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CR1447 in breast cancer

549–556

6:549

Phase I trial of the androgen receptor modulator CR1447 in breast cancer patients

Endocrine CONNECTIONS

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Abstract

CR1447 (4-hydroxytestosterone, 4-OHT) binds to the androgen receptor and has antiproliferative activity in both ER-positive and ER-negative/AR-positive breast cancer cells in preclinical studies. The objective of this first-in man trial was to evaluate the safety and to determine the dose of CR1447, administered as an ointment, for Phase II. Escalating doses (100, 200, 400 mg) of CR1447 were administered topically on a daily basis to patients with ER-positive/AR-positive/HER2-negative advanced breast cancer pretreated with several lines of therapy. 14 patients have been treated for a total of 42 cycles. Two patients, one at dose level 100 mg and one at dose level 200 mg, showed early tumour progression and were replaced. Related adverse events were all ≤ grade 2 and included fatigue, bone and joint pain, stiffness, dry skin and mouth, nausea, sweating, urinary tract infection, rash, headache and distress. No drug-related dose-limiting toxicities (DLTs) were seen. Two patients (17%) achieved stable disease at 3 months. Pharmacokinetic analysis confirmed dose-dependent transdermal uptake of CR1447. 4-OH-androstenedione (4-OHA), a key metabolite of 4-OHT, was undetectable in most of the plasma samples. Urine metabolites of 4-OHT and 4-OHA indicate high exposure of 4-OHT after topical administration. Oestradiol serum concentrations did not increase, confirming preclinical data that CR1447 is not converted to estrogens *in vivo*. In conclusion, CR1447 administered transdermally as an ointment is well tolerated and appears to have single-agent activity in heavily pretreated ER-positive/HER2-negative breast cancer patients. The recommended phase II dose is 400 mg/day.

Introduction

For the treatment of breast cancer (BC), testosterone was already used extensively between the 1930s and 1960s with anecdotal tumour responses (especially in bone metastases) seen in up to 20% of treated women ([1], [2], [3], [4]). Side effects such as hirsutism and other signs of virilisation in treated women and the evidence that

testosterone may be easily converted to estrogens in the body led to the discontinuation of its use (5). These findings suggest that selective androgen receptor (AR) modulators (SARMs) rather than testosterone may be used to treat AR-positive BC.

CR1447 (4-hydroxytestosterone (4-OHT)) is a steroidal small molecule with two distinct properties, acting as a steroidal aromatase inhibitor (AI) and binding to the AR with high affinity (IC_{50} 4.4 nM) (6). *In vitro* studies indicate that human BC cell lines are inhibited by CR1447 in their growth if they express the AR, while knockout of the AR abolishes this effect. A significant proportion of 4-OHT is converted to 4-hydroxyandrostenedione (4-OHA, formestane). *In vivo*, 4-OHT and 4-OHA form a redox reaction. 4-OHA is the 17-beta-oxidized isoform of CR1447. 4-OHA had previously been approved as an AI for the treatment of BC via intramuscular injection but was discontinued due to the development of oral AIs. Unlike testosterone, both compounds, 4-OHA and 4-OHT, are not converted to oestrogens *in vivo* (7, 8).

The combination of two mechanisms of action of CR1447 may result in higher activity. The objective of this phase I trial was to investigate the safety, tolerability, pharmacokinetics and the recommended dose (RD) of CR1447 for phase II in a clinical setting.

Patients and methods

CR1447

CR1447 was supplied in aluminium-coated stick packs as a 4 g ointment containing 2.5% of the active ingredient 4-hydroxytestosterone (4-OHT). CR1447 stick packs had to be stored protected from light at room temperature and should neither be refrigerated or frozen nor heated over 30°C. CR1447 was supplied by CURADIS GmbH, Erlangen, Germany and distributed by Promedipharm GmbH, Germany.

