



# Phase 1 study of TTC-352 in patients with metastatic breast cancer progressing on endocrine and CDK4/6 inhibitor therapy

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## Abstract

**Purpose** TTC-352 is a selective human estrogen receptor (ER) partial agonist developed for treatment of hormone-refractory ER + breast cancer.

**Methods** This was an accelerated dose escalation study with the primary endpoint of maximum tolerated dose that evaluated five dose levels of TTC-352 in breast cancer progressing after at least two lines of hormonal therapy including one in combination with a CDK4/6 inhibitor. The secondary objectives were to determine treatment tolerability, pharmacokinetics of TTC-352, best response, progression-free survival (PFS), and PKC $\alpha$  expression in tumors.

**Results** The study enrolled 15 patients. No dose-limiting toxicity was observed. Patients experienced the following grade 3 toxicities: asymptomatic pulmonary embolism, diarrhea, aspartate transaminase elevation, and myalgia, and one grade 4 toxicity of gamma glutamyltransferase elevation. Pharmacokinetic half-life was 7.6–14.3 h. The intra- and inter-individual variability for AUC<sub>0-∞</sub> hampered assessment of the relationship between dose and AUC<sub>0-∞</sub>. Median PFS was 58 days (95% CI = 28, 112). Higher PKC $\alpha$  expression in tumor stroma was associated with a trend toward longer PFS.

**Conclusions** TTC-352 demonstrates safety and early clinical evidence of antitumor activity against heavily pretreated hormone-refractory breast cancer. Based upon TTC-352 plasma concentrations and tolerability, the 180 mg twice a day is recommended for further testing.

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**Keywords** Estrogen receptor · TTC-352 · Phase 1 study · Refractory breast cancer

## Abbreviations

AI Aromatase inhibitors  
CEE Conjugated equine estrogens

C<sub>max</sub> Maximum plasma concentration  
CTCAE Cancer Institute Common Terminology Criteria for Adverse Events  
DES Diethylstilbestrol  
DLT Dose-limiting toxicity  
E2 17 $\beta$ -Estradiol

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ECOG	Eastern Cooperative Oncology Group
ER	Estrogen receptor
ER+	Estrogen receptor positive
HER2-	Human epidermal growth factor receptor 2 negative
HDE	High-dose estrogens
IHC	Immunohistochemistry
MTD	Maximum tolerated dose
PFS	Progression-free survival
PI3K	Phosphoinositide-3-kinase
PKC $\alpha$	Protein kinase C alpha SEMs
SEMs	Selective estrogen mimics
ShERPA	Selective human ER partial agonist
SRERD	Selective estrogen receptor downregulator
$t_{1/2-\lambda z}$	Terminal half-life
TTF	Time-to-treatment failure

## Background

Though there has been an increase in the number of patients diagnosed with breast cancer over the past several years, overall outcomes have improved for several reasons, including more effective therapies. However, more than 40,000 patients in the USA die from breast cancer annually. Approximately two-thirds of breast tumors are estrogen receptor positive (ER+) and the use of endocrine therapy has improved outcome for patients with all stages of ER+ disease. Unfortunately, endocrine resistance, both de novo and acquired, results in disease recurrence and progression. The addition of CDK 4/6 inhibition to endocrine therapy has led to dramatic improvements in progression-free survival for patients with metastatic hormone receptor positive breast cancer treated in the first-line setting and are now standard of care [1]. Despite the efficacy of endocrine therapy, and the new combinations of AIs and SERD with CDK4/6 inhibitor therapies, all patients with metastatic disease will develop resistance to endocrine therapy ultimately leaving cytotoxic chemotherapy as the only option [2, 3]. Thus, the development of therapeutic alternatives for patients with ER+ endocrine-resistant breast cancer is of critical importance. One drug class that has shown efficacy in the tamoxifen and AI-resistant setting is the novel selective estrogen mimics (SEMs) [4].

TTC-352 [3-(4-fluorophenyl)-2-(4-hydroxyphenoxy)benzo[b]thiophen-6-ol] is one of a subset of SEMs, which acts as a selective human ER partial agonist (ShERPA) in ER+ cell lines [5]. It is argued and supported by animal studies that a ShERPA that mimics the effects of 17 $\beta$ -estradiol (E2) in endocrine-resistant breast cancer cells and tumors, but is a partial agonist in contrast to the full agonists, E2 and diethylstilbestrol (DES), will have an improved side effect profile. Specifically, ShERPAs did not support

the growth of ER+, hormone-dependent cell lines that normally require E2 for growth; nor did they result in significant uterine growth as E2 did in these models [5]. Given these observations, TTC-352 was advanced to drug development for clinical study in patients with endocrine-resistant breast cancer.

