

Phase 2 study evaluating intermittent and continuous linsitinib and weekly paclitaxel in patients with recurrent platinum resistant ovarian epithelial cancer

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HIGHLIGHTS

- First randomized ovarian cancer trial targeting IGF1R pathway in combination with chemotherapy.
- Linsitinib added to paclitaxel either as an intermittent or continuous schedule.
- Addition of linsitinib did not improve outcomes in patients with ovarian cancer.

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ABSTRACT

Background. Linsitinib, an oral, dual inhibitor of insulin-like growth factor-1 receptor and insulin receptor, in combination with weekly paclitaxel, may improve clinical outcomes compared with paclitaxel alone in patients with refractory or platinum-resistant ovarian cancer.

Patients and methods. This open-label phase 1/2 clinical trial (NCT00889382) randomized patients with refractory or platinum-resistant ovarian cancer (1:1:1) to receive either oral intermittent linsitinib (600 mg once daily on Days 1–3 per week) combined with paclitaxel (80 mg/m² on Days 1, 8, and 15; Arm A) or continuous linsitinib (150 mg twice daily) in combination with paclitaxel (Arm B), or paclitaxel alone (Arm C). Primary endpoint was progression-free survival (PFS); secondary endpoints included overall survival (OS), overall response rate (ORR), disease control rate (DCR), and safety/tolerability.

Results. A total of 152 women were randomized to treatment (n = 51 Arm A; n = 51 Arm B, n = 50 Arm C). In combination with paclitaxel, neither intermittent linsitinib (median PFS 2.8 months; 95% confidence interval [CI]: 2.5–4.4) nor continuous linsitinib (median PFS 4.2 months; 95% CI: 2.8–5.1) improved PFS over weekly paclitaxel alone (median PFS 5.6 months; 95% CI: 3.2–6.9). No improvement in ORR, DCR, or OS in either linsitinib dosing schedule was observed compared with paclitaxel alone. Adverse event (AE) rates, including all-grade and grade 3/4 treatment-related AEs, and treatment-related AEs leading to discontinuation, were higher among patients receiving intermittent linsitinib compared with the other treatment arms.

Conclusion. Addition of intermittent or continuous linsitinib with paclitaxel did not improve outcomes in patients with platinum-resistant/refractory ovarian cancer compared with paclitaxel alone.

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1. Background

Ovarian cancer is the second most common gynecologic cancer and the most frequent cause of gynecologic cancer-related deaths in the United States [1]. Although recent treatment advances have improved survival, approximately 85% of patients with advanced ovarian epithelial cancer will experience a recurrence and will eventually develop resistance to chemotherapy [2]. The duration of response following platinum therapy (platinum-free interval) as well as previous platinum therapy response are key considerations when planning for recurrent ovarian cancer management. Several chemotherapy agents, such as pegylated liposomal doxorubicin and topotecan, are active in relapsed platinum-resistant ovarian cancer (defined as a relapse within 6 months of platinum treatment), most with a 10–30% response rate for patients with a treatment-free interval of <6 months [3]. Weekly paclitaxel is a treatment option in platinum-resistant disease with response rates between 25% and 55% [4].

The combination of novel therapies targeting specific pathways involved in ovarian cancer provides the potential to prolong progression-free survival (PFS) in platinum-resistant ovarian cancer [5,6]. One mechanism proposed for platinum and taxane resistance is the presence of abnormalities in the insulin-like growth factor (IGF)-signaling pathway, which play an important role in the growth and survival of multiple human cancers including ovarian cancer. Binding of IGF1 or IGF2 to IGF1 receptors (IGF1Rs) activates the PI3K/AKT pathway, leading to tumor survival and metastasis [7,8]. Increased IGF1R, IGF1, or IGF2 expression, as well as IGF1R activation, correlates with disease incidence, progression, and prognosis in several tumor types [7,9]. Microarray studies in ovarian cancer have shown that upregulation of the IGF-signaling pathway is associated with poor overall survival (OS) [10]. Preclinical studies have demonstrated that inhibition of IGF1R in human tumor xenograft models may reduce tumor growth [11]. IGF1R-mediated pro-survival signaling is an important pathway involved in acquired resistance to chemotherapeutic agents [12,13], including resistance to platinum and paclitaxel in ovarian cancer [13]. Furthermore, inhibition of the IGF-signaling pathway by various IGF or IGF1R blockers, such as metformin, picropodophyllin, or small hairpin RNA, reversed cisplatin and/or paclitaxel resistance in cultured ovarian cancer cells [14–17]. These observations provide a rationale for IGF1R as a treatment target in ovarian cancer.