Patients

Eligible patients were postmenopausal women with locally advanced or metastatic, histologically confirmed breast adenocarcinoma requiring therapy and not suitable for local treatment. Inclusion criteria were immunohistochemical oestrogen receptor (ER) positivity (≥1%) and/or progesterone receptor (PR) positivity (≥1%); in all cases, the tumour had to be human epidermal

growth factor receptor 2 (HER2) negative. Presence of ≥1 measurable or evaluable lesion according to RECIST 1.1 was required. Bone metastases were allowed to be considered as target lesions in case of enhancement on scintigraphy, cytologic/histologic evidence or typical radiographic image. Patients with uncontrolled brain metastases, pulmonary carcinomatous lymphangiosis or liver metastases on >1/3 of the liver were not eligible. All patients gave written informed consent after full explanation of the purpose and nature of all procedures applied.

Study design and procedures

This four centre (Bern University Hospital; Istituto Oncologico della Svizzera Italiana, Bellinzona; Kantonsspital St. Gallen; and Kantonsspital Graubünden, Chur, Switzerland), first-in man, open-label, phase I, dose escalation clinical trial (clinicaltrials.gov ID: NCT02067741) was reviewed by the Swiss Group for Clinical Cancer Research (SAKK) internal review process and approved by the Central and Local Ethics Committees and the Swiss Agency for Therapeutic Products (Swissmedic).

The main objective of the phase I was to determine safety, tolerability and the recommended dose (RD) of CR1447 for phase II, using a standard 3+3 dose escalation design.

Treatment consisted of CR1447 (provided in 100 mg stick packs) topically applied daily to the haunches and thighs until progression. One cycle was defined as lasting 21 days. Three dose escalation steps (100, 200 and 400 mg daily) were performed including 3 patients in each dose cohort, with another 3 confirmatory patients in the last dose cohort or in case of a dose-limiting toxicity (DLT). A DLT was defined as an adverse event (AE), laboratory abnormality or a drug toxicity ≥ grade 3 that was considered to be probably, possibly or definitely related to the trial medication and which occurred during the first cycle of treatment. While 100 and 200 mg were applied once daily, 400 mg were applied as 200 mg twice daily at the same body site. The planned sample size was between 2 and 18 evaluable patients. Since the overall toxicity of CR1447 was estimated to be low, the patients could switch to the next dose level when the next higher cohort of 3 patients had finished first cycle treatment without DLTs. The dose was escalated to the next higher dose level, whenever 0 out of 3 or ≤1 out of 6 patients had a DLT. Patients were evaluated for DLT only at the lowest level

they had been treated. If no DLTs were to occur at the highest dose level, this level was determined to be the recommended dose for phase 2.

Dose modifications were planned for adverse events (AEs) grade 2 (dose reduction by one dose level) and grade 3 or 4 (stop treatment with CR1447 until resolved to grade ≤2 and restart one dose level lower until end of treatment). If an AE grade 3 or 4 occurred on an already reduced dose, CR1447 had to be stopped until the AE resolved to grade ≤2; treatment could then be resumed at one lower dose level.

Patients had to be taken off treatment if they missed ≥7 days of treatment within the 1st cycle or >3 weeks of treatment within subsequent cycles.

Secondary endpoints were disease control at 24 weeks (DC24) i.e. complete remission (CR), partial remission (PR) and stable disease (SD) within the first 24±2 weeks of treatment or stable disease (SD) for at least 24 weeks after treatment start; AEs; pharmacokinetic (PK) analysis of CR1447; pharmacodynamic (PD) analysis i.e. oestradiol levels during treatment; AR and Ki67 expression. Disease control at 12 weeks (DC12) was included as an additional secondary endpoint later on.

Pharmacokinetics (PK) and pharmacodynamics (PD)

Plasma and serum 4-OHT, 4-OHA (for PK) and oestradiol (for PD) concentrations were measured at baseline, after single dose administration at 1, 2, 4, 8, 24 and 72 h (day −7, −6, −5) and during treatment at 1, 8 and 15 days. Urine was collected from 0 to 8 h, and from 8 till 24 h after single administration of CR1447. Urine 4-OHT, 4-OHA (for PK) and oestradiol (for PD) concentrations were assessed at baseline, once at week 2 and once at week 3 of treatment.

High-pressure liquid chromatography with mass spectrometry (HPLC–MS) was used for the determination of 4-OHT and 4-OHA in human plasma, with testosterone-d3 and exemestane being used as internal standards, respectively. The lower limit of quantification of each analyte in plasma was 0.25 ng/mL.

