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Clinical Trial Results

Phase II Study of Dehydroepiandrosterone in Androgen Receptor-Positive Metastatic Breast Cancer

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Trial Information

- **ClinicalTrials.gov Identifier:** NCT02000375
- **Sponsor(s):** None
- **Principal Investigator:** Elisabetta Pietri
- **IRB Approved:** Yes

Lessons Learned

- The androgen receptor (AR) is present in most breast cancers (BC), but its exploitation as a therapeutic target has been limited.
- This study explored the activity of dehydroepiandrosterone (DHEA), a precursor being transformed into androgens within BC cells, in combination with an aromatase inhibitor (to block DHEA conversion into estrogens), in a two-stage phase II study in patients with AR-positive/estrogen receptor-positive/human epidermal growth receptor 2-negative metastatic BC.
- Although well tolerated, only 1 of 12 patients obtained a prolonged clinical benefit, and the study was closed after its first stage for poor activity.

Abstract

Background. Androgen receptors (AR) are expressed in most breast cancers, and AR-agonists have some activity in these neoplasms. We investigated the safety and activity of the androgen precursor dehydroepiandrosterone (DHEA) in combination with an aromatase inhibitor (AI) in patients with AR-positive metastatic breast cancer (MBC).

Methods. A two-stage phase II study was conducted in two patient cohorts, one with estrogen receptor (ER)-positive (resistant to AIs) and the other with triple-negative MBC. DHEA 100 mg/day was administered orally. The combination with an AI aimed to prevent the conversion of DHEA into estrogens. The main endpoint was the clinical benefit rate. The triple-negative cohort was closed early.

Results. Twelve patients with ER-positive MBC were enrolled. DHEA-related adverse events, reported in four patients, included grade 2 fatigue, erythema, and transaminitis, and grade 1 drowsiness and musculoskeletal pain. Clinical benefit was observed in one patient with ER-positive disease whose tumor had AR gene amplification. There was wide inter- and intra-patient variation in serum levels of DHEA and its metabolites.

Conclusion. DHEA showed excellent safety but poor activity in MBC. Although dose and patient selection could be improved, high serum level variability may hamper further DHEA development in this setting. ***The Oncologist*** 2019;24:743–e205

Discussion

Androgen receptors are commonly expressed in BC, but androgens have variable effects in different BC subtypes, and both AR-agonists and AR-antagonists have been studied as anticancer agents in these tumors.

This multicenter, single-arm, two-stage phase II study evaluated the safety and activity of the androgen precursor DHEA, 100 mg/day orally continuously, in combination with

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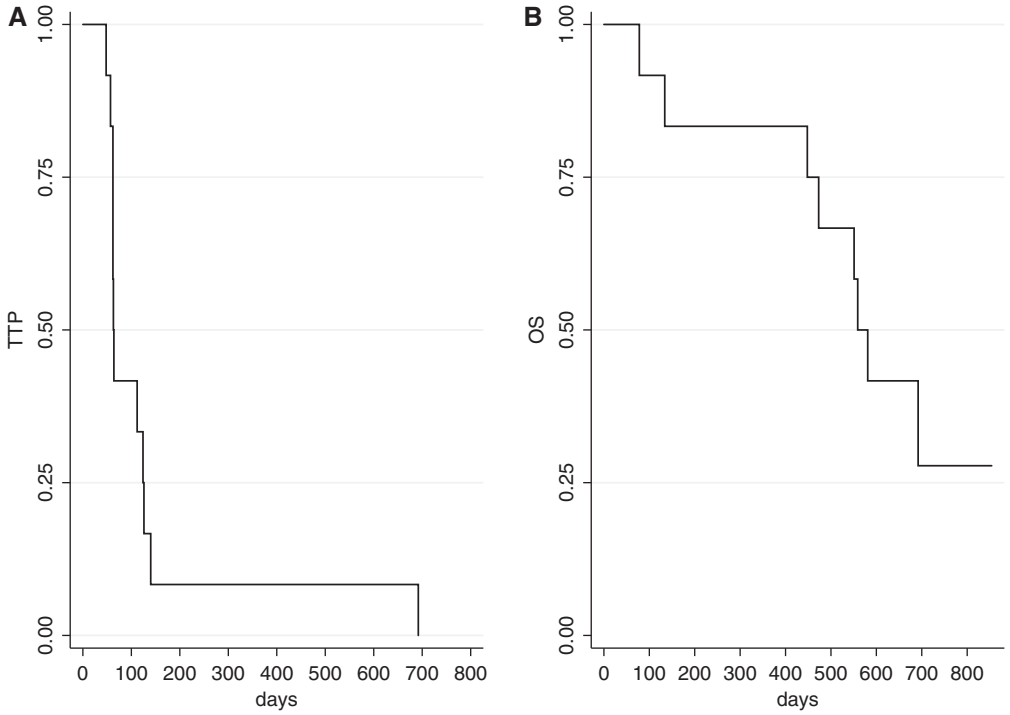