Linsitinib (OSI-906) is an orally active, dual IGF1R and insulin receptor (IR) inhibitor, with anti-proliferative effects in tumor cell lines and in vivo xenograft tumor models [18,19]. By inhibiting both IGF1R and IR, linsitinib offers the potential for enhanced anticancer activity when used in combination with chemotherapy. In a phase 1 study, linsitinib monotherapy demonstrated clinical activity in patients with melanoma and adrenocortical carcinoma [20,21]; however, this activity may be limited. In a phase 3 study, linsitinib, administered as a single agent, did not improve progression-free or overall survival compared with placebo in patients with locally advanced or metastatic adrenocortical carcinoma [22]. Linsitinib, however, has demonstrated antitumor activity in combination with erlotinib in solid tumors [23], including non-small cell lung cancer [24].

The rationale for combining paclitaxel with linsitinib was supported by a body of preclinical evidence indicating the importance of IGF1R inhibition in ovarian cancer. The IGF-signaling pathway plays a critical role in the development, maintenance, progression, survival, and chemotherapeutic response associated with ovarian cancer [25]. IGF1 is overexpressed in serous ovarian carcinoma [26], and IGF1R enhances the proliferation and tumorigenicity of human ovarian cancer cells [27]. Moreover, IGF1R signaling is associated with resistance in ovarian carcinoma [17,28].

Continuous dosing of an oral drug with a relatively short half-life, such as linsitinib, is a logical method to maintain therapeutic plasma levels, but may also lead to increased toxicity. While preclinical studies demonstrate the antitumor activity of intermittent linsitinib, it was

uncertain whether tumor growth inhibition with intermittent dosing would be maintained to the same extent as with continuous dosing. Thus, both intermittent and continuous dosing schedules of linsitinib were used in combination with weekly paclitaxel to identify the optimum dosing regimen that would achieve efficacy while avoiding significant toxicity.

Here, we present the results of the phase 2 portion of a multicenter, randomized, open-label phase 1/2 study, which evaluated intermittent or continuous linsitinib plus continuous weekly paclitaxel versus paclitaxel alone in patients with recurrent/relapsed epithelial ovarian cancer (NCT00889382). The phase 1 portion of the study established the phase 2 recommended doses (combined with weekly paclitaxel) of 600 mg once daily for intermittent linsitinib, and 150 mg twice daily for continuous linsitinib; it also showed preliminary efficacy, with six partial responses and 25 patients with stable disease from a cohort of 58 patients with ovarian cancer [29].

2. Methods

2.1. Study oversight

The study was conducted in accordance with the International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) with the ethical principles of Helsinki, and approved by the independent ethics committee or institutional review board for each study site. All patients provided written informed consent.

2.2. Patients

Patients with histologically or cytologically confirmed ovarian epithelial carcinoma, fallopian cancer, or peritoneal cancer were enrolled. Patients had received prior therapy with platinum plus a taxane, with the taxane administered on a 3-week schedule. A maximum of two prior chemotherapy regimens was permitted. Eligible patients were refractory (progressive disease [PD] during chemotherapy) or resistant (PD within 6 months of completing chemotherapy) to their last platinum-containing chemotherapy regimen. Patients had radiologically confirmed PD by RECIST (v1.1) [3]. Exclusion criteria included prior therapy with weekly paclitaxel, subjects with diabetes mellitus requiring medications, those with brain metastases, and those with an ECOG performance status ≥ 2 (see supplement for full patient criteria).

2.3. Study design

This open-label, multicenter, international study evaluated linsitinib, administered either intermittently (Arm A) or continuously (Arm B), combined with weekly paclitaxel, compared with weekly paclitaxel alone (Arm C); patients were randomized 1:1:1 to each treatment arm. Intermittent linsitinib was administered orally, once daily, at a dose of 600 mg on Days 1–3 of each week of a 21-day treatment period; continuous linsitinib was administered orally, twice daily, at a dose of 150 mg. All patients received weekly intravenous paclitaxel at 80 mg/m² on Days 1, 8, and 15 of each 21-day treatment period.

Dose modifications were allowed at the discretion of the investigator. Re-escalation of dose was not permitted. If a study drug was discontinued, the patient could remain on the other assigned drug. Patients who experienced disease progression on single-agent paclitaxel (Arm C) were allowed to receive a continuous daily dosing of linsitinib.

