AMEERA-1 phase 1/2 study of amcenestrant, SAR439859, in postmenopausal women with ER-positive/HER2-negative advanced breast cancer

Aditya Bardia1,12, Sarat Chandarlapaty2,12, Hannah M. Linden3,12, Gary A. Ulaner4,5, Alice Gosselin6, Sylvaine Cartot-Cotton6, Patrick Cohen6, Séverine Doroumian7, Gautier Paux8, Marina Celanovic8, Vasiliki Pelekanou8,11, Jeffrey E. Ming9, Nils Ternès6, Monsif Bouaboula8, Joon Sang Lee8, Anne-Laure Bauchet6 & Mario Campone10,12 ✉

AMEERA-1 is a Phase 1/2 open-label single-arm study evaluating once-daily (QD) amcenestrant, an orally bioavailable selective estrogen receptor (ER) degrader, in postmenopausal women with ER+/HER2− advanced breast cancer (NCT03284957), who were mostly heavily pretreated (including targeted therapies and fulvestrant). In the dose escalation phase (Part A: \( n = 16 \)), patients received amcenestrant 20-600 mg QD. Based on absence of dose-limiting toxicities, paired functional \(^{18}\)F-fluorodeoxyglucose positron emission tomography, and pharmacokinetics, 400 mg QD was selected as recommended Phase 2 dose (RP2D) for the dose expansion phase (Part B: \( n = 49 \)). No Grade ≥3 treatment-related adverse events or clinically significant cardiac/eye toxicities were reported. The Part B primary endpoint, confirmed objective response rate (ORR) was 3/45 at the interim analysis and 5/46 (10.9%) at the final analysis. The overall clinical benefit rate (CBR) was 13/46 (28.3%). CBRs among patients with baseline wild-type and mutated ESR1 were 9/26 (34.6%) and 4/19 (21.1%), respectively. Paired tumor biopsy and cell-free DNA analyses revealed ER inhibition and degradation, and a reduction in detectable ESR1 mutations, including Y537S. In conclusion, amcenestrant at RP2D of 400 mg QD for monotherapy is well-tolerated with no dose-limiting toxicities, and demonstrates preliminary antitumor activity irrespective of baseline ESR1 mutation status.

1 Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA. 2 Memorial Sloan Kettering Cancer Center, New York, NY, USA. 3 University of Washington Medical Center, Seattle Cancer Care Alliance, Seattle, WA, USA. 4 Hoag Family Cancer Institute, Newport Beach, CA, USA. 5 University of Southern California, Los Angeles, CA, USA. 6 Sanofi, Paris, France. 7 Sanofi, Montpellier, France. 8 Sanofi, Cambridge, MA, USA. 9 Sanofi, Bridgewater, NJ, USA. 10 Institut de Cancérologie de l’Ouest, René Gauducheau, Saint-Herblain, France. 11 Present address: Bayer US-Pharmaceuticals, Cambridge, MA, USA. 12 These authors contributed equally: Aditya Bardia, Sarat Chandarlapaty, Hannah M. Linden, Mario Campone. ✉ Email: Mario.Campone@ico.unicancer.fr
Among women worldwide, breast cancer is the most prevalent form of cancer, accounting for 24.2% of all cancer diagnoses and 15.0% of cancer-related mortality. The majority (68–73%) of women with breast cancer present with hormone receptor-positive (HR+) estrogen receptor-positive [ER+] and/or progesterone receptor-positive [PgR+] and/or progesterone receptor-positive [PgR+] and/or human epidermal growth factor receptor 2-negative (HER2–) disease, with 14–15% presenting with HER2+ disease and 10–12% with triple-negative disease.[3]

Endocrine therapies, including aromatase inhibitors (AIs, e.g., letrozole), selective ER modulators (SERMs, e.g., tamoxifen), and selective ER degraders (SERDs, e.g., fulvestrant) that block ER signaling via ER inhibition, modulation, or degradation, and hence estrogen-promoted tumor growth, are mainstays of treatment for patients with HR+/HER2– breast cancer.[1]

Although 5-year relative survival rates in the US among women with HR+/HER2– breast cancer are high with localized (100%) or regional (90%) disease, approximately 30% of patients will relapse, often with metastatic disease,[2], which is associated with a low 5-year relative survival (30%).[3]

Among women with advanced or metastatic disease, resistance to endocrine therapies commonly occurs, either because of the development of ESR1 mutations in 12–39% of tumors exposed to AIs[4–10] or poor drug bioavailability in tumors treated with fulvestrant[11]. These and other mechanisms of resistance to endocrine therapies in the metastatic setting, including overexpression of ER coactivators and loss of ER dependence via activation of other oncogenic signals such as via NF1[12–14] or ARID1A[15,16] loss, may differentially affect the efficacy of endocrine therapies.[6] Tumors that have developed resistance to one endocrine therapy and continue to express ER but have not lost ER dependence often respond to an alternative endocrine therapy[17] or to a cyclin-dependent kinase (CDK) 4/6 inhibitor, which are downstream effectors[18] and usually given in combination with endocrine therapy for synergy. For example, ESR1 mutations detected after AI therapy for metastatic breast cancer promote AI resistance while retaining sensitivity to ER antagonism via SERMs or SERDs[8,19,20]. However, the in vivo activity of SERMs and SERDs is often compromised by properties of these drugs related to (i) partial agonism of some SERMs[21], (ii) toxicity profiles of certain drugs potentially contributing to discontinuation[22–24], and (iii) poor bioavailability of some compounds compromising target inhibition.[25]. The latter represents a major limitation of the only approved SERD, fulvestrant[26], whose poor oral bioavailability (requiring intramuscular injections[27] and 1 month to reach steady state[28,29]) and pharmacodynamic limitations (the maximum 500 mg dose does not fully occupy the ER[25]) have contributed to therapy failure[30]. This represents a particular problem for the Y537S mutation in ESR1, which is the second most common ESR1 mutation (14%) requiring higher doses of the drug to achieve occupancy and inhibition of the ER ligand-binding domain and is associated with fulvestrant resistance in vivo.[30]. Thus, there remains a need for potent and non-toxic pure ER antagonists to overcome the limitations of existing endocrine therapies.

Amcenestrant (SAR439859) is an orally bioavailable SERD that demonstrates pure ER antagonism in vivo.[31]. Compared with other SERD agents, amcenestrant provides optimal ER antagonism and degradation. Amcenestrant robustly inhibits the ER signaling pathway in multiple ER+ breast cancer cell lines, including fulvestrant- and tamoxifen-resistant lines, as well as cell lines harboring ER mutations.[32–34]. Preclinical data show that amcenestrant degrades the ER with high efficacy (98%) and potency (0.2 nM) comparable to fulvestrant in vitro activity, is metabolically stable, and has no off-target activity (half maximal inhibitory concentration ≤1 μM) in a large in vitro safety screen panel.[35]. Moreover, in a mouse model of subcutaneous MCF7-Y537S mutant ERα + breast cancer, 18F-fluoroestradiol positron emission tomography (18F-FES PET) imaging confirmed that amcenestrant dose-dependently inhibited tumor uptake of 18F-FES, which correlated with immunohistochemical scoring for ERα expression, and 18F-fluorothyroidine (FLT) PET showed a significant decrease in tumoral FLT accumulation when amcenestrant was combined with palbociclib.[33]. Taken together, these findings support the further development of amcenestrant, alone and in combination therapy, for the treatment of ER+ breast cancer.

Here, we report the final results from Arm 1 of the Phase 1/2 study (AMEERA-1) among postmenopausal women with ER+/HER2− advanced breast cancer, which evaluates the safety, antitumor activity, pharmacokinetics, and pharmacodynamics of amcenestrant administered as monotherapy in dose escalation (Part A) and dose expansion (Part B).

Results
The first patient was enrolled on 6 November 2017 and the last patient was enrolled on 26 March 2020. Each patient had data collected until the end of treatment, which was 22–30 days after the last administration of study treatment. All results are reported as of the cutoff date of 30 March 2021.