Protein kinase C alpha (PKC $\alpha$ ) is a member of the serine/threonine kinases and has a role in the transduction of signals for cell proliferation and differentiation [6]. Tumors that develop acquired endocrine resistance, retain ER and show elevated PKC $\alpha$  expression associated with resistance to endocrine therapy [7, 8]. We have previously shown that increased PKC $\alpha$  expression in ER+ breast cancer cell lines with acquired resistance to tamoxifen and AI, correlates with growth inhibition and apoptosis in response to E2 [9, 10]. Growth of multiple MCF-7-derived tamoxifen-resistant tumor cell lines was inhibited by E2, and E2 caused complete regression of xenograft tumors [4], which suggested that PKC $\alpha$  may be predictive of benefit from therapeutic intervention by estrogenic compounds.

The primary objective of this study was to determine the maximum tolerated dose (MTD) for TTC-352 in patients with breast cancer progressing on at least two endocrine therapies including in combination with CDK4/6 inhibitor. The secondary objectives were to evaluate treatment tolerability, to establish the pharmacokinetic profile of TTC-352, to assess best response to treatment, and determine progression-free survival (PFS). An exploratory objective was to evaluate PKC $\alpha$  expression in tumors and correlate it with duration of PFS.

## Methods

### Patients

Enrolled patients had ER+ (defined per ASCO-CAP guidelines) metastatic breast cancer and received and experienced disease progression on at least two lines of endocrine therapy, with one that included a CDK4/6 inhibitor, and for which no effective standard therapy other than chemotherapy was available. Patients with HER2-positive cancer were eligible. Main eligibility criteria were as follows: (a) Eastern Cooperative Oncology Group (ECOG) performance status of  $\leq 1$ ; (b) No impending visceral crisis that requires chemotherapy; (c) No uncontrolled or symptomatic CNS metastases (d) Adequate hepatic, renal, and hematologic functions; and (e) measurable or evaluable disease. The complete list of inclusion and exclusion criteria can be found in the study protocol in Supplementary Material.

The institutional review board at all participating centers approved the study protocol. All patients provided informed consent before study interventions were initiated.

## Treatment plan

TTC-352 was administered as an oral capsule, twice a day. Patients received sequential 28-day cycles of treatment until disease progression, unacceptable toxicity, patient refusal to continue treatment, any other reason to discontinue, or study completion/termination. TTC-352 was supplied in 15 and 60 mg capsules by TTC Oncology, LLC (Edina, MN, USA).

## Study design

A total of five incremental dose levels (15, 30, 60, 120, 180 mg twice a day) were planned. The lower level of tested TTC-352 dose range was determined by results of severely toxic rate (10) in rats (the most sensitive species to TTC-352) being 30 mg/kg. The highest tested dose level of TTC-352 exceeded threefold a level where antitumor activity was observed in two tamoxifen-resistant xenograft models. Cycle was defined as 28 days. Rapid escalation (cohort size of 1 patient) continued until a patient experienced toxicity grade 2 (excluding alopecia or diarrhea), after which enrollment proceeded with cohorts of 3 patients.

A dose-limiting toxicity (DLT) was defined as one of the following probably or possibly related to study medication events occurring during the first cycle: (a) Grade 4 thrombocytopenia of any duration or grade 3 thrombocytopenia with clinically significant bleeding; (b) Grade 3 or greater neutropenia with fever of any duration; (c) Grade 3 or greater clinical non-hematological toxicity (excluding  $\geq$  grade 3 nausea, vomiting, or diarrhea without maximal medical intervention and/or prophylaxis); (d) a pause in treatment of more than 3 weeks after the first 28-day cycle of treatment, due to inadequate recovery of any hematologic toxicity considered related, probably related or possibly related to study medication (adequate hematologic toxicity recovery is defined as a total neutrophil count  $> 1000/\text{mm}^3$  or platelets  $> 100,000/\text{mm}^3$ ); (e) inadequate recovery of any non-hematologic toxicity considered related, probably related or possibly related to study medication (adequate non-hematologic toxicity recovery is defined as improvement to  $\leq$  grade 1, unless the patient entered the study with a grade 2 baseline), disease-related toxicity or a grade 2 baseline neuropathy.

The MTD was defined as the dose associated with DLT in less than or equal to 33% of patients. Once MTD was determined, the MTD dose cohort was expanded to enroll a cohort of 10 patients to assure safety. Treatment was continued until disease progression, unacceptable toxicity, or patient refusal.

## Evaluation of toxicity and response

The US National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 4.03) [11]

were used to evaluate symptoms and toxicity assessment on day 1 of each treatment cycle. Appropriate imaging scans, including CT scans, MRI and bone scans, were performed at the time of study enrollment and every 8 weeks from treatment cycle to assess disease status. Standard clinical measures were used to assess malignancy response including RECIST v1.1 in patients with measurable disease [12]. Secondary efficacy endpoints included best response (complete response, partial response, or stable disease) and PFS and OS measured from time of first administration of drug.