2.4. Assessments

The primary endpoint was PFS based on RECIST version 1.1. Secondary efficacy endpoints included OS, calculated from randomization to death from any cause; CA-125 response; overall response rate (ORR), defined as the proportion of patients with complete response (CR) or partial response (PR) according to RECIST v1.1; and disease control

rate (DCR), defined as CR, PR, or stable disease (SD) for ≥ 6 weeks. Other endpoints were safety profile, pharmacokinetic (PK) parameters, and exploratory biomarker analysis, which included evaluation of Kirsten rat sarcoma viral oncogene homologue (*KRAS*), phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PI3KCA*), and phosphatase and tensin homologue (*PTEN*) mutations in available archival tissue samples. IGF1 plasma concentration assessments were also performed.

All patients were included in the efficacy evaluation (intent-to-treat population). All patients who received at least one dose of treatment were included in safety evaluations, which were performed using National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.02.

2.5. Statistical analysis

Kaplan–Meier survival plots were used to determine the primary endpoint of PFS and the secondary endpoint of OS in each treatment arm. Hazard ratios (HRs) of the treatment effects, along with the 95% confidence intervals (CIs), were calculated using a Cox proportional hazard model. Patients were stratified by the number of prior chemotherapy regimens (one vs two) and the outcome of the most recent platinum-containing chemotherapy regimen (refractory vs resistant). Variables for response rates, CA-125, DCR, and ORR were analyzed using Fisher's exact test. The exact 95% CI (Clopper–Pearson) was calculated for each treatment arm. The CA-125 response rate was summarized based on the CA-125 evaluable population.

3. Results

3.1. Patient demographics

A total of 152 patients were randomized from 46 centers between 2009 and 2014: 51 patients to Arm A, 51 to Arm B, and 50 to Arm C; study flow and patient disposition are depicted in Fig. 1. Baseline patient characteristics were balanced among the three arms (Table 1). Median

age was 58 years (range 18–77 years). A total of 96 patients (63.2%) had serous histology and 17 (11.2%) had clear cell carcinoma. Of the 152 patients, 80 (52.6%) received one prior line of chemotherapy and 72 (47.4%) received two prior lines. A small number of patients had prior radiation therapy (3.9%) or prior hormonal therapy and immunotherapy (5.9%).

3.2. Drug exposure

The median duration of exposure to linsitinib was 62.5 days in Arm A and 103.0 days in Arm B. Median duration of exposure to paclitaxel was 64.0 days in Arm A, 106.0 days in Arm B, and 119 days in Arm C. Study drug treatment was discontinued in most patients (98% in Arm A, 90% in Arm B, and 92% in Arm C; Fig. 1). The most common primary reason for drug discontinuation was disease progression (78% in Arm A, 70% in Arm B, and 61% in Arm C). Linsitinib dose reductions were similar in Arms A and B (40% and 49%, respectively), while 60% of patients had a dose reduction of paclitaxel in Arm A, compared with 43% in Arm B and 35% in Arm C.

3.3. Efficacy

The trial did not meet its primary endpoint. There were no statistically significant differences in median PFS between the three treatment arms (2.8 months for Arm A, 4.2 months for Arm B, and 5.6 months for Arm C; HRs for Arm A and Arm B vs Arm C was 1.3, $P = 0.268$; and 1.2, $P = 0.452$, respectively; Table 2). In addition, there was no difference in PFS according to the number of prior chemotherapy regimens, the outcome of most recent platinum-containing chemotherapy regimen (refractory or resistant), or according to age group (≤ 65 years or > 65 years).

There was no difference in median OS between the three treatment arms, with the median OS for Arm A at 16.0 months (95% CI, 10.1–NR), Arm B at 10.4 months (95% CI, 7.9–14.7), and Arm C at 18.0 months (95% CI, 8.1–NR). In addition, an OS analysis excluding the 10 patients who crossed over to linsitinib after discontinuing paclitaxel alone showed no difference in OS (HR = 0.961, 95% CI, 0.375–2.463; $P = 0.934$).