Figure 1. (A) Time to progression and **(B)** overall survival of the estrogen receptor-positive cohort.
Abbreviations: OS, overall survival; TTP, time to progression.

an AI to prevent its transformation into estrogens, in two cohorts of patients with AR-positive metastatic BC: one with ER-positive/human epidermal growth factor receptor 2 (HER2)-negative and one with triple-negative disease.

Patients were postmenopausal and, when ER-positive, had documented resistance to both nonsteroidal and steroidal AIs. The primary endpoints were safety and activity (clinical benefit rate: proportion of patients with stable disease or objective response after 16 weeks).

From November 2013 to July 2015, 12 patients were enrolled in the ER-positive and 6 in the triple-negative cohort; the last closed early, due to emerging preclinical evidence of tumor stimulation by androgens.

In the ER-positive cohort, the median age was 74 years, Eastern Cooperative Oncology Group (ECOG) performance status 0–2; nine patients had visceral metastases, five were pretreated with 1–2 lines of chemotherapy and all with 1–4 lines of endocrine therapy for advanced disease. The median duration of treatment was 71 days (range 55–697). Seven patients showed progressive disease (PD) at 8 weeks, four had stable disease (SD) at 8 weeks and PD at 16 weeks, and one had SD lasting >16 weeks (692 days). Median time to progression (TTP) was 63 days (95% confidence interval [CI] 57–126) and median overall survival (OS) 559 days (95% CI 134–not reached; Fig. 1). The study closed after the first stage for poor activity. All patients in the triple-negative cohort had PD.

Toxicities deemed to be related to DHEA were (worst grades) G2 fatigue, facial erythema, and increase in transaminases (the last required temporary treatment interruption) and G1 sleepiness and joint/muscular pain. Other toxicities, attributable to AIs or the underlying disease, included four serious adverse events: uncontrolled pain, trauma, seizure, and constipation, and all but the last were considered not treatment related.

There was wide intra- and inter-patient variability in DHEA serum levels.

The patient who experienced prolonged SD was the only one showing AR gene amplification.

The combination DHEA-AI was well tolerated but poorly active in ER-positive metastatic BC. Although dose and patient selection could be further studied, variability in serum levels and in tumor intracrinology (the intracellular formation of sex steroids from DHEA) may hamper further DHEA development in BC.

Trial Information	
Disease	Breast cancer
Stage of Disease/Treatment	Metastatic/advanced
Prior Therapy	More than two prior regimens
Type of Study – 1	Phase II
Type of Study – 2	Single arm
Primary Endpoint	Clinical benefit rate (proportion of patients with stable disease or objective response after 16 weeks of therapy)
Primary Endpoint	Safety

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Secondary Endpoint	Toxicity
Secondary Endpoint	Overall response rate
Secondary Endpoint	Time to progression
Secondary Endpoint	Overall survival
Secondary Endpoint	Correlative endpoint