## Pharmacokinetics

Pharmacokinetics were established for each dose level. The pharmacokinetic profile of TTC-352 was assessed under fasting conditions after the first dose on Day 1 of cycle 1 and after morning administration of the last dose of cycle 1 on Day 28 with evening doses skipped on these days. Blood samples for analysis of TTC-352 plasma concentrations were collected prior to and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24 and 28 (day 28 only) hours following oral administration of TTC-352 on days 1 and 28 of cycle 1. Plasma was assayed for TTC-352 utilizing a validated LC/MS–MS method. The lower limit of quantitation was 0.1 ng/ml. The TTC-352 plasma concentration–time data was analyzed by non-compartmental methods (Phoenix WinNonLin® version 8.1, Certara, L.P., Princeton, NJ).

## PKC $\alpha$ expression evaluation

PKC $\alpha$  expression level in archived tumor biopsies was determined by immunohistochemistry (IHC) at the University of Illinois at Chicago in the laboratory of Dr Elizabeth L. Wiley. IHC analysis was performed on 5  $\mu\text{m}$  sections of formalin-fixed paraffin embedded tissue with the Ventana Benchmark automated staining platform using the iView DAB detection kit according to company protocol using CC1 Standard antigen retrieval. The PKC $\alpha$  antibody (rabbit polyclonal, Santa Cruz Biotechnology, sc-208) was previously validated [13, 14] and used at a dilution of 1:200 and incubated at 37 °C for 30 min. Frequency and intensity of PKC $\alpha$  staining of all tumor cells on each slide were scored on a scale of 0 to 4 without the assessor's knowledge of clinical patient data. Frequency of positive staining in less than 1% of tumor cells was scored as 0, 1%–10% as 1, 11% to 35% as 2, 36%–70% as 3, and over 70% as 4. A composite score is also reported based on the Allred scoring system which is a sum of the frequency and intensity scores yielding numerical values from 0 to 8 [15].

## Statistical analyses

Descriptive statistics were used to describe response rates. PFS curves were estimated using the Kaplan–Meier method. Chi-square tests were used for testing association between PKC $\alpha$  expression and PFS. All statistical tests were 2-sided tests, controlling for Type I error probability of 0.05.

## Results

### Patient characteristics

Fifteen patients were enrolled in the trial between January 30, 2018 and June 9, 2019. All patients were females, with median age of 62 (range: 40–77), had ER + metastatic breast cancer, and all had received multiple lines of prior therapy (median 9, range; 2–15) for metastatic disease. One patient had HER2 positive disease. All patients received prior CDK inhibitor therapy and all, but one, had received cytotoxic chemotherapy for metastatic disease. Two-thirds of patients had received prior therapy with a mTOR inhibitor. Prior therapies are listed in Table 1.

### Dose escalation

There were 2 patients at the first dose level (15 mg twice a day), because the first patient withdrew from the study at Day 22 of the first cycle due to progression of breast cancer metastasis in liver. Therefore, this patient was replaced. In the following dose levels (Table 1), there was one patient per dose. Since in the highest dose level tested of 180 mg twice a day, there was no DLT and MTD was not reached, this cohort was expanded to enroll 10 patients. A total of 47 treatment cycles were administered during the trial. No DLT was observed at dose level 1, 2, 3, 4 or 5.

### Toxicity

Table 2 shows toxicities possibly or probably related to TTC-352. Most common grade 1 and 2 toxicities seen in more than one patient were: diarrhea (87%), hot flashes (27%), abdominal pain (27%), nausea (20%), fatigue (20%), headache (20%), maculopapular rash (20%), dizziness (13%), peripheral neuropathy (13%), vaginal discharge (20%), breast pain (13%), arthralgia (13%), vomiting (13%), QT prolongation (13%), anemia (13%), uterine hemorrhage (13%), and blurred vision (13%). There were four grade 3 toxicities: one of asymptomatic pulmonary embolism, one of diarrhea, one of aspartate transaminase elevation, and one episode of myalgia. There was one grade 4 toxicity of gamma glutamyltransferase elevation. No treatment-related deaths occurred.

## Pharmacokinetics

Pharmacokinetics of TTC-352 were examined in 15 patients following administration of a single-oral dose on Day 1 and 12 patients following TTC-352 twice daily for 28 days. Subjects received either 15 mg, 30 mg, 60 mg, 120 mg, or 180 mg twice a day. The day 28 plasma concentration data from two subjects, 02–002 and 03–006, were not included in the pharmacokinetic analysis. Both subjects experienced adverse events at the 180 mg dose, and, as a result, their TTC-352 doses were lowered from 180 to 150 mg on day 28.

Maximum plasma concentrations ( $C_{max}$ ) typically occurred within 5 h after oral administration. The terminal elimination half-life ( $t_{1/2-\lambda_z}$ ) ranged from 7.23 to 10.23 h in the 15 mg–120 mg groups on day 1, 8.84 to 14.31 h in the 180 mg group on day 1, 9.38 to 20.45 h in the 15 mg – 120 mg groups on day 28 and 4.53 to 20.35 h in the 180 mg group on day 28. Dose and single- versus multiple-dose administration had minimal influence on  $T_{max}$  and  $t_{1/2-\lambda_z}$ . Oral clearance (CL/F) in the  $\leq 60$  mg dose group was lower than in the 120 and 180 mg groups on days 1 (medians: 6505 mL/min vs 11,246 mL/min, respectively) and 28 (median: 2766 mL/min vs. 9333 mL/min, respectively). The CL/F on days 1 and 28 were comparable across dose groups.