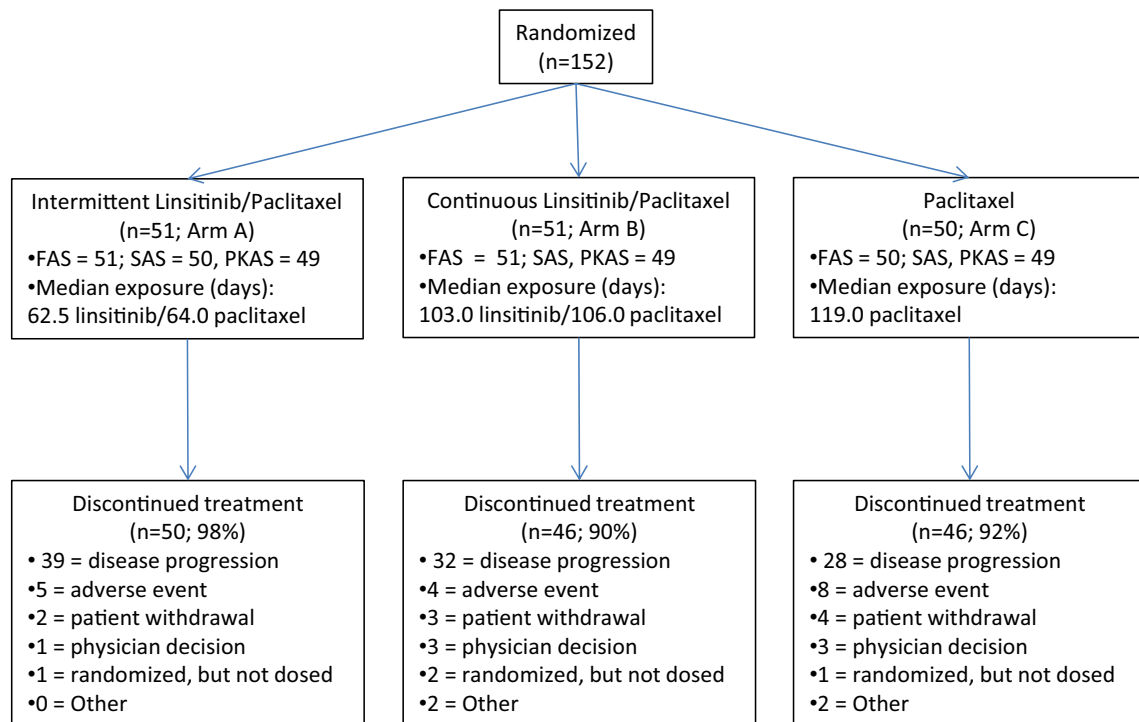


Fig. 1. Study flow chart of patient disposition. Footer: The chart displays the assignment of patients to the three treatment arms. FAS, final analysis set; PKAS, pharmacokinetic analysis set; SAS, safety analysis set.

Table 1
Baseline patient characteristics.

	Intermittent linsitinib/paclitaxel (Arm A; n = 51)	Continuous linsitinib/paclitaxel (Arm B; n = 51)	Paclitaxel (Arm C; n = 50)	Total (n = 152)
Age, median (range)	58.0 (18.0–74.0)	59.0 (37.0–77.0)	56.0 (37.0–76.0)	58.0 (18.0–77.0)
% ≤65 years	76.5	88.2	82.0	82.2
% >65 years	23.5	11.8	18.0	17.8
Race, n (%)				
White	49 (96.1)	47 (92.2)	45 (90.0)	141 (92.8)
Asian	1 (2.0)	1 (2.0)	4 (8.0)	6 (3.9)
Other	1 (2.0)	3 (5.9)	1 (2.0)	5 (3.3)
ECOG performance score, n (%)				
0	31 (60.8)	23 (45.1)	31 (62.0)	85 (55.9)
1	19 (37.3)	28 (54.9)	19 (38.0)	66 (43.4)
2	1 (2.0)	0	0	1 (0.7)
Tumor type, n (%)				
Ovarian – papillary serous	35 (68.6)	27 (52.9)	34 (68.0)	96 (63.2)
Ovarian – endometrioid	0	5 (9.8)	3 (6.0)	8 (5.3)
Ovarian – clear cell	5 (9.8)	6 (11.8)	6 (12.0)	17 (11.2)
Ovarian – mucinous	0	0	2 (4.0)	2 (1.3)
Ovarian – other	8 (15.7)	6 (11.8)	2 (4.0)	16 (10.5)
Fallopian	0	2 (3.9)	2 (4.0)	4 (2.6)
Peritoneal	3 (5.9)	4 (7.8)	1 (2.0)	8 (5.3)
Missing	0	1 (2.0)	0	1 (0.7)
Prior radiation therapy, n (%)	1 (2.0)	2 (3.9)	3 (6.0)	6 (3.9)
Prior disease-related surgery, n (%)	51 (100)	50 (98.0)	50 (100)	151 (99.3)
Prior hormonal therapy and immunotherapy, n (%)	4 (7.8)	4 (7.8)	1 (2.0)	9 (5.9)
Prior chemotherapy, n (%)	51 (100)	50 (98.0) ^a	50 (100)	151 (99.3)
1 regimen	24 (47.1)	29 (56.9)	27 (54.0)	80 (52.6)
2 regimens	27 (52.9)	22 (43.1)	23 (46.0)	72 (47.4)
Platinum-free interval status, n(%)				
Refractory	15 (29.4)	7 (13.7)	14 (28.0)	36 (23.70)
Resistant	36 (70.6)	44 (86.3)	36 (72.0)	116 (76.30)
Best response to prior chemotherapy, n (%)				
Complete response	19 (37.3)	13 (25.5)	18 (36.0)	50 (32.9)
Partial response	17 (9.8)	18 (35.3)	12 (24.0)	47 (30.9)
Stable disease	5 (9.8)	8 (15.7)	9 (18.0)	22 (14.5)
Progressive disease	7 (13.7)	7 (13.7)	10 (20.0)	24 (15.8)
Not evaluable	1 (2.0)	2 (3.9)	0 (0.0029)	3 (2.0)
Unknown	2 (3.9)	3 (5.9)	1 (2.0)	6 (3.9)

