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The oral mTOR inhibitor RAD001 (everolimus) in combination with letrozole in patients with advanced breast cancer: Results of a phase I study with pharmacokinetics ☆

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ABSTRACT

Purpose: To investigate the safety and pharmacokinetics (PK) of combined treatment with letrozole and the oral mTOR inhibitor RAD001 in patients with metastatic breast cancer stable or progressing after ⩾4 months on letrozole alone.

Methods: Eighteen patients received letrozole (2.5 mg/day) and RAD001 at 5 mg/day (cohort 1) or 10 mg/day (cohort 2). In the absence of DLT in cohort 1, cohort 2 was expanded to 12 patients to obtain additional safety and PK data.

Results: Most common adverse events were stomatitis (50.0% of patients), fatigue (44.4%), anorexia and/or decreased appetite (44.4%), diarrhoea (38.9%), headache (33.3%) and rash (33.3%). There was 1 DLT, a grade 3 thrombocytopaenia in cohort 2. No clinically relevant PK interaction was detected. Seven patients received the combination therapy for >6 months. One patient had a complete response, and one had a 28% reduction in liver metastases, both in cohort 2.

Conclusion: Daily therapy with RAD001 plus letrozole is promising: the results suggest antitumour activity with no PK interactions. The overall safety profile of the combination is consistent with that expected for RAD001 monotherapy. A daily dose of RAD001 10 mg is recommended for further trials.

1. Introduction

Endocrine therapy is a mainstay of treatment for patients with hormone receptor-positive (HR^+) early and advanced

☆ Preliminary results from this clinical trial were presented at the 2004 San Antonio Breast Cancer Conference.

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breast cancer. Letrozole (Femara®; Novartis Pharmaceuticals, East Hanover, NJ), a third-generation aromatase inhibitor, has shown superior efficacy over tamoxifen both as early adjuvant therapy[1] and as first-line endocrine therapy for advanced breast cancer.[2] Furthermore, letrozole was superior to placebo when given as extended adjuvant treatment following 5 years of tamoxifen.[3]

With the widespread use of aromatase inhibitors, initial or acquired resistance to these agents poses a major clinical problem. Research into the mechanisms of resistance has shown that various signal transduction pathways are activated and used by breast cancer cells to escape the effect of endocrine therapy. For example, activation of the PI3 kinase/Akt/mTOR pathway is a key adaptive change driving endocrine resistance.[4–6]

Mammalian target of rapamycin (mTOR) inhibition has restored responsiveness to tamoxifen in MCF-7 breast cancer cells constitutively expressing Akt, an effect that occurred partly through blockade of oestrogen receptor α (ERα)–mediated transcription.[7] The role of mTOR in oestrogen-driven breast cancer proliferation suggests that combining an aromatase inhibitor and an mTOR inhibitor could be beneficial to patients with HR^+ tumours.

RAD001 (everolimus) is an orally bioavailable inhibitor of the mTOR pathway. The effects of RAD001 in cancer cells include reduced growth and proliferation and inhibition of protein translation and vascular endothelial growth factor (VEGF) production.[8,9] Consistent with its activity as an inhibitor of mTOR, RAD001 is particularly active in tumour cells with high levels of the active form of Akt, namely pAkt, which is associated with more aggressive tumour cell growth and proliferation.[10]

Early clinical studies of RAD001 alone characterised its pharmacokinetics (PK) and showed most adverse events (AEs) to be of only mild to moderate severity. Dose-limiting toxicities (DLTs) were grade 3 stomatitis, fatigue, hyperglycaemia and neutropaenia. Molecular pharmacodynamic studies of advanced cancer patients before and during RAD001 treatment have shown complete target inhibition at a dose of 10 mg/day. This dose is well tolerated and produces blood levels comparable to those at which anti-tumour effects were demonstrated in pre-clinical models.[11–13]

Treatment of MCF-7/Aro aromatase-expressing breast cancer cells with RAD001 or letrozole has inhibited oestradiol- or androstenedione-induced modulation of the mTOR pathway *in vitro*.[14] RAD001 and letrozole have also inhibited androstenedione-induced proliferation, an effect that was significantly enhanced when the agents were combined ($p < 0.001$). Combination treatment was associated with profound disruption of cell cycle progression, reduced cell viability ($p < 0.01$) and increased apoptosis ($p < 0.05$). A similar effect of the combination on cell proliferation and survival was also observed in T47D/Aro breast cancer cells.[14]

The primary objective of the phase Ib study reported here was to investigate the safety of and potential PK interactions associated with the combination of RAD001 5 or 10 mg/day and letrozole 2.5 mg/day in patients with advanced breast cancer. The secondary objective was to find evidence of anti-tumour activity in patients with no objective response to letrozole alone.