TTC-352  $C_{max}$  in the 180 mg dose cohort ranged from 4.62 to 65.47 ng/mL on day 1 and 25.78 to 93.17 ng/mL on day 28. Area under the plasma concentration–time curve from time 0 to 12 h post-dose ( $AUC_{0-12}$ ) ranged from 35.8 to 272.4 ng-h/ml on day 1 and 154.9 to 975.7 ng-h/mL on day 28. Similar variability occurred at the lower doses. Assessment of dose proportionality was hampered by the small number of patients at the lower doses, and large inter-patient variability in  $C_{max}$ ,  $AUC_{0-12}$  and area under the plasma concentration–time curve from time 0 to infinity ( $AUC_{0-\infty}$ ).

Prediction by  $t_{1/2-\lambda_z}$  of accumulation of TTC-352 plasma concentrations with multiple dosing from day 1 to day 28 underestimated the actual extent of accumulation by approximately two-fold, 1.57 versus 2.64 (day 12 to day 1  $AUC_{0-12}$  ratio). The underestimation of accumulation of TTC-352 plasma concentrations likely represents the failure of  $t_{1/2-\lambda_z}$  to reflect the influence of absorption or enterohepatic recirculation on the functional half-life of TTC-352.

The pharmacokinetics of orally administered TTC-352 at doses of 15 to 180 mg was characterized by high intra- and inter-individual variability.

## Efficacy

Progression of disease was a cause of study treatment discontinuation in 13 (87%) patients. One patient withdrew from the study because of increase in tumor biomarkers (CA 15–3 and CA 27–29), and the other because of gamma glutamyltransferase elevation. Of 15 enrolled, 3 patients were

**Table 1** Patient characteristics

Cohort	TTC 352 dose (twice a day)	15 mg	30 mg	60 mg	120 mg	180 mg
Median (range) age, years		51 (40–62)	54	49	60	67 (54–77)
ECOG PS, <i>n</i> (%)						
0			1	1		6
1	2				1	4
Race, <i>n</i>						
White	2			1	1	10
Asian		1				
Median (range) number of prior lines of therapy	8 (5–12)	15		5	5	9 (2–15)
Type of Prior Therapies						
Cytotoxic Chemotherapy	2	1		1		10
Endocrine therapy						
Non-steroidal AI	2	1		1		9
Steroidal AI	1	1				5
Fulvestrant	1	1		1		10
Tamoxifen	1	1		1		9
Targeted therapy						
CDK4/6 inhibitor	2	1		1		10
mTOR inhibitor	1	1				7
Bone-only disease, <i>n</i>						
Visceral disease, <i>n</i>	1	1		1		8

**Table 2** Drug-related Adverse Events (All Grades) at least possibly or probably related to TTC-352

System organ class <sup>^</sup>	Adverse event preferred term <sup>^</sup>	Number (%) of Patients (n = 15) with Adverse Events by Severity Grade*				Number of Events
		Grade 1	Grade 2	Grade 3	Grade 4	
Possibly or probably related to TTC-352						
Blood and lymphatic system disorders	Anemia		2 (13%)			2
Eye disorders	Vision blurred	2 (13%)				2
Gastrointestinal disorders	Abdominal distension	1 (7%)				1
	Abdominal pain	3 (20%)	1 (7%)			6
	Diarrhea	10 (67%)	3 (20%)	1 (7%)		14
	Nausea	3 (20%)				3
	Vomiting	2 (13%)				2
General disorders	Fatigue	2 (13%)	1 (7%)			3
Investigations	ECG QT prolongation	1 (7%)	1 (7%)			3
	ALT increased		1 (7%)			1
	AST increased			1 (7%)		1
	Alkaline phosphatase increased		1 (7%)			1
	GGT Increased				1 (7%)	2
Metabolism and nutrition disorders	Decreased appetite	1 (7%)				1
	Hypophosphatemia		1 (7%)			1
Musculoskeletal disorders	Arthralgia	2 (14%)				2
	Back pain		1 (7%)			1
	Muscle spasms	1 (7%)				1
	Muscle twitching		1 (7%)			1
	Musculoskeletal chest pain	1 (7%)				1
Vascular disorders	Myalgia	1 (7%)		1 (7%)		3
	Hot flush	4 (26%)				2
	Hypotension	1 (7%)				1
Reproductive system disorders	Vaginal discharge	3 (20%)				3
	Breast pain	2 (13%)				2
	Endometrial thickening	1 (7%)				1
	Uterine hemorrhage	1 (7%)	1 (7%)			2
	Vulvovaginal pruritus	1 (7%)				1
Skin and subcutaneous tissue disorders	Alopecia	1 (7%)				1
	Rash maculopapular	2 (13%)	1 (7%)			3
Respiratory, thoracic and mediastinal disorders	Pulmonary embolism			1 (7%)		1
Nervous system disorders	Dizziness	2 (13%)				2
	Headache	3 (20%)				3
	Peripheral Neuropathy		2 (13%)			2
	Restless leg syndrome	1 (7%)				1
	Somnolence		1 (7%)			1
	Urinary incontinence		1 (7%)			1
	Urinary tract pain	1 (7%)				1