ECOG, Eastern Cooperative Oncology Group.

^a One patient with missing data.

Overall response rate was 17.7% in Arm A, 21.6% in Arm B, and 34.0% in Arm C. No CRs were reported for patients in Arms A or B, while there were three reported for patients in Arm C (6.0%; Table 2). DCR was 58.8% in Arm A, 72.6% in Arm B, and 74.0% in Arm C, and the CA-125 response rate was 35.7% in Arm A, 50.0% in Arm B, and 62.2% in Arm C (Table 2).

3.4. Pharmacokinetics and pharmacodynamics

On both Days 1 and 22, intermittent dosing of 600 mg linsitinib resulted in higher AUC_{0–∞} and C_{max} than the continuous dosing schedule, and median values of the PK parameters were generally consistent between Day 1 and Day 22 for both Arms A and B

Table 2
Summary of efficacy of intermittent linsitinib + paclitaxel (Arm A); continuous linsitinib + paclitaxel (Arm B); paclitaxel only (Arm C).

	Intermittent linsitinib/paclitaxel (Arm A; n = 51)	Continuous linsitinib/paclitaxel (Arm B; n = 51)	Paclitaxel (Arm C; n = 50)
Progression-free survival			
Number of events, n (%)	39 (76.5)	38 (74.5)	33 (66.0)
Median, months (95% CI)	2.8 (2.5, 4.4)	4.2 (2.8, 5.1)	5.6 (3.2–6.9)
HR versus paclitaxel alone; <i>P</i> -value (95% CI)	1.3; 0.268 (0.81–2.09)	1.2; 0.452 (0.75–1.91)	NA
Overall survival			
Number of events, n (%)	20 (39.2)	29 (56.9)	23 (46.0)
Median, months (95% CI)	16.0 (10.1–NR)	10.4 (7.9–14.7)	18.0 (8.1–NR)
HR versus paclitaxel alone; <i>P</i> -value (95% CI)	0.79; 0.451 (0.44–1.45)	1.4; 0.208 (0.82–2.46)	NA
Best response, n (%)			
Complete response	0 (0)	0 (0)	3 (6.0)
Partial response	9 (17.7)	11 (21.6)	14 (28.0)
Stable disease	21 (41.2)	26 (51.0)	20 (40.0)
Progressive disease	12 (23.5)	9 (17.7)	10 (20.0)
Not evaluated	9 (17.7)	5 (9.8)	3 (6.0)
Disease control rate ^a , n (%) 95% CI	30 (58.8) (44.2–72.4)	37 (72.6) (58.3–84.1)	37 (74.0) (59.7–85.4)
Objective response rate ^b , n (%) 95% CI	9 (17.7) (8.4–30.9)	11 (21.6) (11.3–35.3)	17 (34.0) (21.2–48.8)
CA-125 response rate ^c , n (%) 95% CI	15 (35.7) (21.6–52.0)	21 (50.0) (34.2–65.8)	23 (62.2) (44.8–77.5)

CI, confidence interval; HR, hazard ratio; NA, not applicable; NR, not reached.