Additional Details of Endpoints or Study Design

Study design: Simon two-stage design with 10% alpha and beta errors. Assuming an acceptable minimum clinical benefit of 10% and a desirable clinical benefit of 30%, 12 patients were required per cohort in the first stage, with the intent to continue recruitment up to a total of 35 patients per cohort if the number of patients achieving clinical benefit was ≥2 at the first stage, and considering the combination active if the total number of patients achieving clinical benefit was ≥6 in the entire cohort. Descriptive statistics are reported as frequencies and percentages for categorical variables and as median and range for continuous variables. Boxplots are used to represent serum levels of DHEA and glucuronidated metabolites at different time points, and the Friedman nonparametric repeated measure analysis of variance was used to test differences in their distribution over time. TTP and OS curves were estimated using the Kaplan-Meier method.

Correlative endpoints: (a) On formalin fixed, paraffin-embedded tumor samples, we assessed AR expression (AR Cell Marque antibody, clone SP107; Ventana Medical Systems, Oro Valley, AZ) and phosphorylation (Novus Biologicals pSer 650 NBP1-60769 and pSer 210- 213 NB 100-56603 antibodies; Novus Biologicals, Littleton, CO) by immunohistochemistry and AR gene copy number by fluorescence in situ hybridization using Vysis LSI Androgen Receptor Gene (Xq12) SpectrumOrange Probe kit (Abbott Molecular, Des Plaines, IL); (b) measurement of serum levels of DHEA and of its glucuronidated metabolites androstane-3alpha,17beta-diol-3-glucuronide (3-diol-3G), androstane-3alpha,17beta-diol-17glucuronide (3-diol-17G), and androsterone glucuronide (ADT-G) [44].

Investigator's Analysis	Level of activity did not meet planned endpoint

Drug Information

Drug 1	
Generic/Working Name	Dehydroepiandrosterone
Trade Name	Company Name
Drug Type	Androgen precursor
Drug Class	Androgen receptor
Dose	100 mg flat dose
Route	p.o.
Schedule of Administration	100 mg/day continuously
Drug 2	
Generic/Working Name	Anastrozole or exemestane or letrozole
Trade Name	Company Name
Drug Type	Aromatase inhibitor
Drug Class	Estrogen receptor
Dose	1, 25, 2.5 (respectively) mg flat dose
Route	p.o.
Schedule of Administration	1 tablet/day continuously

Patient Characteristics

Number of Patients, Male	0
Number of Patients, Female	18
Stage	Stage IV breast cancer
Age	Median (range): 74 (50–90)
Number of Prior Systemic Therapies	Median (range): 2 (1–4)
Performance Status: ECOG	0 — 14 1 — 3 2 — 1 3 — Unknown —
Cancer Types or Histologic Subtypes	Estrogen receptor-positive, HER2-negative breast cancer, 12 Triple-negative breast cancer, 6

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Primary Assessment Method	
Title	Estrogen receptor-positive, HER2-negative cohort
Number of Patients Screened	13
Number of Patients Enrolled	12
Number of Patients Evaluable for Toxicity	12
Number of Patients Evaluated for Efficacy	12
Evaluation Method	RECIST 1.1
Response Assessment CR	*n* = 0 (0%)
Response Assessment PR	*n* = 0 (0%)
Response Assessment SD	*n* = 5 (42%)
Response Assessment PD	*n* = 7 (58%)
(Median) Duration Assessments TTP	63 days, CI: 57–126
(Median) Duration Assessments OS	559 days, CI: 134–not reached [NR]
(Median) Duration Assessments Duration of Treatment	71 days
Outcome Notes	
Clinical benefit rate (CR or PR or SD at week 16): **one** patient (8%). Enrollment in the triple-negative cohort was closed in advance because of both slow recruitment and preclinical data suggesting that AR may drive tumor progression in some subtypes of triple-negative breast cancer.	
Title	Triple-negative cohort
Number of Patients Screened	7
Number of Patients Enrolled	6
Number of Patients Evaluable for Toxicity	6
Number of Patients Evaluated for Efficacy	6
Evaluation Method	RECIST 1.1
Response Assessment CR	*n* = 0 (0%)
Response Assessment PR	*n* = 0 (0%)
Response Assessment SD	*n* = 1 (17%)
Response Assessment PD	*n* = 5 (83%)
(Median) Duration Assessments TTP	55 days, CI: 13–NR
(Median) Duration Assessments OS	339 days, CI: 63–NR
(Median) Duration Assessments Duration of Treatment	68 days
Outcome Notes	
Enrollment in the triple-negative cohort was closed in advance because of both slow recruitment and preclinical data suggesting that AR may drive tumor progression in some subtypes of triple-negative breast cancer.	