Part A dose-escalation phase: patients. In dose escalation (Part A), a total of 16 patients were treated at five dose levels of amcenestrant once daily (QD) monotherapy with dose escalation proceeding according to criteria described in Fig. 1 and Table 1: 20 mg (n = 3), 150 mg (n = 3), 200 mg (n = 4), 400 mg (n = 3), and 600 mg (n = 3). Among these patients, the median age was 59.5 (range 40–79) years, the Eastern Cooperative Oncology Group (ECOG) performance score (PS) was 0 in 62.5% or 1 in 37.5%, 93.8% had visceral metastases (Table 2). The majority of patients were heavily pretreated: the number of prior lines of therapy in the advanced setting ranged from 1 to 8 (93.8% had received ≥2 prior lines); all had received prior endocrine therapy; 81.3% had received prior targeted therapy; and 56.5% prior fulvestrant (Table 2).

Part A dose escalation phase: treatment exposure. The median duration of treatment was 23.6 weeks (range 4–90 weeks). Median (min–max) relative dose intensity was 100% (86–100%). One (6.3%) patient had a dose reduction to 200 mg at the 400 mg dose level. All 16 patients discontinued the study treatment because of progressive disease.

Although six patients were recruited as planned for the BID regimen, only two patients were DLT-evaluable, including two patients who were unable to have 18F-FES PET scans because sites were unable to perform them due to the coronavirus pandemic. In addition, four patients had early progressive disease occurring before the end of cycle 2. Thus, it was decided not to further pursue exploration of this dosing regimen. A summary of these results is provided in Supplementary Notes: BID dosing regimen.

Part A dose-escalation phase: primary endpoints. No dose-limiting toxicity (DLT) was observed during the DLT observation period (cycle 1) at any amcenestrant dose, no adverse event (AE) met DLT criteria definition in subsequent cycles, and the MTD was not reached. The determination of the recommended Phase 2 dose (RP2D) is described in the Part A pharmacodynamics section.
Part A: Dose escalation phase – monotherapy
Postmenopausal women with ER+/HER2− advanced breast cancer

Amcenestrant QD*
DL1 20 mg
DL2bis 150 mg
DL3 200 mg
DL4 400 mg
DL5 600 mg

Table 1 Pre-specified dose-escalation schedule for amcenestrant monotherapy in Part A.

| Dose level (DL)* | Amcenestrant dose (mg) |
|-----------------|------------------------|
| DL(−1) QD       | 10 once daily          |
| DL1 QD          | 20 once daily          |
| DL1bis QD       | 50 once daily          |
| DL2 QD          | 100 once daily         |
| DL2bis QD       | 150 once daily         |
| DL3 QD          | 200 once daily         |
| DL4 QD          | 400 once daily         |
| DL4bis BID      | 200 twice daily        |
| DL5 QD          | 600 once daily         |
| DL5bis BID      | 300 twice daily        |

* DL2bis is investigated if at least one patient at DL1 shows 30% inhibition of the target by 18F-FES PET or all patients treated at DL2 have >85% inhibition of the target by 18F-FES PET. DL dose level, DLT dose-limiting toxicity, ER+ estrogen receptor positive, 18F-FES PET 18F-fluorodeoxyglucose positron emission tomography, HER2—human epidermal growth factor receptor 2-negative, MTD maximum tolerated dose, RP2D recommended Phase 2 dose, PK pharmacokinetics, QD once daily.

Part A dose-escalation phase: secondary endpoints: safety. The most frequently reported (occurring in at least three patients) treatment-emergent adverse events (TEAEs) specifically related to amcenestrant were as follows: hot flush (n = 5; 31.3%), diarrhea and nausea (n = 4 each; 25.0%), as well as decreased appetite, constipation, night sweats, and asthenia (n = 3 each; 18.8%) (Table 3); of these, most were Grade 1. No TEAE led to treatment discontinuation.

Cardiac non-treatment-related TEAEs were reported in 2 patients (Grade 1 palpitations and Grade 2 prolonged QT interval). An eye disorder treatment-related TEAE was reported in 1 patient (Grade 1 photophobia) with an additional two eye disorder non-treatment-related TEAEs in another patient (Grade 1 photophobia and eye irritation).

Part B: Expansion phase – monotherapy
Amcenestrant at RP2D
400 mg QD (n=49)

A pre-specified interim analysis at n=45 evaluable for response at an earlier data cut-off date did not meet the criterion of 5 objective responses, so further accrual to Part B was stopped, but 4 patients who were already in screening were allowed to continue in the study.

Serious TEAEs associated with disease progression were reported in three patients and were considered unrelated to amcenestrant (Grade 3 back pain in a patient treated at 150 mg QD who later died due to disease progression 22 days after amcenestrant discontinuation; Grade 3 dyspnea in one patient treated at 200 mg QD; and Grade 3 fatigue in one patient treated at 600 mg QD). Three other patients enrolled in Part A died (two due to disease progression 123 and 189 days after amcenestrant discontinuation and one due to an unknown reason 44 days after amcenestrant discontinuation).

Part A dose-escalation phase: secondary endpoints: pharmacokinetics. Pharmacokinetic parameters obtained after single and repeated amcenestrant administrations are presented in Supplementary Tables S1 and S2, respectively. Following a single dose on day 1, the pharmacokinetic concentration-time profile obtained from a total of 16 patients in dose escalation generally showed a moderate absorption rate starting from the 150-mg dose (median time to maximum concentration [tmax] ≈ 3 h) followed by a biphasic elimination profile. After repeated dosing (day 22), median tmax generally remained around 3 h (Fig. 2a). No accumulation was observed on day 22 with amcenestrant at doses ≥400 mg QD (Supplementary Table S3). Exposure increased with dose and did not deviate significantly from dose proportionality between amcenestrant 20–600 mg QD after single or multiple administrations (Supplementary Table S4).

Food intake (moderate fat breakfast) modestly increased exposure (Supplementary Table S5) and median tmax was delayed by ≈1 h. However, this effect was not considered as clinically relevant compared with the overall variability after a single-dose administration.

The 4β-hydroxycholesterol pre-/post-treatment geometric mean ratio, measured after 4 weeks of repeated oral administration of amcenestrant, was consistently above 1, starting from the
200-mg QD dose. This ratio indicated that ≥200 mg QD of amcenestrant can induce cytochrome P450 (CYP) 3 A liver enzyme activity and that this induction effect was higher with increasing daily dose intensity (Supplementary Table S6).

Part A dose-escalation phase: secondary endpoints: pharmacodynamics. Functional pharmacodynamics was assessed using 18F-FES PET/computerized tomography (CT) imaging. Because 18F-FES PET/CT scan results for patients treated at the 20-mg dose level did not meet the criteria for testing the 50-mg and 100-mg doses (Fig. 2b and Supplementary Methods: Dose escalation amcenestrant monotherapy), the next dose level tested was 150 mg. High ER occupancy levels were observed from this dose level (Fig. 2b).

A strong pharmacokinetic/pharmacodynamic relationship was established between amcenestrant plasma concentrations and 18F-FES PET percent occupancy of the ER, with an amcenestrant concentration of 100 ng/mL associated with near 90% occupancy or more (Fig. 2b). As per protocol, because all patients treated at the 150-mg dose showed >90% ER occupancy (Fig. 2b) and no DLTs occurred at any dose without reaching the MTD, amcenestrant 400 mg QD was chosen as the RP2D for the dose-expansion phase. Thus, amcenestrant met its primary endpoints for Part A. Median (range) occupancy was 100% (87–100%) with amcenestrant 400 mg QD and 97% (0–100%) across all dose levels. In addition, 18F-FES avidity in pretreatment scans was detected at multiple tumor sites, including lymph nodes and bone, which was markedly reduced in on-treatment scans (Fig. 2c).