2. Patients and methods

In this phase Ib open-label, escalating-dose trial, sequential cohorts were defined by the dose of RAD001 combined with the registered dose of letrozole 2.5 mg/day. Because RAD001 10 mg/day was previously identified as the optimal biologically active dose, dose escalation was planned to stop after the feasibility of combining RAD001 10 mg/day with letrozole was established, even if the maximum tolerated dose had not been reached. Toxicity was assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE).[15] DLT was defined as any grade 3/4 toxicity occurring within the first 28 days, suspected to be related to study drug (with the exception of grade 3 lymphopaenia, hypercholesterolaemia or hypertriglyceridaemia), or leading to treatment interruption of >2 weeks' duration.

The first cohort of 6 patients received RAD001 5 mg/day. In the absence of DLT, the second cohort received RAD001 10 mg/day. If no more than 1 of 6 patients experienced DLT, an additional 6 could be recruited. In the expanded cohort of 12 patients, the estimated probability of detecting an AE occurring in 10% of individuals was 0.72 and that for an AE occurring in 30% was 0.99. Patients were to remain on combination treatment for as long as they were progression free, in the absence of unacceptable toxicity, or for other reasons that in the investigator's or patient's opinion required termination of participation. The protocol was approved by local ethics committees, and all patients signed informed consent forms before study inclusion.

2.1. *Patient inclusion and exclusion criteria*

Eligible patients were postmenopausal women or men (⩾18 years) with histologically confirmed metastatic or locoregionally recurrent advanced breast cancer who had received first- or second-line endocrine therapy with letrozole (2.5 mg/day) for ⩾4 months without objective response (*i.e.* stable or progressive disease), excluding patients with bulky or symptomatic disease considered an urgent indication for chemotherapy. Other inclusion requirements were a life expectancy of ⩾4 months, World Health Organization (WHO) performance status of 0 or 1, hypercholesterolaemia/hypertriglyceridaemia grade ⩽1 (with lipid-lowering drugs permitted), adequate bone marrow function (absolute neutrophil count [ANC] ⩾ 1.5×10^9/L, platelets ⩾100×10^9/L, haemoglobin ⩾10 g/dL), and normal liver and renal function (serum albumin within reference range, serum bilirubin ⩽1.5 × upper limit of normal [ULN], serum transaminase ⩽3 × ULN, serum creatinine ⩽130 μmol/L or creatinine clearance ⩾60 mL/min by Cockcroft-Gault formula).[16]

Exclusion criteria included treatment with strong CYP3A inhibitors or inducers, known central nervous system metastases, uncontrolled infection, known human immunodeficiency virus infection, anticoagulant medication, lactose intolerance, antineoplastic therapy within 4–6 weeks, radiation therapy within 3 weeks or surgery within 2 weeks prior to the study. Individuals with an active bleeding diathesis were also excluded. Patients with any other concurrent severe

and/or uncontrolled medical disease (*e.g.* diabetes, hypertension, congestive heart failure, ventricular arrhythmias, active ischaemic heart disease, myocardial infarction within 6 months, chronic liver or renal disease, active ulceration of the upper gastrointestinal [GI] tract), impairment of GI function or GI disease that might significantly alter absorption of RAD001 were ineligible.

2.2. Laboratory examinations and safety assessment

Laboratory examinations, vital signs and physical examination were performed locally according to the scheduled visits and included haematology analysis, blood chemistry, and urinalysis (by standard dipstick method). WHO performance status was assessed using standard scales. Abnormal laboratory values were recorded as AEs only if associated with clinical signs and symptoms or required therapy. All reported AEs and serious AEs were recorded and followed up. Serious AEs were followed for 28 days after treatment discontinuation.