Adverse Events

All Cycles

Name	NC/NA	1	2	3	4	5	All grades
Gastrointestinal pain	94%	6%	0%	0%	0%	0%	6%
Fatigue	77%	17%	6%	0%	0%	0%	23%
Anorexia	89%	11%	0%	0%	0%	0%	11%
Dysphagia	94%	6%	0%	0%	0%	0%	6%
Dyspepsia	88%	6%	6%	0%	0%	0%	12%
Nausea	83%	17%	0%	0%	0%	0%	17%
Vomiting	89%	11%	0%	0%	0%	0%	11%
Constipation	94%	6%	0%	0%	0%	0%	6%
Diarrhea	89%	11%	0%	0%	0%	0%	11%
Enterocolitis infectious	94%	0%	6%	0%	0%	0%	6%
Dyspnea	89%	11%	0%	0%	0%	0%	11%
Cough	88%	6%	6%	0%	0%	0%	12%

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Fever	94%	6%	0%	0%	0%	0%	6%
Hot flashes	94%	0%	6%	0%	0%	0%	6%
Agitation	94%	6%	0%	0%	0%	0%	6%
Insomnia	89%	11%	0%	0%	0%	0%	11%
Dizziness	94%	6%	0%	0%	0%	0%	6%
Hypertension	94%	6%	0%	0%	0%	0%	6%
Rash maculo-papular	94%	0%	6%	0%	0%	0%	6%
Skin and subcutaneous tissue disorders - psoriasiform lesions	94%	6%	0%	0%	0%	0%	6%
Localized edema	94%	6%	0%	0%	0%	0%	6%
Aspartate aminotransferase increased	94%	6%	0%	0%	0%	0%	6%
Arthralgia	60%	17%	17%	6%	0%	0%	40%
Cholesterol high	94%	0%	6%	0%	0%	0%	6%

Number of patients experiencing a given toxicity, among 18 patients assessable for toxicity (each patient was registered under the maximum grade experienced for each kind of toxicity).
Abbreviation: NC/NA, no change from baseline/no adverse event.

Serious Adverse Events

Name	Grade	Attribution
Uncontrolled pain	2	Unrelated
Cranial trauma	2	Unrelated
Seizures	2	Unrelated
Constipation and abdominal pain	2	Unlikely

Assessment, Analysis, and Discussion

Completion	Study completed
Investigator's Assessment	Level of activity did not meet planned endpoint

Androgen receptors (AR) are expressed in 60%–90% of breast cancers (BC), mainly in estrogen receptor (ER)-positive tumors [1, 2]. Androgens have variable effects in different BC models [3–5]: often antiproliferative [6–13], mainly in ER-positive tumors; sometimes pro-proliferative [14–18], mainly in triple-negative and human epidermal growth factor receptor 2 (HER2)-positive/ER-negative tumors. Both AR-agonists [19–22] and AR-antagonists are being studied as antitumor agents in BC [23–27]. Dehydroepiandrosterone (DHEA) is a steroid produced mainly by the adrenal cortex and transformed into sex hormones (androgens and estrogens) within peripheral target tissues [28–33]. The action of sex steroids is confined within the cells in which they are synthesized (a process called "intracrinology"), with little or no release into the extracellular spaces or the general circulation. This process also occurs within BC cells, and there is preclinical evidence of antitumor activity of DHEA in BC [34–40]. The administration of an aromatase inhibitor (AI) prevents the conversion of DHEA into estrogens and favors its conversion into androgens.

