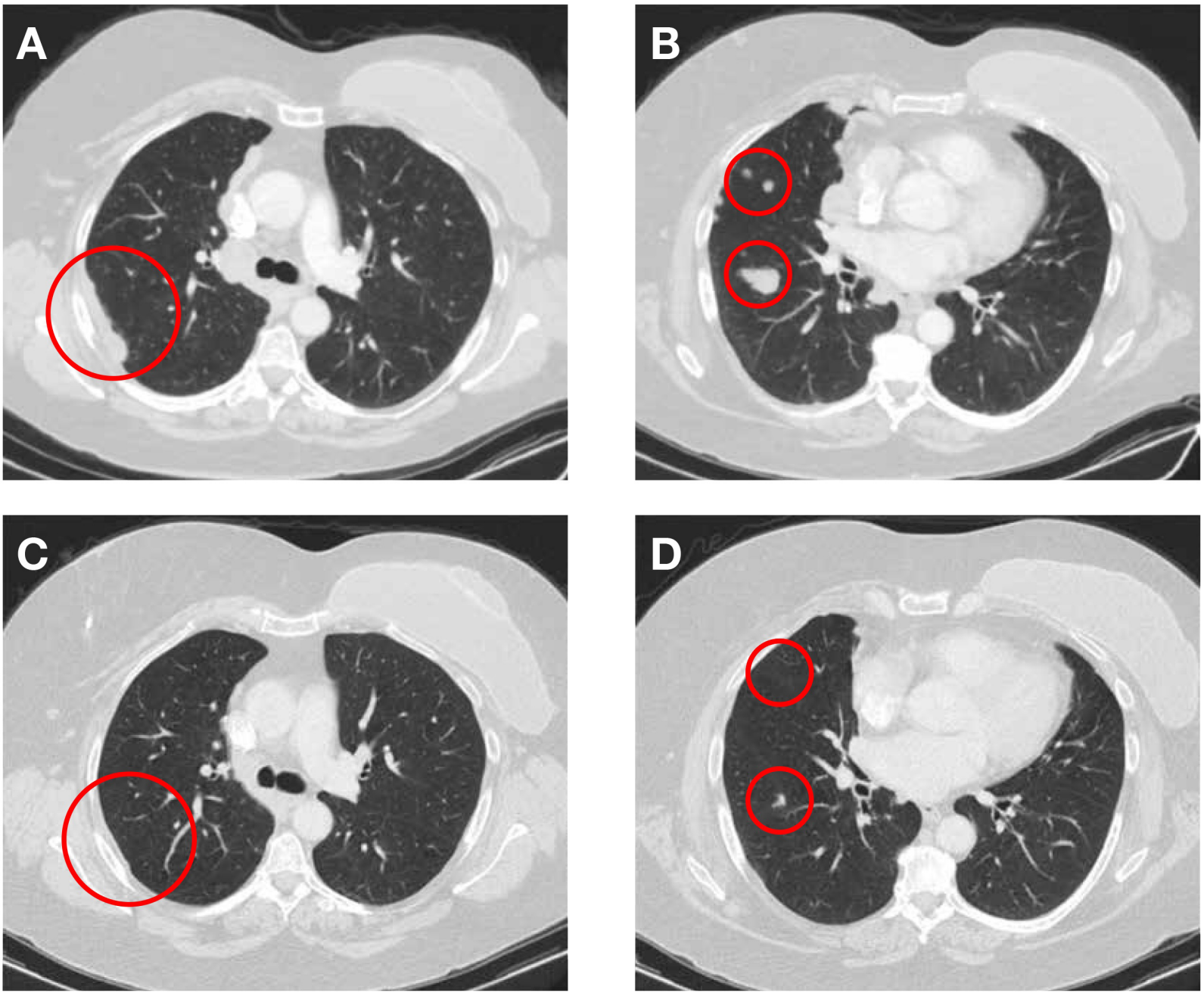
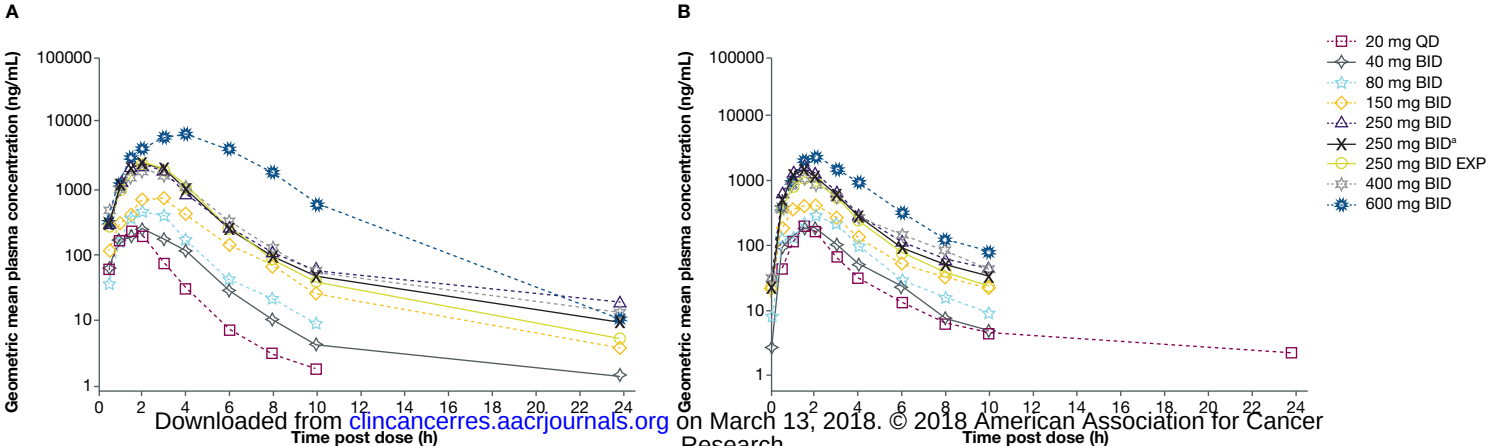


Figure 3





AACR American Association for Cancer Research

Clinical Cancer Research

A first in human study of the new oral selective estrogen receptor degrader AZD9496 for HR+/HER2– advanced breast cancer

Erika P. Hamilton, Manish R. Patel, Anne C Armstrong, et al.

Clin Cancer Res Published OnlineFirst February 13, 2018.