Assessment of androgen receptor status

Central immunohistochemistry staining for the AR of the most recent formalin-fixed paraffin-embedded tissue samples was performed for all patients and scored semi-quantitatively. AR positivity was defined as any positive nuclear staining ≥1% in analogy to the College of American Pathologists' (CAP) recommendations for reporting oestrogen receptor status (9). For every patient's tumour tissue sample, percentage of AR-positive tumour cell nuclei and staining intensity, semi-quantitatively scored from 1 to 3, were determined.

Assessment of response

Best response was assessed by Response Evaluation Criteria in Solid Tumours (RECIST), version 1.1 (10). CT and bone scans were performed within 2 weeks prior to starting treatment at baseline, at week 12 (±8 days) and 24 (±2 weeks). In case of radiologic response (i.e. CR or PR), a confirmatory scan had to be performed after 4 weeks (±8 days).

Data analysis and statistics

The primary DLT and MTD analysis was performed on all evaluable patients who signed the informed consent and who did not miss ≥7 days of treatment during the first cycle of treatment or had a DLT.

Other safety and efficacy data were analysed on all patients who received at least one dose of trial treatment.

Continuous data were summarised using median, minimum and maximum values. Categorical data were summarised using frequency and percentage.

Paired t test was used to compare the body weights of patients at the time of screening vs at the end of treatment.

Results

Between June 2014 and February 2015, fourteen patients were recruited onto the trial. Patient characteristics are listed in Table 1. Most patients presented with bone metastasis and all patients had received prior endocrine therapy. Two patients (one in dose level 1 and one in dose level 2) were not evaluable: one patient had progressive disease after two weeks of treatment; therefore, she was taken off trial since she missed ≥7 days of treatment. The other patient's cancer progressed after two weeks of treatment and was taken off trial. Twelve patients completed the first cycle of therapy, three patients each at dose level 1 and 2 and six (three plus three confirmatory patients) at dose level 3. All fourteen patients were used for the analysis of toxicity and efficacy data.

All patients' tumours stained positive for the AR (Table 2). Percentage of AR-positive nuclei ranged from 70 to 100% and staining intensity from 2 to 3.

Table 1 Patient characteristics for the evaluable patients.*

Characteristics	Count (*n* = 12)
Age (years)	
Median	66
Range	44–80
WHO performance status	
0	8 (67%)
1	4 (33%)
Prior therapy	
Chemotherapy	10 (83%)
Radiotherapy	7 (58%)
Endocrine therapy	12 (100%)
Metastases	
Bone	11 (92%)
Liver	6 (50%)
Lymph nodes	6 (50%)
Lung	3 (25%)
Skin	2 (17%)
Brain	1 (8%)

*Two patients were not evaluable due to early progression within 2 weeks after the start of study treatment and had to be replaced.

Dose escalation and number of cycles administered are summarized in Table 3. Fourteen patients received a total of 42 cycles. Doses had to be modified in one cycle (6%) in the dose level 1 group, in one cycle (20%) in the dose level 2 group and in nine cycles (45%) in the dose level 3 group. The only dose reduction for toxicity reasons was made in one patient in the dose level 3 group for dry skin. Dose modifications are listed in Table 4.

Toxicity

Overall, 92 AEs (including blood count and chemistry changes) grade ≥1 according to NCI CTCAE, v4.0 occurred

Table 2 Androgen receptor status upon immunohistochemistry.

Patient No.	AR positive nuclei (%)	AR staining intensity score
1	100	3
2	99	3
3	100	3
4	99	2
5	95	2–3
6	95	2
7	99	3
8	70	3
9	80	2
10	95	3
11	95	3
12	100	3
13	90	3
14	95	3

during treatment. The numbers of AEs per patient in the safety population that were possibly, probably or definitely drug related are summarised in Table 5.

Haematological toxicity was mild with only two patients experiencing grade 1/2 thrombocytopenia and one patient with grade 1 neutropenia. Anaemia was the most common haematological AE in 50% of patients, of which all were grade 1/2.