To investigate the role of androgens in BC, avoiding the virilizing effects of available androgenic agents, we conducted a two-stage, phase II, prospective clinical study to evaluate the safety and activity of DHEA 100 mg/day in combination with an AI (anastrozole 1 mg/day, letrozole 2.5 mg/day, or exemestane 25 mg/day) in two cohorts of patients with AR-positive metastatic breast cancer: one with ER-positive/HER2-negative (ER-positive cohort) and one with triple-negative (TN cohort) disease.

The DHEA dosage was chosen based on the reported saturation of the enzymatic systems that transform DHEA into sex steroids, occurring at serum levels of about 7 ng/mL [41, 42], and to the reported serum DHEA levels of about 7 ng/mL achieved after oral administration of DHEA 100 mg daily for 6 months [43]. DHEA was produced by the Oncology Pharmacy Laboratory of our institute, whereas AIs were purchased commercially.

Serum levels of DHEA and its glucuronidated metabolites were measured by liquid chromatography-tandem mass spectrometry [44]. The expression of AR and of its main phosphorylated forms (AR 650 and AR 210-213) was assessed by immunohistochemistry and AR gene amplification by fluorescence in situ hybridization.

Patients characteristics are reported in the designated Table. All patients in the ER-positive cohort had developed resistance to both nonsteroidal and steroidal AIs. Seven patients had received an AI as their last line of treatment before entering the trial and, after progressing on the AI, had continued the same AI but with the addition of DHEA. Conversely, five patients received DHEA in combination with an AI to which they had developed resistance in the past, but which was not the last line of therapy they received before entering this trial.

Toxicity is reported in the "adverse events" table. The four serious adverse events reported were not attributed to DHEA. Two patients died within 30 days of the end of therapy, one after 8 days and one after 21 days, all due to tumor progression. No virilizing effects were registered.

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Only one patient had clinical benefit, with stable disease (SD) for almost 99 weeks. She had previously received letrozole for 4 years for a regional relapse and then tamoxifen for 8 months upon progression. Following further progression, she was enrolled in the trial and received letrozole + DHEA.

The three patients whose tumors showed lower AR expression levels (<50% of positive cells and H-score < 100) had disease progression (PD) after 8 weeks, whereas five of the seven patients with higher AR expression showed SD at this time. AR phosphorylation and AR gene copy number were available for 10 patients (Table 2). Remarkably, the patient with clinical benefit was the only one whose tumor harbored an AR gene amplification, with AR gene clusters observed in 20% of tumor cells. All tumor samples showed AR phosphorylation at serine 650 (p650) in variable amounts and at different locations (cytoplasm or nucleus). The two patients with lower p650 H-scores (<100) had PD at 8 weeks, whereas of the eight patients with intermediate/high H-scores, five had SD and three had disease progression at 8 weeks. The patient who experienced prolonged SD had a nuclear expression of p650, whereas in most cases p650 was found in the cytoplasm. AR phosphorylation at serine 210-213 was present, mainly in the nucleus, in only three patients, one of whom was the patient with prolonged SD.

Serum levels of DHEA and its glucuronidated metabolites androstane-3alpha,17beta-diol-3-glucuronide (3α-diol-3G), androstane-3alpha,17beta-diol-17glucuronide (3α-diol-17G), and androsterone glucuronide (ADT-G) were measured at baseline, at 8 weeks, and at the end of treatment in 10 patients. DHEA was assessable at all three time-points in four patients, 3α-diol-3G in two patients, 3α-diol-17G in seven patients, and ADT-G in eight patients. There was wide intra- and interpatient variation in DHEA serum levels (Fig. 2), but no significant changes over time were observed, probably because of the small number of patients with all measurements (p = .333). Only one patient had DHEA values constantly above the target threshold of 7 ng/mL and progressed after 8 weeks. The patient with prolonged disease stabilization had a median DHEA serum level of 4.01 ng/mL. Among the glucuronidated metabolites (Fig. 3), median serum levels of 17α-diol-17G and ADT-G showed significant changes over time (p = .020 and p = .007, respectively, Friedman test). No clear pattern of metabolite levels emerged in relation to response to treatment at 8 weeks.