\*Patients are counted once for multiple occurrences of the same adverse event, according to the worst severity grade [Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0, Published: May 28, 2009 (v4.03: June 14, 2010)]

<sup>^</sup>Coded with MedDRA Version 20.0

unevaluable for radiologic response due to the following: removed due to toxicity prior to starting cycle 3 (N = 1), clinical progression during cycle 1 (N = 1), clinical progression during cycle 2 (N = 1). The percentage change according to RECIST criteria from baseline was evaluated at the time of

best response. There were no complete or partial responses. There were six patients with stable disease, four at 180 mg twice a day dose. Of the 12 evaluable patients, 4 patients had stable disease at 4 months (33%), and 2 patients at 6 months (16% clinical benefit rate (CBR)). Median PFS time was

58 days (95% CI=28,112). Figure 1 shows duration of therapy for each patient. Figure 2A shows Kaplan–Meier PFS curve for all patients.

### PKC $\alpha$ expression evaluation

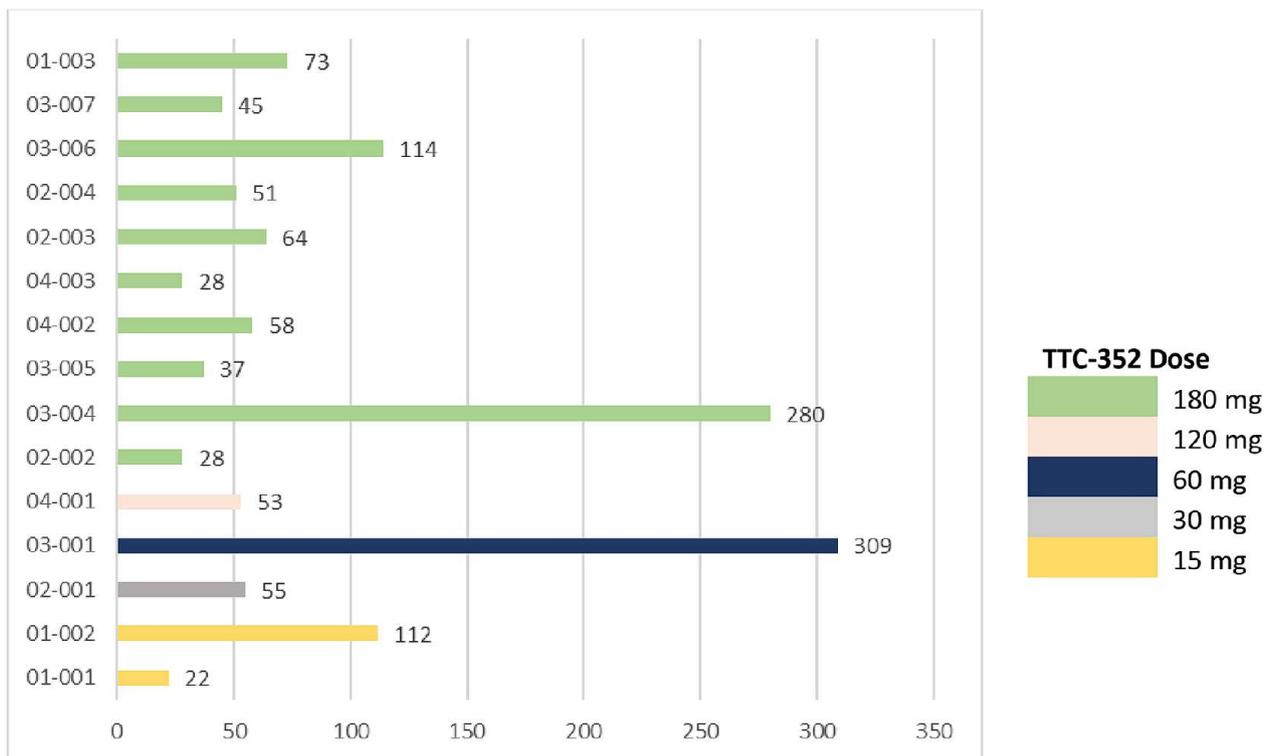
Table 3 shows PKC $\alpha$  expression in archival tumor tissue. There was no tissue available in two patients, and in one patient tissue was inadequate for analysis. Expression of PKC $\alpha$  in tumor cells did not correlate with PFS (HR 1.179, 95% CI=0.772, 1.802),  $p$ -value=0.446), whereas expression of PKC $\alpha$  in tumor stroma had a trend towards weak association with better outcome (HR 0.442, 95% CI=0.164, 1.197),  $p$ -value=0.1082) (Fig. 2b).