| Table 2 Demographic and patient characteristics at baseline in Parts A and B (safety populations) and the biomarker population (Parts A and B excluding the amcenestrant QD 20-mg dose). |
|-----------------|-----------------|-----------------|
| Amcenestrant dose escalation | Amcenestrant 400 mg Part B | Amcenestrant ≥150 mg Biomarkers |
| Part A (N = 16) | Part B (N = 49) | (N = 62) |
| Age, years, median (range) | 59.5 (40–79) | 63.0 (37–88) | 63.0 (37–88) |
| Race, n (%) | | | |
| White | 10/10 (100) | 35/36 (97.2) | 44/45 (97.8) |
| Asian | 0 | 1/36 (2.8) | 1/45 (2.2) |
| Missing | 6 | 13 | 17 |
| ECOG PS, n (%) | 0 | 10 (62.5) | 29 (59.2) | 37 (59.7) |
| 1 | 6 (37.5) | 20 (40.8) | 25 (40.3) |
| ESR1 status at baseline, n (%) | 0 | 5 (31.3) | 27 (55.1) | 31 (50.0) |
| Wild-type | 11 (68.8) | 21 (42.9) | 30 (48.4) |
| Mutated | 0 | 1 (2.0) | 1 (1.6) |
| Visceral metastases, n (%) | 15 (93.8) | 46 (93.9) | 58 (93.5) |
| Prior anti-cancer treatment for advanced or metastatic disease, n (%) | | | |
| Prior chemotherapy | 8 (50.0) | 20 (40.8) | 26 (41.9) |
| Anthracyclines | 2 (12.5) | 2 (4.1) | 3 (4.8) |
| Taxanes | 4 (25.0) | 7 (14.3) | 10 (16.1) |
| Other | 7 (43.8) | 15 (30.6) | 20 (32.3) |
| Prior hormone therapy | 16 (100) | 49 (100) | 62 (100) |
| Aromatase inhibitors | 15 (93.8) | 47 (95.9) | 59 (95.2) |
| SERD (fulvestrant) | 9 (56.3) | 22 (44.9) | 29 (46.8) |
| SARM | 5 (31.3) | 14 (28.6) | 18 (29.0) |
| Prior targeted therapy | 13 (81.3) | 35 (71.4) | 45 (72.6) |
| CDK4/6 inhibitors | 12 (75.0) | 30 (61.2) | 39 (62.9) |
| mTOR inhibitors | 7 (43.8) | 16 (32.7) | 21 (33.9) |
| PI3K inhibitors | 0 | 6 (12.2) | 6 (9.7) |
| Angiogenesis inhibitors | 0 | 1 (2.0) | 1 (1.6) |
| Prior PD-1 inhibitor | 1 (6.3) | 0 | 1 (1.6) |
| Other | 1 (6.3) | 1 (2.0) | 2 (3.2) |
| Prior lines of therapy in advanced settings | | | |
| Minimum-maximum | 1–8 | 1–6 | 1–8 |
| By category, n (%) | | | |
| 1 line | 1 (6.3) | 14 (28.6) | 15 (24.2) |
| 2 lines | 7 (43.8) | 11 (22.4) | 17 (27.4) |
| ≥3 lines | 8 (50.0) | 24 (49.0) | 30 (48.4) |
| Prior lines of hormone therapy in advanced settings | | | |
| Minimum-maximum | 1–6 | 1–6 | 1–6 |
| By category, n (%) | | | |
| 1 line | 3 (18.8) | 17 (34.7) | 20 (32.3) |
| 2 lines | 7 (43.8) | 15 (30.6) | 21 (33.9) |
| ≥3 lines | 6 (37.5) | 17 (34.7) | 21 (33.9) |
| Time from first diagnosis to first study treatment administration, years, median (range) | 9.7 (2.3–22.7) | 6.0 (0.8–24.3) | 6.7 (0.8–24.3) |

CDK 4/6 cyclin-dependent kinase 4 and 6, ECOG PS Eastern Cooperative Oncology Group performance score, ESR1 estrogen receptor 1, PD-1 programmed cell death protein 1, PI3K phosphoinositide 3-kinase, SERD selective estrogen receptor degrader, SARM selective estrogen receptor modulator.

aData on race were not collected for French sites.
Part A dose-escalation phase: secondary endpoints: antitumor activity. All 16 patients were evaluable as per Response Evaluation Criteria In Solid Tumors (RECIST) v.1.1 per investigator/local radiologists review, 1 (6.3%) patient, treated at 150 mg QD, had a confirmed partial response (PR) as best overall response, 8 (50%) patients had stable disease (SD), and 7 (43.8%) patients had progressive disease (PD), giving an objective response rate (ORR) of 6.3% and clinical benefit rate (CBR) of 50.0% (Table 3).

Tumor shrinkage (decrease from baseline in the sum of diameters of target lesions) was observed in 10 (62.5%) patients (Fig. 3a).

Part B dose-expansion phase: Patients. In dose expansion (Part B), an interim analysis was planned after 45 patients were evaluable for response, the results of which stopped accrual to Part B. By the time the 45th patient was enrolled and treated in the study, four patients were already in screening. These four additional patients were allowed to enter the trial, giving a total of 49 patients (Fig. 1). Among these 49 patients, median age was 63.0 (range 37–88) years, 59.2% had ECOG PS of 0, 93.9% had visceral metastases (Table 2). The majority of patients were heavily pretreated: the number of prior lines of therapy in the advanced setting ranged from 1 to 6 (71.4% had received ≥2 prior lines), all had received prior endocrine therapy, 71.4% had received prior targeted therapy, and 44.9% prior fulvestrant (Table 2).

Part B dose-expansion phase: treatment exposure. The median duration of treatment was 10.1 weeks (range 1–117 weeks). Median (min–max) relative dose intensity was 100% (66–105%).

| Table 3 Overview of adverse event profile in Part A and Part B (patients with TEAEs occurring in at least three patients and their associated treatment-related TEAEs)—safety populations. |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| **Part A (N = 16)** | **All grades** | **Grade 1** | **Grade 2** | **Grade ≥3** | **Part B (N = 49)** | **All grades** | **Grade 1** | **Grade 2** | **Grade ≥3** |
| **Any TEAE** | 16 (100) | 5 (31.3) | 7 (43.8) | 4 (25.0) | 13 (81.3) | 10 (62.5) | 3 (18.8) | 0 |
| **Constipation** | 6 (37.5) | 5 (31.3) | 1 (6.3) | 0 | 3 (18.8) | 3 (18.8) | 0 | 0 |
| **Decreased appetite** | 6 (37.5) | 6 (37.5) | 0 | 0 | 3 (18.8) | 3 (18.8) | 0 | 0 |
| **Diarrhea** | 6 (37.5) | 5 (31.3) | 1 (6.3) | 0 | 4 (25.0) | 3 (18.8) | 1 (6.3) | 0 |
| **Hot flush** | 6 (37.5) | 6 (37.5) | 0 | 0 | 5 (31.3) | 5 (31.3) | 0 | 0 |
| **Nausea** | 6 (37.5) | 3 (18.8) | 3 (18.8) | 0 | 4 (25.0) | 3 (18.8) | 1 (6.3) | 0 |
| **Arthralgia** | 5 (31.3) | 2 (12.5) | 3 (18.8) | 0 | 2 (12.5) | 1 (6.3) | 1 (6.3) | 0 |
| **Asthma** | 4 (25.0) | 2 (12.5) | 2 (12.5) | 0 | 3 (18.8) | 3 (18.8) | 0 | 0 |
| **Dyspnea** | 4 (25.0) | 2 (12.5) | 1 (6.3) | 1 (6.3) | 1 (6.3) | 1 (6.3) | 0 | 0 |
| **Fatigue** | 4 (25.0) | 3 (18.8) | 0 | 1 (6.3) | 2 (12.5) | 2 (12.5) | 0 | 0 |
| **Urinary tract infection** | 4 (25.0) | 0 | 3 (18.8) | 1 (6.3) | 0 | 0 | 0 | 0 |
| **Back pain** | 3 (18.8) | 2 (12.5) | 0 | 1 (6.3) | 1 (6.3) | 1 (6.3) | 0 | 0 |
| **Hypoesthesia** | 3 (18.8) | 3 (18.8) | 0 | 0 | 0 | 0 | 0 | 0 |
| **Night sweat** | 3 (18.8) | 3 (18.8) | 0 | 0 | 0 | 0 | 0 | 0 |
| **Part B (N = 49)** | 49 (100) | 11 (22.4) | 22 (44.9) | 16 (32.7) | 26 (53.1) | 21 (42.9) | 5 (10.2) | 0 |
| **Any TEAE** | 49 (100) | 12 (24.5) | 2 (4.1) | 0 | 3 (6.1) | 2 (4.1) | 1 (2.0) | 0 |
| **Constipation** | 14 (28.6) | 10 (20.4) | 3 (6.1) | 1 (2.0) | 4 (8.2) | 4 (8.2) | 0 | 0 |
| **Vomiting** | 14 (28.6) | 8 (16.3) | 2 (4.1) | 2 (4.1) | 2 (4.1) | 2 (4.1) | 0 | 0 |
| **Fatigue** | 12 (24.5) | 8 (16.3) | 2 (4.1) | 0 | 2 (4.1) | 2 (4.1) | 0 | 0 |
| **Abdominal pain** | 11 (22.4) | 9 (18.4) | 1 (2.0) | 1 (2.0) | 2 (4.1) | 2 (4.1) | 0 | 0 |
| **Nausea** | 11 (22.4) | 9 (18.4) | 1 (2.0) | 1 (2.0) | 2 (4.1) | 2 (4.1) | 0 | 0 |
| **Arthralgia** | 10 (20.4) | 7 (14.3) | 3 (6.1) | 0 | 4 (8.2) | 3 (6.1) | 1 (2.0) | 0 |
| **Asthma** | 10 (20.4) | 7 (14.3) | 3 (6.1) | 0 | 2 (4.1) | 2 (4.1) | 0 | 0 |
| **Diar...
**Fig. 2** Part A dose escalation: amcenestrant plasma concentration-time profiles, pharmacokinetic/pharmacodynamic relationships, and 18F-FES PET/CT images. 