The poor activity of DHEA in our study may partly be due to heavy pretreatment, which may have compromised hormone sensitivity. Variability in adrenal function [45], in DHEA disposition after oral administration especially in elderly patients [46–59], and in BC cells intracrinology may further be involved [60].

The AR gene amplification present in the only patient who showed a prolonged clinical benefit is intriguing, prompting to hypothesize the potential value of AR gene amplification as a predictive biomarker of response to androgenic treatments in breast cancer. However, the small number of patients involved in the study and the low rate of clinical benefit prevents any definitive conclusions from being drawn. Similarly, the role of phosphorylated AR remains to be ascertained.

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(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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Figures and Tables

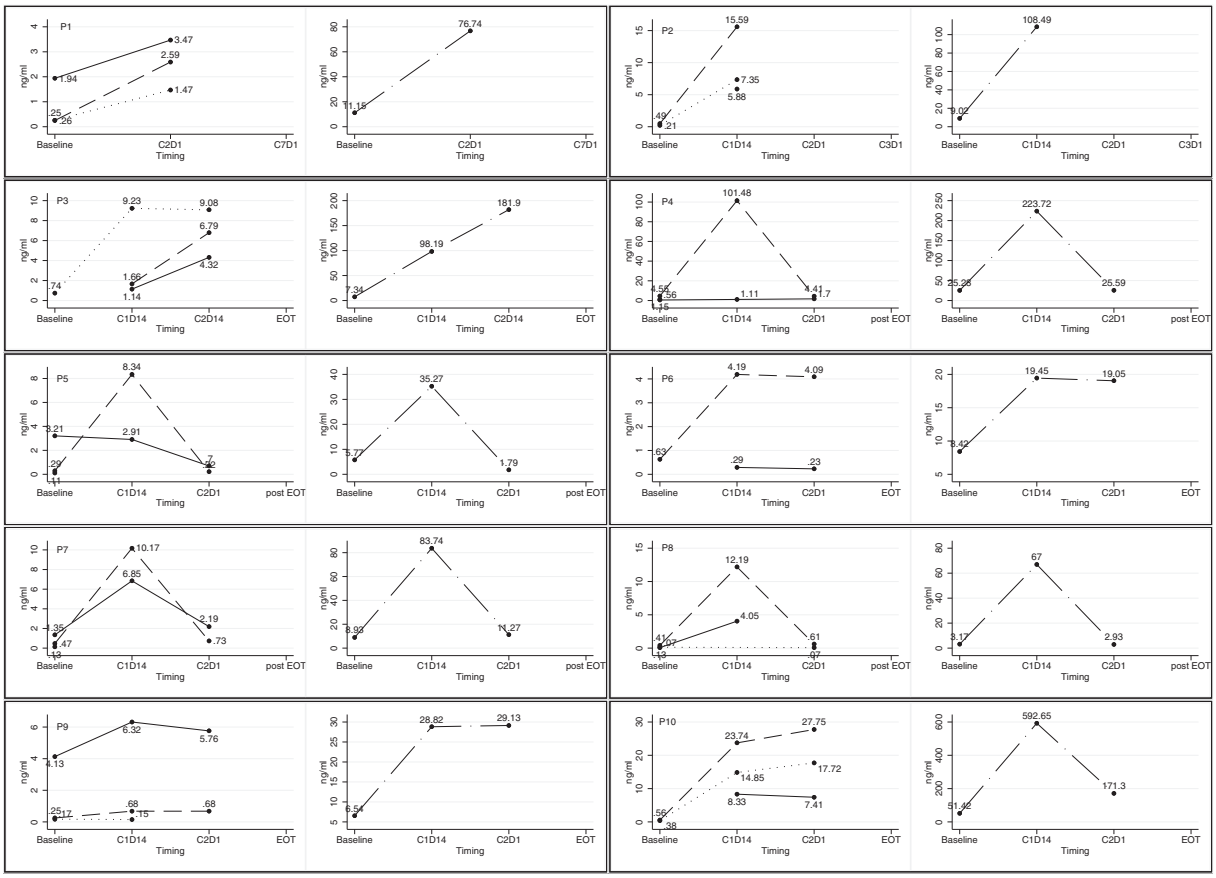

Figure 2. Individual serum concentrations of dehydroepiandrosterone (DHEA) and metabolites in 10 patients from the estrogen receptor-positive cohort. The following are reported for each patient: Left panels: serum concentrations of DHEA, androstane-3alpha,17beta-diol-3-glucuronide (3α-diol-3G), and androstane-3alpha,17beta-diol-17glucuronide (3α-diol-17G) at different time points during treatment. Right panels: serum concentrations of androsterone glucuronide (ADT-G) at different time points during treatment. Solid line: DHEA levels; dotted line: 3α-diol-3G levels; dashed line: 3α-diol-17G levels; dash-dotted line: ADT-G levels. Abbreviations: Baseline, before starting treatment; C1D14, cycle 1 day 14; C2D1, cycle 2 day 1; EOT, end of treatment.