^a Disease control rate = complete response + partial response + stable disease.^b Overall response rate = complete response + partial response.^c CA-125 responders were those patients who had two consecutive post-baseline visits (at least 28 days apart) with a 50% decrease from baseline.

(Table 3). In Arm C, Day 1 and Day 22 paclitaxel reached maximum concentrations at approximately 1 h (end of infusion). Pharmacokinetic parameters were generally consistent for paclitaxel between Day 1 and Day 22 (Table 3). Co-administration of linsitinib in Arms A and B did not alter the pharmacokinetics of paclitaxel, compared with Arm C.

In Arms A and C, pre-dose median plasma concentrations of IGF1 remained similar from Day 1 through Day 43 (Table 4). Plasma IGF1 levels were higher by Day 8 in patients receiving continuous linsitinib dosing (Arm B); these levels remained elevated and nearly constant from Days 15–43. The increases in plasma levels of IGF1 were not associated with improved efficacy compared with paclitaxel alone.

KRAS, *PIK3CA*, and *PTEN* mutations were evaluated in patients for whom samples were available, which was approximately 50% of patients in each arm. Mutations were present in ≤5 patients in each treatment arm: *KRAS*, Arm A (n = 1), Arm B (n = 1); *PIK3CA*, Arm A (n = 4), Arm B (n = 2); *PTEN*, Arm A (n = 4), Arm B (n = 5); therefore, further analysis was not performed.

3.5. Safety

Treatment-emergent AEs were reported in almost all patients, including grade 3/4 AEs in 72.0% of patients in Arm A, 40.8% in Arm B, and 20.8% in Arm C. Serious AEs were reported in 36.0% of patients in Arm A, 36.7% in Arm B, and 34.7% in Arm C. All-grade treatment-related AEs were reported in a total of 94.6% of patients, with the highest proportion of grade 3/4 treatment-related AEs reported in Arm A (52.0% in Arm A, 32.7% in Arm B, and 28.6% in Arm C; Table 5). The most common treatment-related AEs were fatigue (42% in Arm A, 44.9% in Arm B, and 51.0% in Arm C) and nausea (56.0%, 32.7%, and 42.9%, respectively). Overall, there were more patients in Arm A who discontinued treatment due to a primary cause of a drug-related AE (30.0% of patients in Arm A, 14.3% in Arm B, and 16.3% in Arm C). In Arm A, 60% of patients had no linsitinib reduction, 40% had no paclitaxel reduction. In Arm B, 51% and 57.1% of patients had no reduction of linsitinib and paclitaxel,

respectively, compared with 65.3% of patients in Arm C who did not have paclitaxel dose reduction.

Adverse events of special interest to linsitinib include hyperglycemia, hypoglycemia, and prolonged QTc interval. Hyperglycemic events considered to be treatment related were observed in 2.0% of patients in Arm A, 10.2% in Arm B, and 2.0% in Arm C. Grades 3/4 hyperglycemic events were reported in 2.0% of patients in Arm A, 6.1% of patients in Arm B, and no patients in Arm C. One hyperglycemic event (in Arm A) was deemed serious; none of the events warranted discontinuation from the study. Cardiac AEs included peripheral edema and prolonged QT intervals by electrocardiography. Two patients in Arm B reported serious cardiac events. One patient experienced cardiac arrest on Day 16 of the study, which was not considered to be related to study drug (by the investigator). The other patient experienced a grade 2 myocardial infarction, which was considered by the investigator to be related to paclitaxel; the dose was interrupted, but not modified, in response to this AE. All-grade QT prolongation was observed in a higher proportion of patients receiving the 600 mg intermittent schedule (32.0% in Arm A, 4.1% in Arm B, and 0% in Arm C).

There were 13 deaths due to AEs; of which 11 occurred while the patient was on treatment or within 30 days of the last dose, and the remaining two occurred during follow-up. In Arm B, one patient progressed to grade 5 pulmonary embolism, possibly related to linsitinib and paclitaxel, and grade 5 pneumonitis, possibly related to linsitinib and probably related to paclitaxel. The other causes of death were: in Arm A, respiratory arrest (n = 1); in Arm B, cancer recurrence/progression (n = 4), sepsis (n = 1), and ileus (n = 1); and, in Arm C, intestinal obstruction (n = 2) and cardiovascular insufficiency (n = 1). Two additional deaths due to intestinal obstruction, one in Arm B and one in Arm C, occurred >30 days after the last dose.