**a** Mean amcenestrant plasma concentration-time profiles observed following repeated oral administration (cycle 1, day 22) under fasting conditions; **b** pharmacokinetic/pharmacodynamic relationship between plasma concentrations of amcenestrant measured just before 18F-FES radioisotope administration and individual mean percent occupancy of estrogen receptors (mean percent reduction in 18F-FES SUV\textsubscript{max}(cor)); **c** representative 18F-FES PET; and **d** coronal CT images at screening and on-treatment with amcenestrant 150 mg. 18F-FES PET/CT scans were performed 16–24 h after the previous amcenestrant dose, except for two patients whose scans occurred 8 h after the previous dose. Of 16 patients, 14 patients had scans at baseline and between 11–15 days after first amcenestrant administration (as per protocol) and two patients had post-baseline scans on days 10 and 28, respectively. The patient with a post-baseline scan on day 10 was included in the pharmacodynamic analysis because the scan occurred after ≥8 days of continuous treatment, but the patient receiving amcenestrant 200 mg with a post-baseline scan on day 28 was excluded from pharmacodynamic analysis and dose escalation decisions. **c** Annotated example of the reduction in SUV\textsubscript{max}(cor) for the first index lesion in the 5th lumbar vertebra; physiologic 18F-FES avidity is noted in the liver, intestines, and bladder. **d** Annotated example of the reduction in tumor diameter of a right pelvic lymph node in the associated CT scan.
Five (10.2%) patients had dose reductions to 200 mg QD and 2 (4.1%) patients had at least 7 consecutive days of dose omission. A total of 46 (93.9%) patients discontinued amcenestrant, including 43 (87.8%) due to progressive disease, 1 (2%) for a non-treatment-related TEAE (pneumonia), and 2 (4.1%) for other reasons (HER2 amplification detected, chest wall resection). Three (6.1%) patients remained in treatment at the date of data cutoff.

**Part B dose-expansion phase: primary endpoint.** At the interim analysis of antitumor activity, which followed the Simon 2-stage design, 45 (91.8%) participants in Part B were assessed for an interim futility analysis at the data cutoff of 30 May 2020. At that time, an objective response based on independent central review (ICR) was observed in 3/45 (6.7%) participants, which did not meet the prespecified criterion of at least five responders; therefore, the primary endpoint for Part B was not met and accrual to Part B was stopped, excepting four patients who were already in treatment when the study was stopped. The primary endpoint for Part B was not met and accrual to Part B was stopped, excepting four patients who were already in treatment when the study was stopped.

A total of 46/49 (93.9%) patients were evaluable as per RECISTv.1.1 in Part B dose expansion. The ORR by ICR was 5/46 (10.9%), comprising 1 confirmed complete response (CR) and 4 confirmed PRs (Table 4).

**Part B dose-expansion phase: secondary endpoints: safety.** All patients experienced at least one TEAE (all grades) during Part B, regardless of the relationship with amcenestrant, and one Grade ≥3 TEAE of pneumonia led to treatment discontinuation. The most frequently reported TEAEs (in at least three patients) specifically related to amcenestrant were as follows: hot flush (10.2%), vomiting and arthralgia (8.2%), and constipation and gastroesophageal reflux disease (6.1%) (Table 3); of these, most were Grade 1. At least one Grade ≥3 TEAE occurred in 16/49 (32.7%) patients; however, none of these events were considered related to amcenestrant (Table 3).

One cardiac non-treatment-related TEAE was reported (Grade 1 sinus bradycardia). Three eye disorder non-treatment-related TEAEs were reported in two patients (Grade 2 eyelid erythema and Grade 1 visual impairment in one patient, and Grade 1 macular edema in the other patient).

Serious TEAEs occurred in 13/49 (26.5%) patients (Supplementary Table S7) and none were considered related to amcenestrant by the investigator. Of the 13 patients with serious AEs, two died at 1 and 4 days after amcenestrant discontinuation.

The first patient died due to pneumonia (clinical context: on amcenestrant from days 1–10, disease progression observed from day 1, Grade 3 TEAE of pneumonia on day 5, despite treatment for pneumonia the patient died on day 11), and the second due to an unknown cause (clinical context: on amcenestrant from days 1 to 64, pleural target lesion and bone non-target lesion found on CT on day 11, pleural and bone lesions had progressed and a new liver lesion was found on CT on day 53, amcenestrant was discontinued due to disease progression on day 64, the patient died on day 68). Two other patients enrolled in Part B died during the follow-up period at 32 and 69 days after amcenestrant discontinuation, both due to disease progression.

**Part B dose-expansion phase: secondary endpoints: pharmacokinetics.** Pharmacokinetic parameters obtained after single and repeated QD administrations of amcenestrant 400 mg are presented in Supplementary Table S8 and were consistent with those measured observed between day 1 and day 22 with geometric accumulation ratios of 0.93 (90% confidence interval [CI]: 0.75–1.15) and 0.81 (90% CI: 0.64–1.01) based upon the area under the curve over 24 h (AUC_0-24h) and maximum concentration (C_{max}), respectively. After repeated amcenestrant 400 mg dose QD administration, moderate total variability was observed on day 22 (coefficients of variation [CVs] for C_{max} and AUC_0-24h were 28.0% and 37.5%, respectively), apparent total clearance of the drug from plasma at steady state (CLss/F) was low (10.6 L/h), and geometric mean (CV) trough concentration (C_{trough}) reached was 466 ng/mL (87.3%).

Amcenestrant excreted in urine over the 24-h interval after QD administration represented <0.1% of the administered dose, indicating no renal clearance of the parent drug.

Results for the 4β-hydroxycholesterol pre-/post-treatment geometric mean ratio were similar to those observed in Part A (Supplementary Table S6).

**Part B dose-expansion phase: secondary endpoints: antitumor activity.** ORRs by baseline ESR1 status (wild-type and mutated) were 15.4% and 5.3%, respectively, by ICR review; corresponding values for CBR were 34.6% and 21.1%, respectively (Table 4).

Median time to first response was 8.1 weeks by investigator/local radiologists review.

Tumor shrinkage occurred in 21/41 (51.2%) patients (Fig. 3a by ICR).

Duration of treatment up to 117 weeks were observed (Fig. 3b).

The ORR by investigator/local radiologists review was 4/46 (8.7%) with a median time to first response of 8.1 weeks and with three patients still under treatment at the date of data cutoff. Clinical Benefit was achieved in 12/46 (26.1%) patients according to the investigator/local radiologists review.

**Part B dose-expansion phase: post hoc exploratory subgroup analyses of antitumor activity.** Because most patients recruited to the study were heavily pretreated with up to eight lines of prior therapy in the advanced setting (76.9% ≥2 prior lines), 73.8% had received prior targeted therapies, and 47.7% prior fulvestrant, we conducted three post hoc exploratory analyses for the Part B primary endpoint of antitumor activity. Among patients who had received ≤3 prior lines of therapy in the metastatic setting (n = 27), the ORR was 18.5% and the CBR was 44.4%; among patients who had not received prior CDK4/6 inhibitors (n = 19), the ORR was 26.3% and CBR 52.6%; and among patients who had not received prior mTOR or CDK4/6 inhibitors or fulvestrant (n = 11), the ORR was 36.4% and CBR was 54.5% (Table 5).

**Post hoc exploratory biomarker analyses.** In order to maximize the sample size and exposure to a pharmacodynamically active amcenestrant dose for post hoc exploratory biomarker analyses, we selected a pooled population of patients from Parts A and B who had received an amcenestrant dose ≥150 mg. We chose this dose cutoff because doses ≤150 mg showed high levels of ER occupancy on 18F-FES PET scans, whereas ER occupancy was very low for two of the three patients receiving amcenestrant 20 mg (Fig. 2b).