### Discussion

We conducted the first in-human phase 1 study of a novel ShERPA, TTC-352, in patients with ER + metastatic breast cancer previously treated with at least two prior hormonal therapies, one of which was given in combination with a CDK4/6 inhibitor.

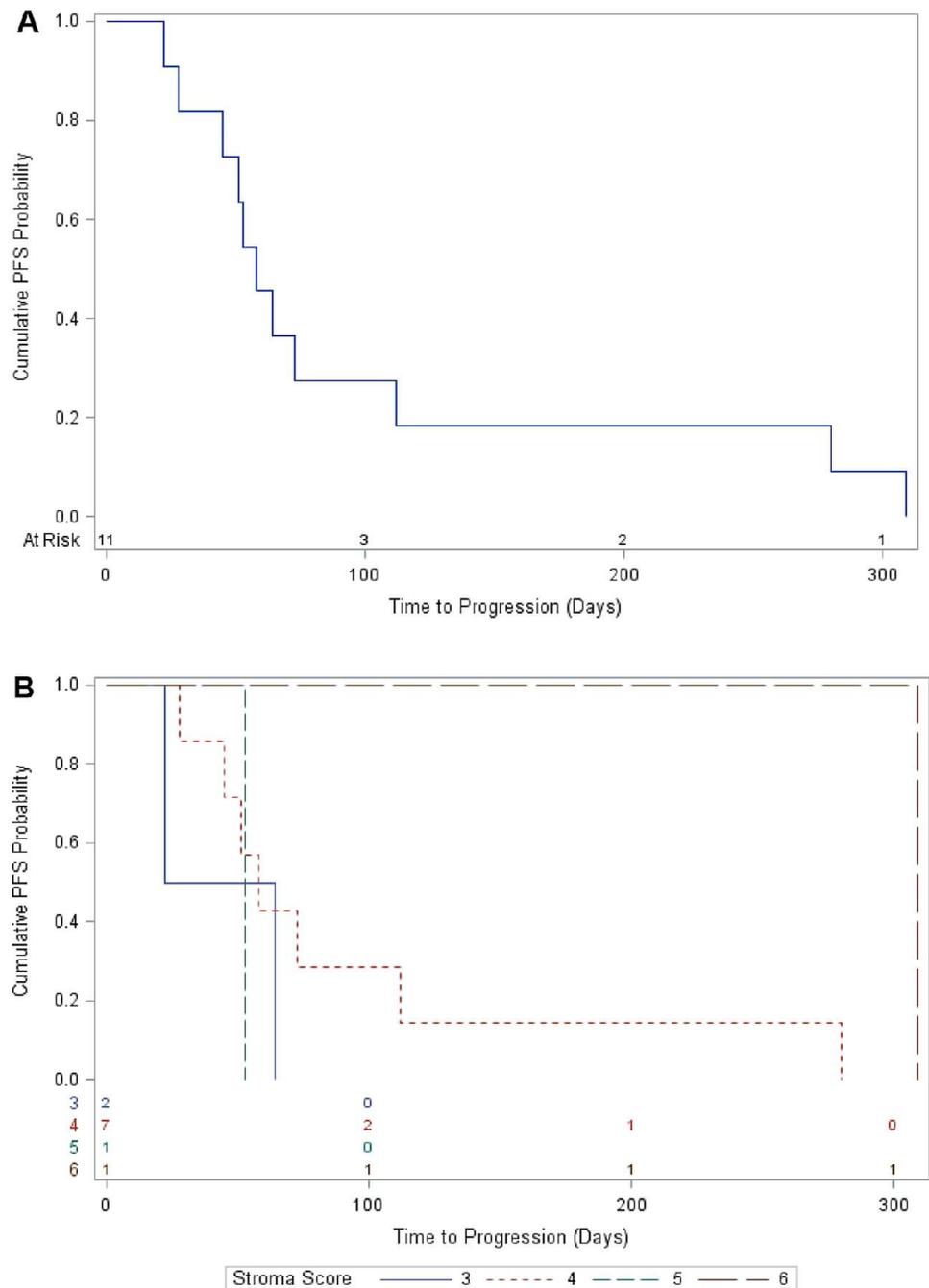
The approval of cyclin-dependent kinase CDK4/6 inhibitors in combination with AIs or the SERD, fulvestrant, has transformed the treatment of hormone receptor (HR)-positive, HER2 negative metastatic breast cancer. Pivotal studies of the three approved CDK4/6 inhibitors in combination with an AI or fulvestrant support combination therapy as the preferred therapeutic approach for most patients HR positive, HER2-negative metastatic breast cancer dramatically improving PFS by approximately 50% and improving overall survival in several trials. After disease progression on CDK4/6 inhibition, at present, there are no studies supporting continuation of CDK4/6 inhibitor therapy [16], though this approach is being evaluated in ongoing clinical trials. Following endocrine therapy and CDK inhibition, options for further therapy include mTOR inhibition, or PI3-kinase inhibition in tumors with PI3-kinase mutations, or chemotherapy, but therapeutic resistance ultimately occurs with resultant disease progression [17]. Therefore, with the goal of improving survival while maintaining quality of life, there is a critical need for new therapies for endocrine-resistant metastatic breast cancer.