2.3. Pharmacokinetic assessment

PK parameters were derived from concentration–time data with standard non-compartmental analysis methods and included peak concentration (C_{max}), time to reach the C_{max} (t_{max}), trough (pre-dose) concentration (C_{min}) and area under the curve over the dosing interval (AUC_{last}). Letrozole was measured in plasma using automated solid-phase extraction and liquid chromatography with fluorescence detection. Heparinised blood (5 mL) was frozen at −0 °C within 20 min of collection. Samples were processed using automated solid-phase extraction and liquid chromatography with fluorescence detection. The calibration range was 1–200 nmol/L, with a lower limit of letrozole quantification of 1 nmol/L, using 1 mL of plasma. RAD001 blood concentrations were determined by liquid/liquid extraction followed by liquid chromatography and mass spectroscopy. EDTA blood (2 mL) was stored at −20 °C within 60 min of collection. The lower limit of RAD001 quantification was 0.3 ng/mL.

Blood samples were taken for 24-h PK profiling of both RAD001 and letrozole at steady state (day 15), immediately pre-doses, and 1, 2, 5, 8 and 24 h thereafter. On day 0, prior to administration of RAD001, the baseline PK profile for letrozole alone was obtained. No RAD001-only baseline was obtained, as the interruption of letrozole for adequate washout was considered unethical. Steady-state RAD001-only PK data in patients with advanced cancers were obtained in another phase I study with RAD001 10 mg/day. Single, pre-dose blood samples were collected on days 8 and 29 for determination of letrozole and RAD001 trough levels.

2.4. Efficacy assessment

Tumour response was assessed every 2 months by standard radiography, computed tomography, magnetic resonance imaging or clinical examination according to Response Evaluation Criteria in Solid Tumors (RECIST).[17]

2.5. Statistical analysis

Data were analysed based on intent-to-treat analysis. Descriptive statistics were used for patient data and laboratory values. The effects of RAD001 on letrozole PK and *vice versa* were assessed.

3. Results

Eighteen eligible Caucasian patients (17 females, 1 male) with a median age of 64.5 years (range 32–75) and breast cancer metastases to bone, lung, liver, kidney, superficial lymph nodes and/or skin were enrolled. Five patients had a WHO performance status of 0, 13 had a status of 1 (Table 1). All patients received letrozole before study entry. Thirteen patients also received chemotherapy (4 of 6 in RAD001 5 mg/day cohort, 9 of 12 in the RAD001 10 mg/day cohort). All patients received at least one dose of study medication. Seven patients remained on therapy for >6 months, and 6 received study medication for $\geqslant$1 year (2 patients in the RAD001 5 mg/day cohort, 4 patients in RAD001 10 mg/day cohort).

3.1. Safety results

The combination was well tolerated at both RAD001 dose levels. Detailed safety results appear in Tables 2 and 3. One patient in the RAD001 10 mg/day cohort experienced a DLT (grade 3 thrombocytopaenia) and grade 3 neutropaenia, recovering within 2 weeks of RAD001 and letrozole interruption. The four most frequent AEs were stomatitis (grades 1 and 2) in 3 of 6 patients in the RAD001 5 mg/day cohort and in 6 of 12 patients in the RAD001 10 mg/day cohort, fatigue in 2 patients in the 5 mg/day cohort and in 6 patients in the 10 mg/day cohort, diarrhoea in 7 patients in the 10 mg/day cohort, and anorexia and/or decreased appetite in 8 patients in the 10 mg/day cohort—3 of whom had a concomitant GI disturbance (diarrhoea, irritable bowel syndrome, GI infection) not considered related to the study drug. Non-dose-limiting thrombocytopaenia (grade 1) occurred in 1 of 6 patients receiving RAD001 5 mg/day and in 7 of 12 patients receiving RAD001 10 mg/day. In these patients, platelet counts intermittently returned to normal levels despite ongoing treatment with RAD001. No grade 1 neutropaenia was observed in patients receiving RAD001 in either cohort. Grade 2 neutropaenia was observed in 3 patients receiving RAD001 10 mg/day, and grade 3 neutropaenia was observed in a patient receiving RAD001 10 mg/day who also experienced grade 3 thrombocytopaenia. Non-dose-limiting increases in hepatic enzymes were common with grade 1 elevations in alkaline phosphatase in 69.2%, SGOT (AST) in 47.1%, and SGPT (ALT) in 75% of patients. Grade 2 increases in these enzymes occurred in 11.8%, 16.7% and 5.6% of patients, respectively. Bilirubin levels remained within the normal range for all patients.