The biomarker population comprised 62 patients: 13 received amcenestrant QD ≥150 mg during Part A and 49 during Part B. Among these patients, median age was 63 (range 37–88) years, 59.7% had ECOG PS of 0, 93.5% had visceral metastases (Table 2), and the majority were heavily pretreated; the number of prior lines of therapy in the advanced setting ranged from 1 to 8 (48.4% had received ≥3 prior lines), all had received prior endocrine.
**Fig. 3 Waterfall and swimmer plots.** a Waterfall plot of best relative change from baseline in the sum of diameters of target lesions in the response-evaluable populations of Part A by local investigators/radiologists review ($n = 16$) and Part B by independent central review ($n = 41$; three patients were missing relative change data and two patients had a non-evaluable best overall response) and b Swimmer plot of duration of treatment in the safety populations of Part A ($n = 16$) and Part B ($n = 49$) with overall responses assessed by local investigators/radiologists review. CDK4/6i cyclin-dependent kinase 4/6 inhibitor, chemo chemotherapy, ICR independent central review, Local local investigators/radiologists review, mTORi mammalian target of rapamycin inhibitor, SERD selective estrogen receptor degrader. Checkboxes correspond to baseline characteristics.
therapy, 72.6% had received prior targeted therapy, and 46.8% prior fulvestrant (Table 2).

In ER degradation/pathway inhibition analyses, amcenestrant demonstrated robust on-target activity as shown by overall reductions in ER expression (ER degradation) (Fig. 4a; median relative change from screening was −58% [range −93 to −44%] among patients with non-zero values at screening and available data at cycle 2, day 28; n = 6), reduction in PgR expression (Fig. 4b; median relative change from screening was −88% [range −100 to −38%]; n = 3), reduction in Ki67 protein expression (Fig. 4c; median change from screening in percentage of Ki67 positive cells was −8% [range −30 to +30%]; n = 7), and reduction in ER activation score by gene set variation analysis (GSVA) (Fig. 4d; median change from screening was −0.4 [range −1.1 to +0.4]; n = 5). ER or PgR nucleus H-scores decreased at cycle 2, day 28 in all patients with non-zero screening values. Among patients with ER or PgR nucleus H-scores equal to zero at screening, one patient had an increase in PgR nucleus H-score at cycle 2, day 28. ER activation score decreased or remained stable only among patients who showed clinical benefit (median change from screening was −0.6 [range −1.1 to 0]; n = 4), whereas ER activation score increased for the patient with no clinical benefit (change from screening: +0.4).

Among response-evaluable patients in the biomarker population with ESR1 mutations at cycle 1, day 1 (28/58; 48.3%) detected by multiplex droplet digital polymerase chain reaction (ddPCR) in circulating free DNA (cfDNA), the ORR was 1/28 (3.6%) detected by ddPCR in circulating free DNA (cfDNA). The ORR to amcenestrant was 1/28 (3.6%) detected by ddPCR in circulating free DNA (cfDNA) and 0/28 (0.0%) detected by ddPCR in circulating free DNA (cfDNA) in patients with ESR1 mutations.

Among response-evaluable patients in the biomarker population with ESR1 mutations at cycle 1, day 1 (28/58; 48.3%) detected by multiplex droplet digital polymerase chain reaction (ddPCR) in circulating free DNA (cfDNA), the ORR was 1/28 (3.6%) detected by ddPCR in circulating free DNA (cfDNA) and 0/28 (0.0%) detected by ddPCR in circulating free DNA (cfDNA) in patients with ESR1 mutations (Table 6).

Among response-evaluable patients in the biomarker population with available data for ddPCR at cycle 1, day 1 and at cycle 2, day 28 (n = 31), 14/31 (45.2%) patients had at least one ESR1 mutation at cycle 1, day 1 (total number of mutations = 27). Of these 14, 13/14 (92.9%) demonstrated decreases in allele frequency from cycle 1, day 1 to cycle 2, day 28 for at least one mutation during amcenestrant treatment for both patients with and without clinical benefit (Fig. 4e).

Amcenestrant therapy

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**Table 4 Prespecified analyses of antitumor activity in the response-evaluable populations of Parts A and B.**

| Population | Part A | Part B | Part B | Part B |
|------------|--------|--------|--------|--------|
| Review method | Local | ICR | ICR | ICR |
| Endpoint | Secondary | Interim | Primary | Wild-type | Mutated |
| Subgroup |  |  |  |  |
| Best overall response, n (%) |  |  |  |  |
| Number | 16 | 45 | 46 | 26 |
| Complete response (CR)a | 0 | 1 | 2 | 1 |
| Partial response (PR)a | 1 | 4 | 8 | 7 |
| Stable disease (SD) | 8 | 50.0 | 19 | 42.2 |
| Progressive disease (PD) | 7 | 43.8 | 19 | 42.2 |
| Not evaluable (NE) | 0 | 2 | 4 | 4 |
| Objective response rate, n (%) | 1 | 0.3-26.4 | 5 | 11.1 |
| [90% CI]b | 5 | 10.9 | 4.4-22.0 | 4.4-21.0 |
| Clinical benefit rate, n (%) | 8 | 27.9-72.1 | 13 | 28.9 |
| [90% CI]a | 18.0-42.0 | 13 | 28.3 | 17.6-41.1 |
|  | 9 | 34.6 | 19.4-52.6 | 4 | 21.1 |

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**Table 5 Post hoc subgroup analyses by <3 prior lines of therapy in the metastatic setting, by no prior CDK4/6 or mTOR inhibitors or fulvestrant, and by no prior CK4/6 inhibitors in the Part B response-evaluable population by independent central review.**

| ≤3 prior lines in the metastatic settingd | No prior CDK4/6i, mTORi, or fulvestranfe | No prior CDK4/6if |
|----------------------------------------|---------------------------------|-----------------|
| Best overall response, n (%) | 27 | 11 | 19 |
| Number | 1 | 4 | 4 |
| Complete response (CR)a | 1 | 0 | 1 |
| Partial response (PR)a | 4 | 4 | 4 |
| Stable disease (SD) | 12 | 44.4 | 37.0 |
| Progressive disease (PD) | 10 | 37.0 | 27.3 |
| Not evaluable | 0 | 0 | 0 |
| Objective response rate, n (%) | 5 | 18.5 | 35.1 |
| [90% CI]b | 5 | 13.5-65.0 | 5 | 26.3 |
| Clinical benefit rate, n (%) | 5 | 28.0-61.8 | 5 | 26.3 |
| [90% CI]c | 5 | 27.1-80.0 | 5 | 26.3 |
| [90% CI]f | 10 | 52.6 | 32.0-72.6 |

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**Table 3 Post hoc subgroup analyses by <3 prior lines of therapy in the metastatic setting, by no prior CDK4/6 or mTOR inhibitors or fulvestrant, and by no prior CK4/6 inhibitors in the Part B response-evaluable population by independent central review.**
decreased the number of most ESR1 mutations (including known resilient alleles D538G and Y537S). At cycle 2, day 28, 10/27 (37.0%) of these mutations were no longer detectable (Fig. 4f).

**Discussion**

In the current AMEERA-1 study, among postmenopausal women with metastatic ER+/HER2− breast cancer treated with amcenestrant ≥20 mg QD as monotherapy, we observed an absence of DLTs without reaching the maximum tolerated dose (MTD), a favorable safety profile, and a strong pharmacokinetic/pharmacodynamic relationship between plasma amcenestrant concentration and saturation of ER occupancy in multiple tumor sites by 18F-FES PET/CT imaging at doses ≥150 mg as well as evidence of antitumor activity. On this basis, amcenestrant 400 mg QD was chosen as the monotherapy RP2D for the dose-expansion phase.

Amcenestrant demonstrated overall median ER occupancy of 97% on 18F-FES PET/CT scans across all doses (n = 15) and in
multiple tumor sites, including lymph nodes, lung, and bone. Median occupancy was 100% at the RP2D of amcenestrant 400 mg QD (n = 3; range 87–100%). In contrast, fulvestrant 500 mg has shown median ER occupancy levels of 85% (n = 16; range –60% to 99%), with lower levels of ER occupancy being associated with an increased risk of disease progression.26,27.