Although clinical trials have supported the re-emergence of E2 as a possible treatment strategy for patients with endocrine-resistant breast cancer [18], this therapy is



**Fig. 1** Number of days that a subject was on therapy grouped by TTC-352 dose cohort. Each bar represents a subject and color-coded dose increases from the lower bars of the graph to the highest bars

**Fig. 2** **a** Kaplan–Meier progression-free survival (PFS) curve for all patients. **b** Stroma score and progression-free survival



associated with the potential for serious side effects such as an increased risk of endometrial cancer and thromboembolic events, which has deterred the clinical community from adopting it as a treatment strategy. Prior to the introduction of tamoxifen, high-dose estrogen (HDE) and DES were used to treat metastatic breast cancer in the 1960s and 1970s [19]. Randomized trials compared tamoxifen to HDE/DES and found similar or slightly inferior efficacy, but better tolerability with tamoxifen [20, 21]. More recently, the preclinical evidence for the switch

from E2 promoting growth in breast epithelial cells to causing growth inhibition was observed in both cell lines and xenograft models and is often described as the cyclical response to estradiol [22]. This laboratory observation was later validated coincidentally in the Women's Health Initiative study reporting that women treated with conjugated equine estrogens (CEE) post-hysterectomy had reduced risk of breast cancer incidence and mortality [23]. Later, Ellis et al. addressed the tolerability issue by comparing low-dose estrogen versus HDE in patients with resistance

**Table 3** PKC $\alpha$  expression by immunohistochemistry in tumor cells and tumor stroma and progression-free survival of patients in study

Subject	% tumor cells	Intensity tumor	Tumor score	% stroma	Intensity stroma	Stroma Score	PFS (Days)	Drug dose
01-001	1-10	3+	4	11-50	1+	3	22	15
01-002	11-50	2+	4	11-50	2+	4	112	15
03-001	0	0	0	> 50	3+	6	309	60
04-001	1-10	1+	2	> 50	2+	5	53	120
03-004	1-10	1+	2	11-50	2+	4	280	180
04-002	1-10	1+	2	11-50	2+	4	58	180
04-003	0	0	0	11-50	2+	4	28	180
02-003	0	0	0	1-10	2+	3	64	180
02-004	1-10	1+	2	1-10	3+	4	51	180
03-007	1-10	1+	2	11-50	2+	4	45	180
01-003	0	0	0	11-50	2+	4	73	180

PFS progression-free survival; PKC $\alpha$  protein kinase C alpha

to AIs and discovered that the lower dose provided a similar clinical benefit as HDE but with fewer side effects [18]. Another report by Zucchini et al. showed similar clinical benefit with low-dose E2 valerate [24]. A recent review of clinical data suggests that HDE should be considered a valuable alternative to chemotherapy in selected patients [25].

The premise for development of a ShERPA is to preserve the antitumoral activity of E2, but with less toxicity due to partial agonist activity [5]. TTC-352 was selected for its activity in tamoxifen-resistant cell lines and efficacy in inducing tumor regression, comparable to E2, in PKC $\alpha$ -overexpressing and other tamoxifen-resistant xenografts. While endometrial thickening was seen in estradiol-treated animals, none was seen in TTC-352-treated animals [4, 5].

In this first in-human clinical trial with TTC-352, no DLT was observed, and only 4 events of grade 3 toxicity were observed: asymptomatic pulmonary embolus discovered incidentally on restaging computed tomography, diarrhea, increase in aspartate transaminase level, myalgia, and one grade 4 toxicity of gamma glutamyltransferase elevation (7% each). This is in contrast to the known toxicity of E2, which is associated with nausea and vomiting, hyponatremia, pleural effusions, pain, serious infections, and thromboembolism [18]. In summary, TTC-352 use was associated with mild toxicity and compared favorably to that of E2, making it an attractive agent for further use in endocrine-resistant ER + breast cancer.

Pharmacokinetics of TTC-352 demonstrated variability and multiple peaking of TTC-352 plasma concentrations across dose cohorts following single and multiple doses. Enterohepatic recycling of TTC-352 may explain the large variability in TTC-352 pharmacokinetics, multiple peaking, and failure of  $t_{1/2-\lambda_z}$  to predict accumulation and fluctuation of plasma concentrations with multiple dosing [26–28].