Amongst the 6 patients receiving RAD001 5 mg/day, 4 discontinued because of disease progression and 2 because of suspected AEs (see Table 2): 1 patient developed grade 3 oedema, and the other experienced a worsening of pre-existing chronic hepatitis (enrollment of this patient in the study was noted as a protocol violation). The most common AEs

Table 1 – Key demographic and background characteristics by treatment group

	All patients	RAD001 5 mg/day	RAD001 10 mg/day
Total number of patients			
Intent-to-treat population	18	6	12
Dose-determining population[a]	17	6	11
Median age, y (range)	64.5 (32–75)	68.5 (58–72)	61.5 (32–75)
Performance status			
0	5	1	4
1	13	5	8
Prior antineoplastic therapy (*n*)			
Hormonal therapy total[b] (letrozole/tamoxifen)	18 (18/15)	6 (6/4)	12 (12/11)
Chemotherapy total[b] (adjuvant/metastatic)	13 (10/4)	4 (4/0)	9 (6/4)
Time since diagnosis			
<2 years	3	2	1
⩾2 years	15	4	11
Localisation of metastases[c] (target and non-target lesions)			
Bone	9	1	8
Lung (with or without mediastinum)	5	1	4
Liver	7	3	4
Kidney	1	1	0
Superficial lymph node	1	1	0
Skin	1	0	1

a One patient withdrew consent prior to the end of the dose-finding period.
b A patient with both hormonal therapies or both chemotherapy indications is counted only once in each total.
c A patient with several localisations is counted once for each localisation.

Table 2 – Key study parameters

	All patients	RAD001 5 mg/day	RAD001 10 mg/day
Total number of patients			
Treated	18	6	12
Discontinued	18	6	12
Exposure (mo)			
Median duration (range)	3.91 (0.5–29.5)	3.66 (1.6–21.7)	3.96 (0.5–29.5)
Discontinuations due to			
Adverse events (AEs)	4	2	2
Disease progression	13	4	9
Withdrew consent	1	0	1
AEs			
Total *n* with AEs	18	6	12
Total *n* with AEs grade 3/4[a]	6	2	4
Total *n* with AEs causing dose adjustment/interruption	4	0	4
Total *n* with serious AE	4	2	2
Total *n* with serious AE with suspected			
Relation to study drug	1	0	1

a No grade 4 AEs occurred during this study. Grade 3 AEs in the RAD001 5 mg/day cohort were peripheral oedema and a mass in the right breast, each in 1 patient; grade 3 AEs in the RAD001 10 mg/day cohort were fatigue in 2 patients, thrombocytopaenia/neutropaenia in 1 patient, and anorexia, diarrhoea, hypokalaemia and *Campylobacter jejuni* enteritis in 1 patient.

in the RAD001 5 mg/day cohort were stomatitis (50.0%) and fatigue, nausea and aesthenia (each 33.3%) (see Table 3).

Amongst the 12 patients given RAD001 10 mg/day, 9 discontinued treatment because of disease progression, 1 withdrew consent, and 2 discontinued because of suspected AEs (1 had grade 1 bone pain, and 1 experienced recurrent general weakness) (see Table 2). The most common AEs reported in the RAD001 10 mg/day cohort were anorexia and/or decreased appetite (66.7%), diarrhoea (58.3%), stomatitis and fatigue (each 50.0%), headache and rash (each 41.7%), peripheral oedema (33.3%) and dry skin (25.0%) (see Table 3). In 4 patients in the 10 mg/day cohort, RAD001 was reduced

Table 3 – Adverse events (AEs) in patients on RAD001 and letrozole by treatment group (>10% in all patients)