4. Discussion

In this randomized phase 2 study of weekly paclitaxel combined with either intermittent or continuous dosing of linsitinib, neither

Table 3
(A) Pharmacokinetics of linsitinib (Arms A and B) and (B) pharmacokinetics of paclitaxel (Arm C).

A	Intermittent linsitinib/paclitaxel (Arm A) – Linsitinib data		Continuous linsitinib/paclitaxel (Arm B) – Linsitinib data	
	Day 1	Day 22	Day 1	Day 22
<i>C</i> _{max} (ng/mL)				
Evaluable, n	48	42	48	35
Median (Range)	4515.0 (903.0–14,500.0)	3670.0 (0.0–7030.0)	1475.0 (220.0–4530.0)	2110.0 (19.3–5200.0)
<i>t</i> _{max} (h)				
Evaluable, n	48	39	48	35
Median (Range)	2.0 (1.0–8.1)	4.0 (1.0–8.0)	1.1 (0.0–8.0)	2.0 (0.0–8.0)
<i>AUC</i> _{0–∞} (h*ng/mL)				
Evaluable, n	48	42	48	35
Median (Range)	22,667.9 (3330.3–79,718.2)	18,622.6 (0.0–38,225.3)	4630.2 (906.7–14,059.1)	9564.7 (22.6–26,471.4)
B				
	Paclitaxel (Arm C)			
	Day 1	Day 22		
<i>C</i> _{max} (ng/mL)				
Evaluable, n	47	45		
Median (Range)	2500.0 (159.0–207,000.0)	2350.0 (182.0–20,000.0)		
<i>t</i> _{max} (h)				
Evaluable, n	47	45		
Median (Range)	1.0 (0.0–8.0)	1.1 (0.2–4.0)		
<i>AUC</i> _{0–∞} (h*ng/mL)				
Evaluable, n	41	38		
Median (Range)	3578.7 (318.0–10,276.6)	3214.2 (610.0–16,094.5)		

Table 4

Pre-dose plasma concentrations of insulin-like growth factor-1 (IGF1) (full analysis set).

	Intermittent linsitinib/paclitaxel (Arm A)		Continuous linsitinib/paclitaxel (Arm B)		Paclitaxel (Arm C)	
	n	Median (range), ng/mL	n	Median (range), ng/mL	n	Median (range), ng/mL
Day 1	46	44.03 (11.97–81.11)	45	35.10 (4.91–111.71)	44	39.21 (2.67–111.84)
Day 8	46	47.90 (13.83–118.48)	42	52.83 (3.27–128.08)	43	40.75 (4.67–119.41)
Day 15	43	50.22 (10.06–90.24)	40	72.16 (16.92–170.63)	44	40.33 (7.07–114.08)
Day 22	43	48.20 (10.18–91.40)	43	61.87 (17.47–184.80)	44	45.36 (10.49–97.09)
Day 43	29	49.33 (12.04–101.54)	34	71.38 (21.54–157.99)	33	44.96 (5.76–114.33)

dosing regimen led to improvement in PFS or OS over paclitaxel alone in patients with platinum-resistant or -refractory epithelial ovarian cancer. Weekly paclitaxel is a common standard treatment option for women with platinum-resistant/refractory ovarian cancer. The median PFS is approximately 4.0–7.0 months, demonstrating an urgent need to develop agents that can delay taxane resistance [30,31]. The addition of antiangiogenic agents, such as bevacizumab (a VEGFA antibody), pazopanib (a multi-targeted tyrosine kinase inhibitor of VEGF receptors), and trebananib (an anti-angiopoietin), to weekly paclitaxel significantly improves PFS [6,32,33]. Bevacizumab is currently approved in combination with paclitaxel in platinum-resistant ovarian cancer [6,34,35]. However, antiangiogenic agents are not suitable for all patients with platinum-resistant/refractory ovarian cancer. Therefore, the development of other novel drug–chemotherapy combinations is needed. In light of the evidence suggesting that the IGF1R-signaling pathway reversibly confers resistance to taxanes and platinum in patients with ovarian cancer, inhibiting this pathway appears to be a promising target to develop drugs that could restore sensitivity to chemotherapeutic agents [14,16,17]. This background prompted us to investigate the therapeutic effects of combining linsitinib, a novel oral IR/IGF1R inhibitor, with paclitaxel. Several monoclonal antibodies that inhibit this pathway are also being studied, including ganitumab (AMG 479), a fully human monoclonal antibody against IGF1R, that has shown synergistic and additive effects with carboplatin or paclitaxel in ovarian cancer cell lines [36]; and dalotuzumab, a

humanized anti-IGF1R antibody that in combination therapy in patients with high IGF1 expression ovarian cancer resulted in two patients achieving stable disease who remained on treatment for at least 4 months [37].