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Table 1. Patient and tumor characteristics

Variable	Cohort 1 (*n* = 12), *n* (%)	Cohort 2 (*n* = 6), *n* (%)
Median age, years (range)	74 (58–90)	76 (50–86)
Performance status (ECOG)		
0	9 (75)	5 (83)
1	2 (17)	1 (17)
2	1 (8)	—
Hormone receptors[a]		
Androgen-positive	12	6
Estrogen-positive	12	—
Estrogen-negative		6
Progesterone-positive	9 (75)	—
Progesterone-negative	3 (25)	6
Negative HER2 status[a]	12	6
Number of metastatic sites		
1	2 (16.67)	1 (17)
2	2 (16.67)	2 (33)
3	6 (50.00)	2 (33)
4	2 (16.67)	1 (17)
Sites of metastases		
Soft tissues (only)	2 (17)	0
Bone (± soft tissue)	1 (8)	1 (17)
Viscera (± other)	9 (75)	5 (83)
Previous lines of hormone therapy for MBC		
1	1 (8)	—
2	6 (50)	—
3	3 (25)	—
4	2 (17)	—
Previous lines of chemotherapy for MBC		
0	7 (58)	1 (17)
1	3 (25)	1 (17)
2	2 (17)	2 (33)
3	—	2 (33)
Chosen aromatase inhibitor		
Exemestane	6 (50)	4 (67)
Anastrozole	4 (33)	2 (33)
Letrozole	2 (17)	—
Last line of therapy before enrollment into this clinical trial		
Same AI, continued within this study	7 (58)	
Other treatment	5 (42)	

[a]Based on the most recent tumor biopsy performed (Cohort 1: six primary tumors and six metastases; Cohort 2: three primary tumors and three metastases).

Abbreviations: —, no data; AI, aromatase inhibitor; ECOG, Eastern Cooperative Oncology Group; HER2, human epidermal growth receptor 2; MBC, metastatic breast cancer.