	All patients, *n* (%)	RAD001 5 mg/day, *n* (%)	RAD001 10 mg/day, *n* (%)
AEs			
Total number of patients with AEs	18 (100.0)	6 (100.0)	12 (100.0)
Stomatitis	9 (50.0)	3 (50.0)	6 (50.0)
Anorexia, decreased appetite	8 (44.4)	0	8 (66.7)[a]
Fatigue	8 (44.4)	2 (33.3)	6 (50.0)[b]
Diarrhoea	7 (38.9)	0	7 (58.3)[a]
Headache	6 (33.3)	1 (16.7)	5 (41.7)
Rash	6 (33.3)	1 (16.7)	5 (41.7)
Oedema, peripheral	5 (27.8)	1 (16.7)[a]	4 (33.3)
Nausea	4 (22.2)	2 (33.3)	2 (16.7)
Aesthenia	3 (16.7)	2 (33.3)	1 (8.3)
Dry skin	3 (16.7)	0	3 (25.0)
Pruritus	3 (16.7)	1 (16.7)	2 (16.7)
Arthralgia	2 (11.1)	0	2 (16.7)
Bone pain	2 (11.1)	0	2 (16.7)
Constipation	2 (11.1)	0	2 (16.7)
Neuropathy	2 (11.1)	0	2 (16.7)
Dry mouth	2 (11.1)	1 (16.7)	1 (8.3)
Dyspnoea	2 (11.1)	1 (16.7)	1 (8.3)
Epistaxis	2 (11.1)	1 (16.7)	1 (8.3)
Hypercholesterolaemia	2 (11.1)	1 (16.7)	1 (8.3)
Pharyngitis	2 (11.1)	0	2 (16.7)
Pyrexia	2 (11.1)	0	2 (16.7)
Weight, decreased	2 (11.1)	0	2 (16.7)

a One grade 3 AE.
b Two grade 3 AEs; no grade 4 AEs.

to 5 mg/day because of AEs, either following interruption (in 1 patient with stomatitis and 1 with general weakness and thrombocytopaenia) or without interruption (in 1 patient with stomatitis and 1 with lower-limb neuropathy).

3.2. *Pharmacokinetic evaluation*

Thirteen patients provided blood samples for PK analysis; 12 were evaluable for letrozole PK with RAD001, and 10 were evaluable for RAD001 PK (Table 4). Data for steady-state RAD001-only PK were taken from 6 patients with advanced cancers involved in another unpublished phase I study (CRAD001C2101) who received RAD001 10 mg/day as monotherapy. The PK of letrozole did not change in the presence of RAD001 (see Table 4 and Fig. 1). The co-administration-to-baseline ratios in 12 patients ranged from 0.88 to 1.67 for C_{max} and from 0.92 to 1.39 for AUC_{last}. The PK of RAD001 in the presence of letrozole was generally comparable to that in

Table 4 – Letrozole and RAD001 pharmacokinetic parameters

Parameter[a]	Alone	With RAD001 5 mg/day	Ratio	Alone	With RAD001 10 mg/day	Ratio
Letrozole						
n	4	3	3	9	9	9
t_{max} (h)	1.5 (1–2)	1 (1–1)	N/A	1 (1–2)	2 (0–24)	N/A
C_{max} (nmol/L)	406 ± 134	341 ± 96	0.96 ± 0.12	495 ± 300	509 ± 247	1.09 ± 0.23
C_{min} (nmol/L)	308 ± 87	306 ± 115	1.08 ± 0.11	409 ± 272	418 ± 232	1.12 ± 0.27
AUC_{last} (nmol h/L)	7872 ± 2676	7149 ± 2426	1.02 ± 0.03	10 103 ± 6539	10 605 ± 5713	1.10 ± 0.14

Parameter[a]	RAD001 5 mg/day with letrozole	RAD001 10 mg/day with letrozole	RAD001 10 mg/day (from CRAD001C2101)
RAD001			
n	1	9	6
t_{max} (h)	1	1 (1–5)	1 (1–6)
C_{max} (ng/mL)	31	52 ± 13	61 ± 17
C_{min} (ng/mL)	18	13 ± 5	13.2 ± 7.2
AUC_{last} (ng/mL h)	298	541 ± 211	514 ± 231

N/A, not applicable.
a Values are mean ± standard deviation except t_{max}, which is median (range).