Elevated triglycerides were the most common blood chemistry AE in 57% of patients, of which all were grade 1/2. Elevated liver function tests were seen in up to 50% of patients, including grade 3/4 toxicity. Renal function tests were elevated in 29% of patients, of which all were grade 1/2.

Drug-related non-haematological toxicity included rash and burning sensation of the skin in 14% of patients and dry skin in 21% patients. General symptoms included nausea and fatigue in 14% of patients each, sweating, headache, dry mouth, hot flashes and arthralgia in 7% of patients each. Bone pain and decreased joint range of motion occurred in 14% of patients each. One patient experienced a urinary tract infection and one patient suffered from agitation. All non-haematological AEs were grade 1/2. Occurrence of hirsutism was not observed. Body weight remained unchanged during treatment with CR1447. Mean body weight at screening was 69.36 kg (s.d. 15.93) compared to 69.71 kg (s.d. 15.34) at the end of treatment ($P = 0.54$).

Two serious adverse events (SAEs) were seen in this study. A 70-year-old patient in the dose level 2 group was admitted to the hospital with abdominal pain. Trial medication was discontinued and the patient died ten days later. Tumour progression was deemed the cause of death and the event was judged unrelated to the study medication. The second patient, a 54-year-old woman with brain metastases and a history of deep vein thrombosis, developed intracranial haemorrhage under anticoagulation with enoxaparin one month after starting on the trial. CR1447 was temporarily discontinued and enoxaparin was stopped. The patient recovered with residual symptoms of hemiplegia, but her tumour progressed and she was taken off trial. The event was judged unrelated to the study medication.

Response

Of the 14 patients, none achieved disease control (DC) at 24 weeks. Two (14%) patients showed s.d. at 12 weeks; the first patient with s.d. at 12 weeks had previous treatment

Table 3 Dose escalation and number of cycles administered.

	All patients (*n* = 14)			Evaluable patients (*n* = 12)		
	DL 1 (100 mg)	DL 2 (200 mg)	DL 3 (400 mg)	DL 1 (100 mg)	DL 2 (200 mg)	DL 3 (400 mg)
No. of cycles	4	4	6	3	3	6
1	1 (25%)	3 (75%)	1 (17%)	–	2 (67%)	1 (17%)
2	–	1 (25%)	3 (50%)	–	1 (33%)	3 (50%)
3	–	–	–	–	–	–
4	2 (50%)	–	–	2 (67%)	–	–
5	–	–	1 (17%)	–	–	1 (17%)
6	–	–	–	–	–	–
7	–	–	–	–	–	–
8	1 (25%)	–	1 (17%)	1 (33%)	–	1 (17%)

with two lines of endocrine treatment (letrozole for 5 years in the adjuvant setting, followed by tamoxifen for 1 year in the palliative setting) and two lines of chemotherapy (cyclophosphamide, methotrexate and 5-fluorouracil, followed by epirubicin and cyclophosphamide); the second patient with s.d. at 12 weeks had two lines of endocrine treatment (tamoxifen for two years, followed by anastrozole for 7 months, both in the palliative setting). Eleven (79%) patients showed progressive disease and 1 (8%) patient's response could not be assessed due to withdrawal of consent beforehand.

Pharmacokinetics

A total of 140 study test samples were analysed in order to determine 4-OHT and 4-OHA plasma concentrations.

Plasma concentrations after single dose administration of 100 mg CR1447 are shown in Fig. 1A. 4-OHT plasma concentrations were in the range of blq (below the lower limit of quantification) to 1.88 ng/mL. The C_{max} and AUC values showed high inter-individual variability. Median T_{max} ($n = 12$) for 4-OHT was 16 h (range: 1.0–72.0), median C_{max} ($n = 14$) was 0.63 ng/mL (range: 0.0–1.88) and the median $AUC_{0–72}$ ($n = 12$) was 27.2 h*ng/mL (range: 0.0–69.8).

The 4-OHA plasma concentrations were below the lower limit of quantification in all samples after single dose except for one sample tested.