Although 18F-FES PET/CT imaging can assess ER occupancy to its ligand estrogen, it does not constitute formal proof that amcenestrant degrades ERs in tumors; however, our paired tumor biopsy analyses confirmed that amcenestrant achieved on-target activity (ER degradation/pathway inhibition) among women with metastatic ER+/HER2− breast cancer whose tumors carried multiple ESR1 mutations (Fig. 4a–d). Of note, although all patients were ER+ at the diagnostic biopsy, which may have been conducted before enrollment or screening, some patients had ER nucleus H-scores of zero at the screening biopsy, which may have arisen because of ER degradation from prior exposure to fulvestrant or to tissue sampling bias.

Various ESR1 mutations were detected at baseline, including D538G, Y537S, and Y537N. The proportion of patients with ESR1 mutations at baseline in the current study (49.2%) was higher than that generally reported in advanced/metastatic breast cancer (36.4%)10, but was consistent with other published studies among patients with hormone-receptor-positive metastatic breast cancer receiving monotherapy with fulvestrant or oral SERDs where rates as high as 65% have been reported.35–38. This could suggest that patients with difficult-to-treat ESR1 mutations tend to be overrepresented in the clinical trial setting. On treatment at cycle 2 day 28, amcenestrant reduced the number of most of these ESR1 mutations in cfDNA, and in 37% of cases, these mutations were no longer detectable, including known resilient Y537S and D538G mutations (Fig. 4f). Moreover, amcenestrant showed clinical benefit independent of ESR1 mutation status, demonstrating activity in tumors with ESR1 mutations known to be associated with endocrine resistance. Thus, mechanisms other than ESR1 mutations appear to drive resistance to amcenestrant among these patients.

Amcenestrant is orally administered QD and has a favorable pharmacokinetic profile, whereas fulvestrant, the only approved SERD, has low oral bioavailability and must be administered as two large-volume intramuscular injections with up to 1 month to reach a steady state.28. Amcenestrant showed limited accumulation; and exposure increased proportionally with doses up to 600 mg after repeated oral administration. At the 400 mg dose, exposure showed moderate total variability (<40%) associated with a low systemic clearance (10.6 L/h), and no elimination in the urine. The increase in exposures when administered with food was considered a moderate effect when compared with the larger overall variability of pharmacokinetic exposures, allowing amcenestrant to be administered regardless of food status. The mean C_{trough} concentration obtained at the 400 mg dose level (geometric mean 466 ng/mL, Supplementary Table S8) was well above the minimum concentration associated with near 90% occupancy or more of ERs (Fig. 2b). This C_{trough} was about 40 times higher (in molar concentration) than that for fulvestrant (12.5 ng/mL) at steady state after 500 mg intramuscular administration (day 1, 4 of cycle 1, 500 mg monthly from cycle 2).29

In dose expansion, the primary endpoint evaluating antitumor activity showed an ORR by ICR of 5/46 (10.9%), comprising 1 CR and 4 PRs, and a CBR of 13/46 (28.3%). It is important to note that most patients were heavily pretreated with up to eight lines of therapy in the advanced setting, including up to six lines of hormonal therapies, and prior treatment with chemotherapy, fulvestrant, and cyclin-dependent kinase 4 and 6 (CDK4/6), phosphoinositide 3-kinase (PI3K), and mammalian target of rapamycin (mTOR) inhibitors.

Although cross-trial comparisons should be conducted with caution and the current trial sample size was small, we indirectly compared ORR and CBR with methodologically similar studies investigating the recommended 500 mg dose of fulvestrant monotherapy in the second or third-line setting among postmenopausal women (or where pre- or perimenopausal women had received a gonadotropin-releasing hormone analog prior to randomization that continued throughout the study) with advanced/metastatic ER+/HER2− breast cancer and whose tumor responses were confirmed as per REGIST v1.1 in populations with measurable disease. Four studies met these criteria. The PALOMA-340 and FAKTION41 studies recruited women who had received up to three prior lines of endocrine therapy and the SOLAR-142 and SANDIPPER43 studies with up to two prior lines. The PALOMA-3 study excluded women who had received prior fulvestrant and CDK4/6, PI3K, and/or mTOR inhibitors. The FAKTION, SOLAR-1, and SANDIPPER studies excluded women who had received prior fulvestrant and PI3K inhibitors, and the SOLAR-1 and SANDIPPER studies also excluded women who had received prior mTOR inhibitors. All four comparator studies investigated less heavily pretreated patients than those in AMEERA-1. In these studies, the fulvestrant monotherapy ORR ranged from 10.9 to 16.2% and CBR ranged from 36.0 to 44.1%40–43.

Our post hoc exploratory subgroup analyses assessed the impact of prior therapies on antitumor activity with amcenestrant. Among women who had received ≤3 prior advanced lines in the metastatic setting (n = 27), the ORR was 18.5% and CBR was 44.4%, which appeared numerically comparable to historical results with fulvestrant monotherapy.40–43. Among women who had not received prior CDK4/6 inhibitors (n = 19), the ORR was 26.3% and CBR 52.6%. Among women who had not received prior CDK4/6 or mTOR inhibitors or fulvestrant (n = 11), the ORR was 36.4% and CBR was 54.5%. Given that endocrine therapy plus a CDK4/6 inhibitor is now standard of care for the first-line management of patients with ER+/HER2− advanced/metastatic breast cancer,44 which was not established practice at the time AMEERA-1 was designed, we recognize that patients who had not received a prior CDK4/6 inhibitor (35.4% in the current study) had a greater antitumor response to amcenestrant than patients who had received a prior CDK4/6 inhibitor.
However, because AMEERA-1 was a first-in-human Phase 1 study, it was important to evaluate the safety and antitumor activity of amcenestrantr in a broad population of patients with advanced/metastatic breast cancer regardless of prior therapy use.

Amcenestrantr demonstrated a favorable safety profile and treatment was well-tolerated. No TEAEs led to treatment discontinuation and most were Grade 2 or less. No serious AEs were considered treatment-related by investigators and no treatment-related TEAEs of Grade ≥3 occurred. Among listed serious AEs (Supplementary Table S7), no clinically significant cardiac or eye safety findings were observed, whereas these AEs have been reported with other orally administered SERDs in development3,24. Study strengths include the combined evaluation of safety, antitumor activity, and pharmacokinetics/pharmacodynamics demonstrating robust target engagement and ER degradation/pathway inhibition in ER+/HER2−- advanced breast cancer. Study limitations of this Phase 1/2 trial include the small sample size because the prespecified interim analysis results after at least four cycles of amcenestrantr therapy led to stopping accrual to Part B. With continued follow-up and monitoring, patients who had responded at the final analysis cutoff date of 30 March 2021. Had these responses been observed earlier than the cutoff date of 30 May 2020, Part B accrual would have continued. However, the knowledge that amcenestrantr could be associated with ongoing responses after prolonged therapy was lacking at the time of the design of the study. Given that prolonged responses with amcenestrantr were observed, including encouraging clinical benefit rates, and with a very promising safety profile, we considered it unnecessary to reopen the study because sufficient information on the compound had been gained to justify continuing with the amcenestrantr clinical development program in Phase 2 and Phase 3 trials. Additional limitations included, a lack of racial diversity, not all patients having paired tumor biopsies, and the absence of a control arm, requiring validation of these results in larger comparative studies.

Amcenestrantr development plan studies in specific populations include: the ongoing AMEERA-1 investigating amcenestrantr in combination with palbociclib (Parts C and D), alpelisib (Parts F and G), everolimus (Parts H and I), or abemaciclib (Parts J and K); AMEERA-3 (NCT04059484), a randomized Phase 2 trial investigating amcenestrantr monotherapy versus physician’s choice of endocrine therapy among patients with ER+/HER2−- locally advanced or metastatic breast cancer with prior exposure to hormonal therapies; AMEERA-4 (NCT04191382), a window of opportunity study investigating amcenestrantr at two dose levels versus letrozole on change in Ki67 (percentage of positive tumor cells) among newly diagnosed preoperative postmenopausal women with ER+/HER2−- breast cancer; and AMEERA-5 (NCT04478266), a randomized, multicenter, double-blind, Phase 3 study, investigating amcenestrantr plus palbociclib versus letrozole plus palbociclib among patients with ER+/HER2−- breast cancer who have not received prior systemic anti-cancer treatment for advanced disease.