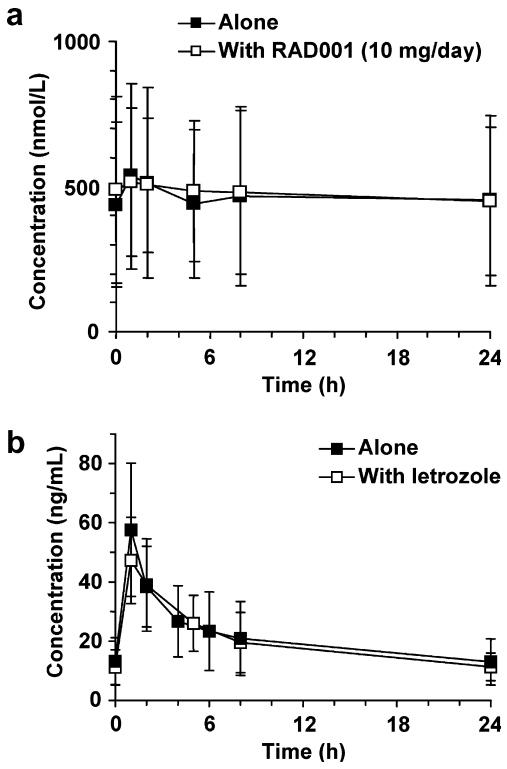

Fig. 1 – (a) Mean letrozole plasma concentration (nmol/L) ± standard deviation in patients with letrozole 2.5 mg/day alone or with RAD001 10 mg/day. (b) Mean RAD001 whole blood concentration (ng/mL) ± standard deviation in patients with RAD001 10 mg/day alone or with letrozole 2.5 mg/day. RAD001-alone data were obtained in a previous study.

the absence of letrozole (see Table 4 and Fig. 1). No significant alterations in plasma and whole blood levels of letrozole and RAD001, respectively, were observed when the agents were co-administered.

3.3. *Efficacy evaluation*

For most patients, best response to single-agent letrozole was reported as stable disease (Table 5). The best response to combined treatment with letrozole and RAD001 was evaluated in 18 patients. One patient with lesions of the skin, the sole site of her disease, and receiving RAD001 10 mg/day experienced a durable complete response >22 months in duration. The disease had been progressing with letrozole monotherapy. Seven months after RAD001 10 mg/day was added, a complete response was observed. The patient had been treated with letrozole for 7 weeks before the addition of RAD001 10 mg/day.

No partial responses were observed. In one patient receiving RAD001 10 mg/day, there was a 28% reduction in liver metastases, just short of the RECIST definition of partial response (reduction of 30%). This reduction was observed 46 days after the first dose of RAD001, and the patient met the RECIST criteria for stable disease for 13 months. The patient had been receiving letrozole for 2 years prior to the study. The liver metastases had been enlarging over a 12-month period during letrozole monotherapy (without meeting the criteria for progressive disease). In addition, prolonged stable disease (>12 months from study entry) was observed in 2 patients receiving RAD001 5 mg/day and in 2 patients receiving RAD001 10 mg/day (see Table 5).

Table 5 – Disease status under letrozole alone and best response to combined treatment with RAD001 and letrozole

	All patients (n = 18)	RAD001 5 mg/day (n = 6)	RAD001 10 mg/day (n = 12)
Disease status on letrozole alone			
Duration of letrozole alone median (range) (mo)[a]	15.0 (1.7–49.2)	12.4 (2.3–27.7)	26.0 (1.7–49.2)
Best response on letrozole alone (n)			
Stable disease	15	5	10
Progressive disease	1	0	1
Unknown	1	0	1
Not applicable	1	1	0
Best response to combined treatment			
Overall response (CR or PR) (%)	1 (5.6)	0	1 (8.3)
Complete response (%)	1 (5.6)	0	1 (8.3)
Duration (mo)[b]			22.3
Partial response (%)	0	0	0
Stable disease (%)	9 (50.0)	4 (66.7)	5 (41.7)[c]
Stable disease >6 mo (%)	6 (33.3)	2 (33.3)	4 (33.3)
Duration (mo)[d]		14, 21	8, 13, 17, 23
Progressive disease (%)	5 (27.8)	2 (33.3)	3 (25.0)
Unknown (%)	3 (16.7)[e]	0	3 (25.0)

CR, complete response; PR, partial response.
a There were 3 patients with less than 4 months of letrozole therapy before inclusion (1 patient in the 5 mg cohort with 2.3 months and 2 in the 10 mg cohort with 1.7 and 3.8 months, respectively).
b Time from first confirmed CR to progression.
c Including 1 patient with 28% reduction in liver metastasis.
d Time from first treatment to progression.
e Three patients could only be classified as 'unknown': 2 had no post-baseline tumour assessments reported before discontinuation, and 1 had stable disease reported at day 42 (*i.e.* minimum follow-up of >6 weeks required to classify best response as 'stable disease' was not reached).