Table 4 Dose deviations (all patients, *n* = 14).

|	DL 1	DL 2	DL3
Cycles (*n*)	17	5	20
No deviation	16 (94%)	4 (80%)	11 (55%)
Physician's choice	–	–	2 (10%)
Patient's choice	–	–	6 (30%)
Toxicity	–	–	1 (5%)
Other*	1 (6%)	1 (20%)	–

*Patients forgot taking the medication.

Plasma concentrations after multiple dose administration of CR1447 are shown for 4-OHT in Fig. 1B and for 4-OHA in Fig. 1C. 4-OHT plasma concentrations on day 8 and day 15 were in the range of 0.37–2.81 ng/mL for dose level 100 mg, 0.72–5.61 ng/mL for dose level 200 mg and 2.06–10.05 ng/mL for dose level 400 mg. 4-OHA plasma concentrations on day 8 and day 15 were in the range of blq to 0.34 ng/mL for dose level 100 mg, blq to 0.89 ng/mL for dose level 200 mg and blq to 0.83 ng/mL for dose level 400 mg. 4-OHT and 4-OHA plasma concentrations increase with rising dose levels. No conclusions with regard to dose linearity can be drawn from these data, due to the high inter-individual variability and the limited number of data.

Table 5 Worst drug-related toxicity per patient (all cycles).

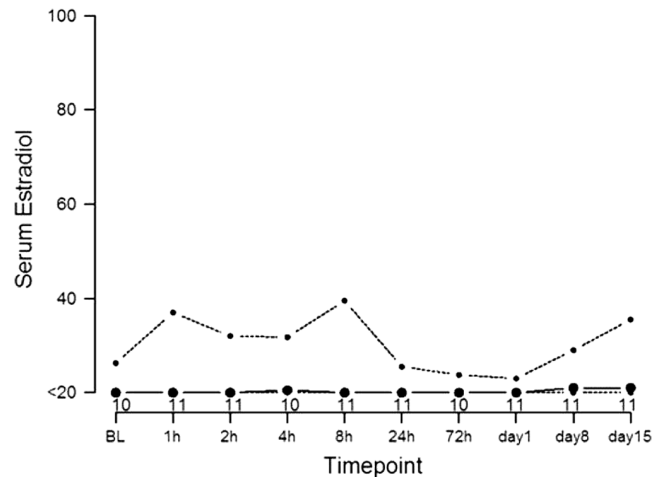| Adverse event	Grade 1/2	Grade 3/4
Haematological and blood chemistry toxicity		
Elevated triglycerides	57% (8)	–
Elevated AST	29% (4)	21% (3)
Anaemia	50% (7)	–
Elevated AP	29% (4)	14% (2)
High creatinine	29% (4)	–
Elevated ALT	21% (3)	7% (1)
Elevated bilirubin	7% (1)	14% (2)
Thrombocytopenia	21% (3)	–
Neutropenia	7% (1)	–
Rash, burning sensation	21% (3)	–
Dry skin	21% (3)	–
Nausea	14% (2)	–
Fatigue	14% (2)	–
Joint range of motion decreased	14% (2)	–
Bone pain	7% (1)	–
Dry mouth	7% (1)	–
Sweating	7% (1)	–
Urinary infection	7% (1)	–
Headache	7% (1)	–
Agitation	7% (1)	–
Hot flashes	7% (1)	–
Arthralgia	7% (1)	–
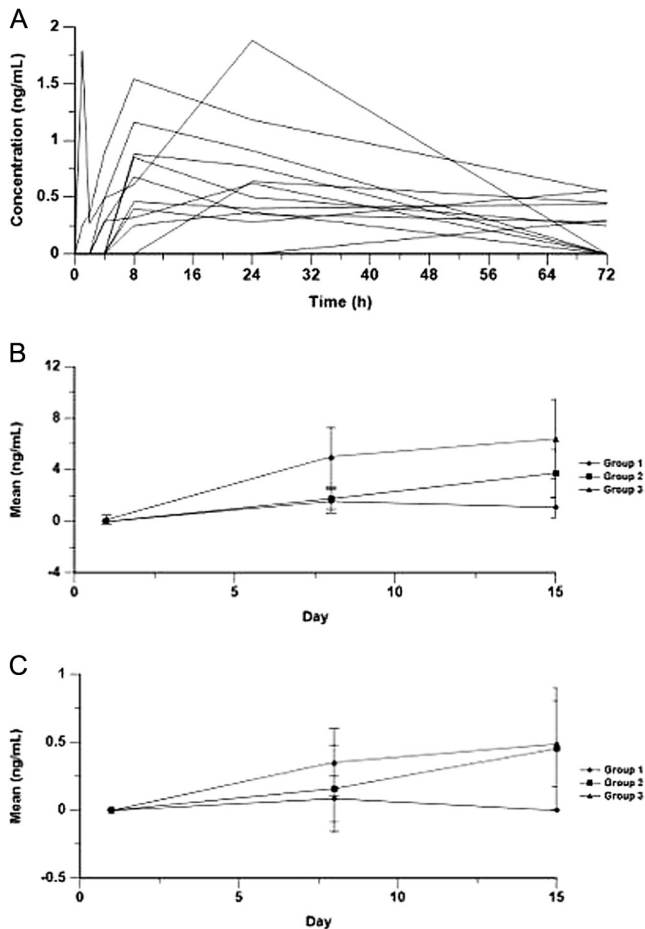
Figure 1