Therapy following hormonal therapy and CDK inhibition failure has limited benefit in the majority of patients. Analysis of outcomes of subsequent therapies after progression following palbociclib or to palbociclib and endocrine therapy in the randomized TReND trial revealed that overall time-to-treatment failure (TTF) was only 3.8 months (95% CI=3.5, 4.8). In addition, TTF was similar in patients receiving subsequent hormonal therapy (3.7 months (95% CI=2.8, 4.8)) or chemotherapy (4.6 months (95% CI=3.5, 5.8)) [29]. In this context preliminary data from our phase 1 study with median PFS of 58 days across all dose levels in more heavily treated patients than in the TReND study is encouraging. Especially intriguing is the fact that there were two patients in our study with disease control of more than 6 months (280 and 309 days). Both patients had more than 3 lines of prior therapy, including prior SERD and chemotherapy. One patient had received prior tamoxifen, anastrozole, letrozole with palbociclib, exemestane and everolimus, and multiple chemotherapy regimens. The second patient had prior therapy with tamoxifen, letrozole with everolimus, fulvestrant with palbociclib, fulvestrant with capecitabine, fulvestrant with abemaciclib, megestrol acetate, anastrozole, exemestane, then several lines of chemotherapy with vinorelbine, then paclitaxel, and a single agent capecitabine. Both women had performance status of 0, one of them had breast cancer with mutation in the *ESR1* gene (D538G) and bone metastases only disease (Table 4). Tumor genomic testing was not mandated and results where available are presented in Table 4.

Activation of PKC $\alpha$  is associated with endocrine-resistance and tumor aggressiveness in the clinic [7, 8]. Tonetti et al. found that lack of responsiveness to tamoxifen was associated with upregulation of PKC $\alpha$  signaling [4, 10, 13, 30]. TTC-352 was designed to induce an inhibitory effect in PKC $\alpha$ -expressing breast cancer models [3, 4].

**Table 4** TTC-352 dose, progression-free survival, PKC $\alpha$  expression, and tumor characteristics

Dose level	Best response	PFS	PKC tumor	PKC stroma	HER2	ESR1	PIK3CA	BRCA2	CHEK2	Bone mets	Visceral mets	ECOG
180	PD	28	UNK	UNK	NEG						X	1
180	SD	280	2	4	NEG	D538G				X		0
180	PD	37	UNK	UNK	NEG						X	0
180	PD	58	2	4	UNK	Y537N					X	0
180	PD	28	0	4	NEG		X				X	1
180	SD	64	0	3	NEG						X	1
180	PD	51	2	4	NEG						X	1
180	SD	114	UNK	UNK	NEG					X		0
180	SD	45	2	4	NEG						X	0
180	SD	73	0	4	NEG		X				X	0
120	PD	53	2	5	POS			X			X	1
60	SD	309	0	6	NEG						X	0
30	PD	55	UNK	UNK	NEG				X		X	0
15	PD	22	4	3	NEG						X	1
15	SD	112	4	4	NEG						X	1

PFS progression-free survival in days, PKC, protein kinase C

Therefore, we hypothesized that expression of PKC $\alpha$  in tumor tissue would be predictive of benefit from TTC-352. We collected archived diagnostic breast cancer tissue from patients participating in this study and correlated PKC $\alpha$  with PFS. Although the study sample was small (only 11 tissue samples were available and adequate for analysis), expression of PKC $\alpha$  in tumor stroma appeared to be associated with a longer PFS. While stromal expression of PKC $\alpha$  was unexpected based on our preclinical findings, PKC $\beta$  expression was reported in the tumor microenvironment in a breast cancer cohort suggesting linkage to tumorigenesis [31]. Further, there is evidence of stromal PKC $\alpha$  expression in the Human Protein Atlas <https://www.proteinatlas.org/> [32]. Given the small size of the study sample, it is necessary to further examine PKC $\alpha$  expression and localization in future studies to determine whether this biomarker can guide patient selection for TTC-352 therapy.

## Conclusions

In summary, this phase 1 trial established the dose of TTC-352 for future testing in phase 2 studies. TTC-352 has a favorable toxicity profile and an encouraging signal of efficacy in breast cancer refractory to at least two prior hormonal therapies and CDK4/6 inhibitor. Next steps include defining the role of PKC- $\alpha$  expression in tumor stroma as a predictive biomarker and validation of its efficacy in a phase 2 clinical trial in patients with ER + breast cancer who have been less heavily pretreated.

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**Data availability** For the data supporting the presented results please contact the corresponding author. Supplementary information is available at the British Journal of Cancer's website.

## Compliance with ethical standards

**Conflicts of interest** AZD serves as Chief Medical Officer and obtained honoraria from TTC Oncology and Vanquish Oncology. AZD has received honoraria for consulting work from EMD Serono. AZD has equity in TTC Oncology, IGF Oncology, Squarex, and Martell Diagnostic Laboratories. AZD reports research funding to institutions from Merck, and Eli Lilly. DAT and GRJT have equity in TTC Oncology. RPV has received honoraria from TTC Oncology. RMO serves as an advisor for Lilly, PUMA, Novartis, Genomic Health and Biotheranostics and receives grant support from PUMA, Novartis, Seattle Genetics, and Eisai. All other authors declare no competing interests.

**Ethical approval** This clinical trial was approved by the Institutional Review Board at the participating institutions. It was conducted in accordance with the Declaration of Helsinki.

**Informed consent** All participants provided written informed consent.

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