4. Discussion

Results of this phase Ib study indicate that the combination of daily oral doses of the novel mTOR inhibitor RAD001 with the third-generation aromatase inhibitor letrozole involves no PK interactions. Overall tolerability of the combination was satisfactory, with 22.2% of patients discontinuing because of an AE. The safety profile of the combination is generally consistent with that observed for RAD001 monotherapy and with published safety data for temsirolimus, another mTOR inhibitor, as monotherapy in advanced breast cancer patients.[18]

Most thrombocytopaenia and neutropaenia events occurred within 2 weeks of treatment. Subsequent studies of the combination should therefore include a blood count at day 15. Not all patients who experienced toxicity needed to stop treatment with RAD001. Of the 4 patients in the RAD001 10 mg/day cohort who had their dose interrupted or adjusted because of AEs, 2 continued for >18 months on a reduced dose (RAD001 5 mg/day). Dose reduction or delay algorithms will be included in subsequent studies of the combination therapy with the aim of developing evidence-based recommendations for the management of RAD001 toxicity. Amongst patients who did not experience toxicity leading to dose reduction or interruptions within the first 4 weeks, the combination treatment was frequently feasible over a prolonged time period. Of the 18 patients, 7 were treated with the combination for >6 months, and 6 were treated for >12 months.

The study results suggest that the combination of RAD001 with letrozole has anti-tumour activity. There was one complete response for 22 months, and one near partial response for 13 months (both in the RAD001 10 mg/day cohort).

Based on the clinical findings of this study, an oral dose of RAD001 10 mg/day in combination with letrozole is recommended for further trials. This same dose has been identified as providing complete target inhibition in initial PK-pharmacodynamic modelling and subsequent molecular pathology studies of the tumours of treated patients.[11–13]

In the future, the challenge will be to identify patients most likely to benefit from the combination of RAD001 and letrozole. Letrozole and the other third-generation aromatase inhibitors are known for their excellent tolerability. Combining an mTOR inhibitor with an aromatase inhibitor does add side effects, particularly mucositis, but also fatigue, skin, and GI AEs. The results of a phase III trial of the mTOR inhibitor temsirolimus (administered at an oral dose of 30 mg/day for 5 days every 2 weeks) combined with letrozole, in comparison to letrozole alone, were recently reported.[19] In an unselected population of HR^+ postmenopausal women with advanced breast cancer, the combination of temsirolimus and letrozole did not improve progression-free survival. These findings illustrate the need to investigate ways of distinguishing patients who benefit from the addition of the mTOR inhibitor. Subsequent clinical development must accept the complexity of well-conducted correlative studies to help identify predictive biomarkers.

A double-blind, randomised, placebo-controlled phase II study is ongoing to compare the efficacy of letrozole monotherapy with that of the combination of letrozole and RAD001 10 mg/day for neoadjuvant breast cancer treatment. Tumour biopsies are being performed at baseline, at day 15, and after 4 months of treatment. Major study objectives are to assess whether molecular characteristics of the tumour predict benefit from the combination therapy and to identify molecular markers that may serve as patient selection parameters in RAD001 phase III development. Amongst others, markers directly reflecting mTOR inhibition by RAD001 (p-S6) and those associated with mTOR activation (p-Akt, PI3 kinase mutations, Cyclin D1) will be analysed.

This study has established the feasibility of combining RAD001 with letrozole. No PK interactions were observed, and the safety profile appears satisfactory. Side effects compromising tolerability are characteristic for the class of drug, similar to those of other targeted therapies, and are manageable and acceptable for an oral therapy that sufficiently benefits patients. A daily dose of oral RAD001 10 mg/day is recommended for further study in combination with letrozole. With this combination and at this dose level, signals suggestive of anti-tumour activity were seen in patients not achieving an objective response to letrozole alone. Further development of this combination must focus on identifying a predictive biomarker to allow selection of patients who benefit from this novel treatment approach.

Conflict of interest statement

Jutta Steinseifer, Carine Wouters, Chiaki Tanaka, Ulrike Zoellner and Pui Tang are employees of Novartis Pharma AG or its Belgian or US subsidiary. Jutta Steinseifer, Chiaki Tanaka and Ulrike Zoellner have stock holdings in Novartis Pharma AG.

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