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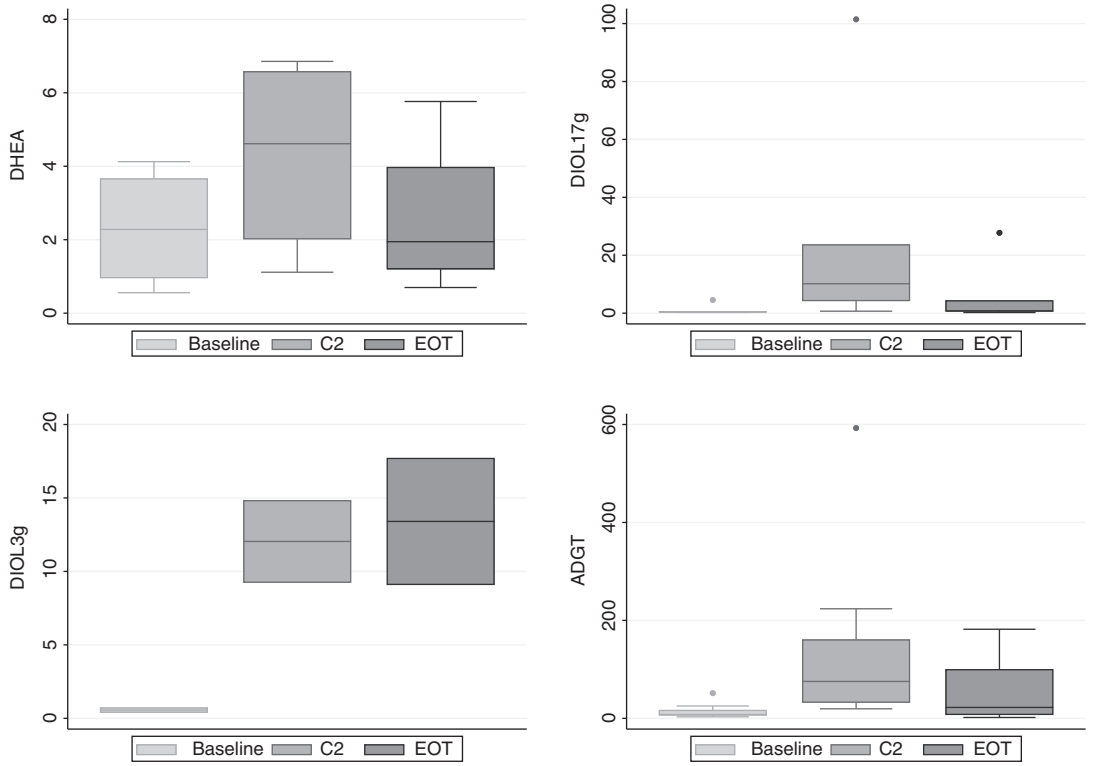

Figure 3. Boxplots of serum concentrations of DHEA and metabolites. Box and whisker plots, showing the median, interquartile range, and the highest and lowest values for each analyte at three time points (baseline, cycle 2 day 1, and end of treatment). Abbreviations: C2, cycle 2 day 1; DHEA, dehydroepiandrosterone; EOT, end of treatment.

Table 2. Androgen receptor expression, phosphorylation, and gene amplification

		AR (nuclear)			AR	AR p650					AR p210-213					Response
Patient	Site	%	Int	H	FISH	%	N	C	Int	H	%	N	C	Int	H	at 8 weeks
1	M (chest wall skin)	70	3	210	+	45	+	–	2-3	135	10	+	–	1	10	SD
2	P	90	3	270	–	90	–	+	3	270	0					PD
3	P	90	3	270	–	80	+	+	3	240	35	+	+	1	35	SD
4	P	30	2	60	–	95	–	+	3	285	0					PD
5	P	90	3	270	–	50	–	+	2-3	150	30	+	–	1	30	PD
6	M (chest wall skin)	95	3	285	–	100	–	+	3	300	0					SD
7	P (relapse)	85	3	255	–	90	–	+	3	270	0					SD
8	M (mediastinum)	80	3	240	–	90	–	+	3	270	0					SD
9	P	25	2	50	–	70	+	–	1	70	0					PD
10	P	30	3	90	–	30	+	+	2	60	0					PD

Abbreviations: %, percentage of stained cells; AR, androgen receptor; AR FISH, AR gene amplification by fluorescence in situ hybridization; C, cytoplasm; H, H-score (= % * Int); Int, staining intensity; M, metastasis; N, nuclear; P, primary tumor; p650, phosphorylation at serine 650; p210-213, phosphorylation at serine 210-213; PD, progressive disease; SD, stable disease.

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