Plasma concentrations of 4-hydroxyandrostenedione (4-OHA) and 4-hydroxytestosterone (4-OHT) after a single transdermal application of 100 mg 4-OHT (72 h-pharmacokinetics, $n = 12$) (A). Plasma concentrations of 4-OHT after transdermal administration of 4-OHT at 100 mg/day (Group 1), 200 mg/day (Group 2) and 400 mg/day (Group 3) (B). Plasma concentrations of 4-OHA after transdermal administration of 4-OHT at 100 mg/day (Group 1), 200 mg/day (Group 2) and 400 mg/day (Group 3) (C).

The cumulated amount of each analyte excreted in the urine (U_{cum}) was evaluated based on the individual urine concentrations of 4-OHT and 4-OHA (both, unchanged analyte, and following cleavage of the metabolite conjugates) and the total excreted volume within the sampling period following administration. The cumulated amounts ($n = 14$) are shown as medians in Table 6.

Figure 2

Serum oestradiol concentrations (pmol/L) over the time of treatment with CR1447. The serum concentrations from baseline (BL) until 72 h were measured after single dose administration of 100 mg 4-hydroxytestosterone (4-OHT) in 12 patients. The subsequent concentrations on days 1, 8 and 15 were measured during daily application of 4-OHT at three different doses, 100 mg ($n = 3$), 200 mg 4-OHT ($n = 3$) and 400 mg 4-OHT ($n = 6$), respectively. Means (solid line) are given for all 12 patients at each time point. Dotted lines indicate 1st and 3rd quartiles (the dotted 1st quartile line overlaps mostly with the solid mean line).

Pharmacodynamics of oestradiol

Serum oestradiol concentrations (pmol/L) after single dose administration of 100 mg 4-OHT (baseline to 72 h, $n = 12$) and during daily application of 100 mg 4-OHT ($n = 3$), 200 mg 4-OHT ($n = 3$) and 400 mg 4-OHT ($n = 6$), respectively (day 1, 8 and 15) are shown in Fig. 2. Median oestradiol serum concentrations did not increase over the time of 15 days.

Discussion

In preclinical studies, CR1447 has been shown to inhibit human BC cell lines in their growth if they express the AR, while knockout of the AR abolishes this effect. The antiproliferative activity of 4-OHT was assessed in nearly 100 different experimental settings using different cell lines expressing the AR. Downregulation of the AR receptor by siRNA technology leads to a significant

Table 6 Urine PK of 4-OHT and 4-OHA ($n = 14$).

	$U_{cum\ 0–24}$ (4-OHT) (nmol)	$U_{cum\ 0–24}$ (4-OHA) (nmol)	$U_{cum\ 0–24}$ (sum 4-OHT) (nmol)	$U_{cum\ 0–24}$ (sum 4-OHA) (nmol)
Median (range)	2.42 (0.00–39.5)	0.49 (0.00–1.93)	8.72 (3.75–53.5)	34.5 (0.00–192)

U_{cum}, cumulative concentration in the urine; 4-OHT, 4-hydroxytestosterone; 4-OHA, 4-hydroxyandrostenedione.

reduction of the antiproliferative effects of 4-OHT (11). In this first-in man phase I trial, we investigated the toxicity, pharmacokinetics and pharmacodynamics of CR1447 in patients with ER-positive/AR-positive/HER2-negative BC.