In conclusion, amcenestrantr, an oral SERD, at RP2D of 400 mg QD for monotherapy showed no DLTs and a favorable safety profile with no clinically significant cardiac or eye safety findings, and demonstrated preliminary antitumor activity irrespective of baseline ESR1 mutation status among postmenopausal women with metastatic ER+/HER2−- breast cancer, most of whom were heavily pretreated with targeted therapies and/or fulvestrant. Amcenestrantr exhibited pharmacodynamic evidence of ER degradation/pathway inhibition on biomarker analysis with functional imaging and paired tumor biopsies, and decreased ESR1 mutations, including known resilient alleles D538G and Y537S.

**Methods**

**Study design.** AMEERA-1 (NCT03284957) is a multinational, open-label, non-randomized, dose-escalation and dose-expansion, safety, antitumor activity, pharmacokinetics, and pharmacodynamic study consisting of five arms, each with two parts as follows: Arm 1, amcenestrantr administered orally as monotherapy in 28-day cycles in dose escalation (Part A) and dose expansion (Part B) cohorts; Arm 2, amcenestrantr combined with palbociclib in dose escalation (Part C) and dose expansion (Part D) cohorts; Arm 3, amcenestrantr combined with everolimus in dose run-in (Part F) and dose expansion (Part G) cohorts; Arm 4, amcenestrantr plus everolimus in dose escalation (Part H) and dose expansion (Part I) cohorts; and Arm 5, amcenestrantr plus abemaciclib in dose-escalation (Part J) and dose expansion (Part K) cohorts. Here, we describe the final analysis results with amcenestrantr monotherapy from Arm 1 (Parts A and B). Clinical data were collected in electronic case report forms using Medidata Rave v2020.3.2.

**Patients.** The first patient was enrolled on 6 November 2017 and the last patient was enrolled on 26 March 2020. Key inclusion criteria were as follows: postmenopausal women; histological or cytological proven diagnosis of ER+/HER2−- breast adenocarcinoma; measurable disease by RECIST v1.1; ≥6 months prior endocrine therapy for advanced breast cancer; ≤3 prior chemotherapy regimens for dose escalation eligibility and ≤1 prior chemotherapy regimen for dose expansion eligibility; ECOG PS 0 or 1. Key exclusion criteria were as follows: known brain metastases, leptomeningeal carcinomatosis, and/or spinal cord compression; prior treatment with another SERD, except fulvestrant following a ≥20-week washout period. Full inclusion/exclusion criteria, safety, and pharmacokinetic parameters are detailed in the online Supplementary Methods (Supplementary Table S9 for the names of the boards/committees who provided approval) and complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws. All patients provided written informed consent and no compensation was offered.

**Treatment.** During dose escalation, treatment was initiated on day 1 in the fasting state in cohorts starting at a dose of 20 mg orally QD, escalating in subsequent cohorts to planned doses of 50–600 mg. For all cohorts, treatment was dosed daily on day 3 after a moderate fat breakfast, and was administered orally QD for advanced breast cancer. Dose escalation was based on the occurrence of DLT together with target saturation by 18F-FES PET/CT scans and pharmacokinetic parameters during the first 28-day cycle, and according to a 3+3 design. For a detailed description of methods for dose escalation and selection of the RP2D, see Supplementary Methods: Dose escalation amcenestrantr monotherapy. A twice-daily (BID) dosing regimen of 6 patients receiving amcenestrantr 300 mg BID was also planned. During dose expansion, patients were treated at the RP2D. Patients were treated until disease progression, unacceptable toxicity, withdrawal of patient consent, or other reasons determined by the investigator (poor compliance with the protocol or intercurrent illness).

Treatment exposure was assessed using relative dose intensity (%), defined as (actual dose intensity – planned dose intensity) / 100 where actual dose intensity was defined as the total cumulative dose in mg + (duration of treatment in weeks × 7) – 1 and planned dose intensity in mg/day was defined as the planned dose at cycle 1 day 1.

**Primary endpoints.** In Part A, the dose-escalation phase, the primary endpoints were the incidence of treatment-related DLTs during cycle 1 (days 1–28), defined as the occurrence of any TEAEs related to the study therapy from a predefined list of hematological, non-hematological, and hepatic AEs as per the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.03 (see Supplementary Methods: Definition of DLTs for a list of DLT-defining TEAEs); and the determination of the MTD, defined as the highest dose level at which ≤1/6 evaluable patients experienced a DLT, and the RP2D.

In the absence of DLTs, the RP2D was defined considering pharmacokinetics after repeated administration, level of inhibition of target occupancy measured by 18F-FES PET/CT imaging, and pharmacokinetics/pharmacodynamics on ER occupancy. The RP2D should be at least two dose levels above the dose level showing >90% decrease in maximum standardized uptake value (average of all ROIs’ maximum 18F-FES uptake standardized body weight and corrected for background 18F-FES uptake; SUV_max(bodywt)) on 18F-FES PET/CT imaging (considered to represent inhibition of estrogen ligand binding or complete ER degradation), unless there were DLTs at this dose, in which case the RP2D could be any dose where >90% decrease in standardized uptake was reached. In Part B, the dose-expansion phase, the primary endpoint was the confirmed ORR by ICR.

Because most patients recruited to the study were heavily pretreated with up to 8 lines of prior therapy in the advanced setting (76.9% ≥2 prior lines), 73.8% had received targeted therapies, and 47.7% prior fulvestrant, we conducted two exploratory subgroup analyses for the Part B primary endpoint among patients who had received ≥3 prior lines of therapy in the metastatic setting, and among patients who had not received prior CDK4/6 or mTOR inhibitors or fulvestrant.
These subgroup analyses based upon prior treatments were performed to match baseline patient characteristics to historical studies with fulvestrant for indirect antitumor activity comparison. In addition, we conducted a third exploratory subgroup analysis among patients who had not received a prior CDK4/6 inhibitor.

Secondary endpoints. Overall safety in Parts A and B was assessed by tabulating TEAEs, coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 22.1, and graded according to NCI-CTCAE v4.03 criteria. TEAEs were classified as related or unrelated to amenestrant therapy based upon the local investigator’s clinical judgment.

To evaluate pharmacokinetics in Part A, venous blood samples were collected pre-dose and at regular intervals on cycle 1, day 1 (up to 48 h), on day 3 (up to 24 h), and on day 22 (up to 24 h). Amencestrant concentrations were determined in plasma using a validated liquid chromatography-tandem mass spectrometry method. The validated range was 5 ng/mL to 50,000 ng/mL. Plasma pharmacokinetic parameters, including \( t_{max} \), \( C_{max} \), and AUC(24 h), were determined after a single dose on cycle 1 day 1 in the fasting state and on day 3 in the fed state (moderate fat breakfast). After multiple-dose administration, \( t_{max} \), \( C_{max} \), AUC(0-24 h), trough, and CL/F on day 22 were determined on day 22 in the fasting state.

In Part B, venous blood samples were collected pre-dose and at regular intervals on cycle 1, day 1 (up to 24 h), and on day 22 (up to 24 h). Plasma pharmacokinetic parameters (\( t_{max} \), \( C_{max} \), AUC(24 h), on day 1 and day 22, and in addition, \( C_{ trough} \) and CL/F on day 22) were determined regardless of food status in a subset of patients participating with repeated pharmacokinetic sampling. On day 22, 24 h urine fraction was collected to determine the amount of amcenestrant eliminated by renal clearance.

The potential induction/inhibition effect of amenestrant on CYP3A using 4β-hydroxycholesterol was assessed for both Parts A and B.

To evaluate pharmacodynamics in Part A, \( ^{13} \)F-FES PET/CT imaging was performed at baseline and between Days 11 and 15 of the first cycle (16-24 h after the previous amcenestrant dose) to evaluate the percent of residual ER availability at a steady state of ER occupancy (after ≥8 continuous days of treatment). ER occupancy was calculated as the percentage reduction in \( ^{13} \)F-FES SUV max (corrected between the on-treatment and screening scans. In addition, a blood sample was collected just before \( ^{13} \)F-FES radiotrace isotope administration on the day of \( ^{13} \)F-FES PET/CT (day 11-15) to assess the correlation between the plasma concentration of amenestrant and scan results. For detailed \( ^{13} \)F-FES PET/CT imaging and image analysis methods see Supplementary Methods: \( ^{13} \)F-FES PET/CT imaging and image analysis.

In Part A, preliminary antitumor activity was assessed using the ORR, defined as a complete or partial response, combined on a second evaluation point of at least 4 weeks apart, as per RECIST v1.1, as well as the CBR (CR = confirmed CR, PR, or SD ≥ 24 weeks) by investigator/local radiologist review.

In Part B, the ORR and CBR by baseline ESR1 gene mutational status (wild-type and mutated), overall ORR and CBR by investigator/local radiologists review as well as time to first tumor response (CR or PR) were assessed.

Post hoc analyses. Post hoc exploratory biomarker analyses were conducted. In order to maximize sample size and exposure to a pharmacodynamically active amenestrant dose for exploratory biomarker analyses, we selected a pooled population of patients from Parts A and B who had received an amenestrant dose ≥150 mg. We chose this dose cutoff because doses ≥150 mg showed high levels of ER occupancy on \( ^{13} \)F-FES PET scans, whereas ER occupancy was very low for two of the lower doses. Minimum amenestrant dose was 150 mg (Fig. 2b).

To analyze ER degradation/pathway inhibition, tumor biopsies were collected at screening and at the end of cycle 2 (cycle 2, day 28) in patients from Part B who provided consent for their collection. For each biopsy, formalin-fixed paraffin-embedded (FFPE) tissue sections were collected of 5 μm each for immunohistochemistry (IHC) analysis, and of 10 μm each for RNA extraction and subsequent RNA-seq analysis. IHC ER, PgR, and Ki67 protein expression slides were assessed by two expert pathologists (V.P., and A.-E.L.). IHC staining was performed on the Ventana Discovery XT IHC platform using anti-ER clone SP1 (CONFIRM Anti-Estrogen Receptor, Roche, ref. 790–4253), anti-PgR clone 1C2 (CONFIRM Anti-Progesterone Receptor, Roche, ref. 790–4286). Stained slides were evaluated by standard light microscopy using a manual scoring system. Staining was evaluated in tumor cells only. For ER and PgR expression, H-scores were calculated. For Ki67 protein expression, the percentage of positive tumor cells was calculated.

Total RNA was extracted from FFPE tissue (slides of 10 μm). A targeted-enrichment RNA-seq approach was applied utilizing KAPA RNA HyperPrep and whole exome SeqCap kits (Roche) followed by the generation of sequencing reads with NextSeq 500 (Illumina). RNA-seq FASTQ files were processed with STAR aligner and Cufflinks to generate gene-level fragments per kilobase of transcript per million mapped reads (FPKM)\(^ {47,48} \). Those FPKM values were converted to the gene-level estimation of expression in transcripts per million (TPM). The TPM data were then quantile-normalized and log2-transformed. GSVA was employed to calculate the ER activation score by using the ER activity signature (87 genes)\(^ {33} \).

To evaluate mutant ESR1 status in cfDNA was determined by ddPCR detecting 12 ESR1 pathogenic single-nucleotide variants in the ligand-binding domain of ESR1 using the OncoBEAM™ platform by Sysmex Inostics (Baltimore, MD, USA)\(^ {35} \).

Statistical considerations. Sample size considerations. A minmax Simon 2-stage design with a 5% one-sided Type 1 error rate was used to test the null hypothesis of a 10% response rate. Under the alternative assumption of 20% response rate, a total of 78 patients were needed to guarantee at least 80% power, accounting for the binding futility interim analysis. In the first stage, 45 patients were to be accrued in Part B and were evaluated for tumor responses after they had had two tumor assessments (i.e., after at least four cycles of amenestrant therapy) or early progression or an end-of-treatment tumor assessment, whichever occurred first. If ≤4 responders by ICR in these 45 patients were observed, accrual to Part B would be stopped. The null hypothesis would be rejected if ≥13 responders were observed in the 78 patients.

Analysis populations and associated statistical methods. The DLT-evaluable population during Part A was planned to include all patients who had received a first complete cycle, who had received at least 75% of the intended dosing (unless the patient discontinued treatment before cycle 1 completion because of a DLT), and had evaluable \( ^{13} \)F-FES PET/CT scans at baseline and between day 11 and day 15 of the first cycle. Results were analyzed using descriptive statistics.

The safety population included all patients exposed to at least one dose of the study treatment. Results were analyzed using descriptive statistics.

The response-evaluable population was defined as treated patients with measurable disease at study entry who had an evaluable baseline and at least one post-baseline evaluable tumor assessment. Patients with an early progression as per RECIST v1.1 or who died from disease progression were evaluable for response. Results were analyzed using mostly descriptive statistics; for ORR and CBR, 90% CIs were calculated using the exact method of Clopper-Pearson.

The pharmacokinetic population evaluable for non-compartmental pharmacokinetic analysis (NCA) was defined as all patients from the all-treated population without any major deviations related to study treatment administration (e.g., early vomiting just after drug administration, food status), and who had adequate blood samples enabling determination of at least one pharmacokinetic parameter. NCA was performed using Phoenix® software.

The biomarker-evaluable population was defined as all patients with available biomarker data from the response-evaluable pooled population of patients from Parts A and B who had received an amenestrant dose ≥150 mg excluding the pharmacodynamically inactive 20 mg dose. Only paired samples (screening or cycle 1, Day1 and available cycle 2, day 28 samples) were considered for assessment of the evolution of biomarkers over time. Results were analyzed using descriptive statistics.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Researchers, such as any researcher holding a faculty appointment or research position at an institution of higher education, a research organization, or a nonprofit organization, may request access to patient-level data and related study documents including the clinical trial protocol, study protocols with any amendments, blank case report form, statistical analysis plan, and dataset specifications for legitimate research purposes, as evaluated by an independent scientific review board. Patient-level data will be anonymized and study documents will be redacted to protect the privacy of our trial participants. Access to data may be restricted in cases where data collected are subject to contractual obligations (for example, data obtained by Sanofi from independent institutions where the data-sharing contract prohibits onward sharing to third parties) or require patient consent for sharing (for example, if patients do not consent for their data to be shared with third parties or to be deposited in repositories). Data use agreements will be used for approved data requests. Access to data is expected to take on average 2–5 months, and will depend on various factors. These factors include the number of data requests, the number of days of availability of the requestor to respond to comments, the ability and align with the data use agreement, and if the data from the trial have already been anonymized. Further details on Sanofi’s data-sharing criteria, eligible studies, and process for requesting access can be found at: https://www.vivri.org/. Patients in this study did not give consent for their genomic data to be shared or deposited in repositories. Thus, in accordance with Sanofi’s commitment to honoring patient preferences and protecting patient privacy, patient-level genomic data, including RNA-seq data, are not available. Although Parts A and B of this study are complete, which comprise the data presented in this manuscript, the other parts of the study (Parts C through K) are ongoing. As such, at the time of publication, the study is not eligible for posting on the Vivri platform until all parts of the study have been completed. However, requests for data of unlisted studies can be placed via the following link: https://vivri.org/members/enquiries-about-studies-not-listed-on-the-vivri-platform/.

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A.B., S.C., H.M.L., G.A.U., A.G., P.C., G.P., M. Celanovic, V.P., J.E.M., and M. Campone contributed to the conceptualization of the study. A.B., S.C., H.M.L., G.A.U., and M. Campone were study investigators and contributed to the recruitment and supervision of the study. G.A.U., A.G., S.C.C., S.D. G.P., and N.T. contributed to the methodology and formal analysis of data. M.B., J.S.L., and A-L.B. contributed to the methodology and investigational aspects of the study. All authors contributed to the writing, reviewing, and editing of the manuscript.

Competing interests
A.B. has provided consulting or advisory roles for AstraZeneca/Daiichi Sankyo, Lilly, Foundation Medicine, Gilead, Novartis, Genentech, Pfizer, Spectrum Pharmaceuticals, bioTheranostics, Merck, Radius Health, Immunomedics, Sanofi, and Puma Biotechnology, Siemens, Zionexa, and Lantheus. A.G., S.C-C., P.C., S.D., G.P., M. Celanovic, V.P., J.E.M., N.T., M.B., J.S.L., and A-L.B. are employees of Sanofi and may hold shares and/or stock options in the company. M. Campone has provided consulting, advisory, or speaker roles for AbbVie, Accord Healthcare, AstraZeneca, Daiichi Sankyo, G1 Therapeutics, Lilly, Novartis, Pfizer, Pierre Fabre Oncology, Sandoz, Sanofi, Seagen, and Servier; and has received honoraria for travel from AstraZeneca, Novartis, Pfizer, and Roche.

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Correspondence and requests for materials should be addressed to Mario Campone.

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