Toxicity in general was mild. Anaemia was the most common haematological toxicity, while blood chemistry changes were observed mostly for triglycerides, liver and kidney function tests. While six out of seven patients who experienced liver and kidney function test impairment also suffered from liver metastasis, the finding of altered lipid profiles is not surprising. Based on previous experience with AIs, one might assume that CR1447 with its known AI activity may also elicit similar side effects (12). However, none of those led to dose modifications or disruptions regarding the treatment with CR1447 in our study. There is some controversy on the impact of AIs on the lipid profile. Lipid profile changes will need attending in future trials with CR1447.

Non-haematological toxicities included skin reactions/dry skin, which lead to a dose reduction in one patient only. These symptoms and other, less frequent ones such as nausea, fatigue, sweating, headache, bone and joint pain, dry mouth and hot flashes might fit well to the antihormonal effects of CR1447. All drug-related non-haematological toxicities were of grade 1/2 only.

The two serious adverse events (SAEs) seen in this study (death due to tumour progression and intracranial haemorrhage under anticoagulation) were judged unrelated to the study medication. No DLTs were seen.

The observation of two patients (17%) with stable disease after 12 weeks of treatment suggests some single-agent activity, given that all patients received prior endocrine therapy, and 83% of these patients also had prior chemotherapy.

Recently, there has been a growing interest in targeting the AR in the treatment of BC. Clinical trials report a median progression-free survival of 12 weeks with the non-steroidal AR antagonists bicalutamide (13) and orteronel (14), but even complete responses have been observed (15). In a combination trial of the non-steroidal AR antagonist enzalutamide with the oestrogen antagonist fulvestrant, 45% of patients still remained on treatment after 24 weeks (16). The non-steroidal AR modulator GTx-024 demonstrated clinical benefit in 35% of patients with metastatic BC at 6 months (17). Disease control at 12 weeks in our study is comparable with the results of the other studies cited. However, there are concerns that a pure antagonist could foster virilisation. Therefore, a partial antagonist, or selective androgen receptor modulator (SARM) such as the steroidal AR antagonist CR1447 might offer an advantage over the non-steroidal AR antagonists.

PK results confirmed a relatively quick absorption of 4-OHT. One could have speculated that 4-OHT might be rapidly converted to 4-OHA *in vivo*, a drug which has been used previously as an AI (formestane, Lentaron). Formestane had to be given by intramuscular injection because of fast metabolisation if taken orally (18). Interestingly, our PK data show that the ratio of 4-OHT:4-OHA in the serum is roughly 10:1 even after two weeks of daily administration. However, the ratio of 4-OHT:4-OHA in urine is 1:5, possibly indicating metabolisation of 4-OHT to 4-OHA prior to and/or during elimination.

Serum oestradiol concentrations did not change during treatment with CR1447, indicating no relevant conversion of the drug to oestrogen.

The dose recommended for phase 2 trials must take account of the RECIST and toxicity data. As, due to the steroidal nature of the molecule, we did not anticipate issues with dose-related toxicity, we chose three different dose levels on the basis of preclinical studies and extrapolations from previous experience with 4-OHA. A relatively high number of cycles could be evaluated for toxicity (16 in DL1 vs 4 in DL 2 and 20 in DL3). CR1447 was well tolerated at all dose levels, including the highest dose level at 400 mg/day.

In summary, CR1447 administered transdermally as an ointment was well tolerated and appears to have single-agent activity in heavily pretreated ER-positive/AR-positive/HER2-negative BC patients. The next step will be a phase II trial in order to test the hypothesis if CR1447 might offer the possibility of non-chemotherapy-based endocrine therapy to the limited treatment options in triple-negative (TN)/AR-positive BC. The recommended phase II dose is 400 mg/day.

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