Our results showed PFS was longer in the weekly paclitaxel only arm (Arm C), compared with both linsitinib combination arms (Arms A and B). Grades 3 and 4 AEs were more frequent in intermittent linsitinib dosing than continuous dosing; this may be due patients being exposed to higher peak drug concentrations arising from higher single doses in intermittent dosing. The decreased tolerability to the intermittent dosing schedule leading to drug discontinuations and dose reductions may partly explain the shorter PFS observed for this arm compared with the continuous dosing arm and to paclitaxel alone arm. Patients receiving the intermittent schedule of linsitinib with paclitaxel had a decreased median exposure to paclitaxel treatment compared with the other arms, and it is possible that these patients had a shorter PFS due to a lower exposure to paclitaxel. Importantly, coadministration of linsitinib with either intermittent or continuous dosing did not alter the concentration profile of paclitaxel compared with patients receiving paclitaxel alone, suggesting an absence of inhibition of paclitaxel by linsitinib.

The median PFS and response rate in the weekly paclitaxel alone arm of this randomized trial was consistent with previous recent studies in this patient population [34,38]. Disappointingly, the SaPPROC trial, a randomized, placebo-controlled trial of weekly paclitaxel and a Src/

Table 5

All-grade treatment-related AEs ≥10% patients in either treatment and grade 3/4 treatment-related AEs.

Adverse event, n (%)	Intermittent linsitinib/paclitaxel (Arm A; n = 50)		Continuous linsitinib/paclitaxel (Arm B; n = 49)		Paclitaxel (Arm C; n = 49)		Total (n = 148)	
	All-grade	Grade 3/4	All-grade	Grade 3/4	All-grade	Grade 3/4	All-grade	Grade 3/4
Any treatment-related AE	50 (100)	26 (52.0)	43 (87.8)	16 (32.7)	47 (95.9)	14 (28.6)	140 (94.6)	56 (37.9)
Fatigue	21 (42.0)	2 (4.0)	22 (44.9)	1 (2.0)	25 (51.0)	1 (2.0)	68 (45.9)	4 (2.7)
Nausea	28 (56.0)	0	16 (32.7)	1 (2.0)	21 (42.9)	0	65 (43.9)	1 (0.7)
Alopecia	10 (20.0)	0	15 (30.6)	0	19 (38.8)	0	44 (29.7)	0
Anemia	9 (18.0)	3 (6.0)	10 (20.4)	0	17 (34.7)	3 (6.1)	36 (24.3)	6 (4.1)
Diarrhea	17 (34.0)	1 (2.0)	9 (18.4)	1 (2.0)	10 (20.4)	0	36 (24.3)	2 (1.4)
Peripheral neuropathy	7 (14.0)	0	7 (14.3)	1 (2.0)	17 (34.7)	2 (4.1)	31 (20.9)	3 (2.0)
Vomiting	14 (28.0)	2 (4.0)	5 (10.2)	2 (4.1)	9 (18.4)	0	28 (18.9)	4 (2.7)
Constipation	6 (12.0)	0	10 (20.4)	1 (2.0)	10 (20.4)	0	26 (17.6)	1 (0.7)
Nail disorder	5 (10.0)	0	5 (10.2)	1 (2.0)	11 (22.4)	1 (2.0)	21 (14.2)	2 (1.4)
Neutropenia	12 (24.0)	10 (20.0)	3 (6.1)	3 (6.1)	4 (8.2)	3 (6.1)	19 (12.8)	16 (10.8)
Asthenia	10 (20.0)	1 (2.0)	4 (8.2)	0	4 (8.2)	1 (2.0)	18 (12.2)	2 (1.4)
Drug eruption	9 (18.0)	0	4 (8.2)	0	5 (10.2)	0	18 (12.2)	0
Prolonged QT electrocardiogram	16 (32.0)	2 (4.0)	2 (4.1)	0	0	0	18 (12.2)	2 (1.4)
Paresthesia	6 (12.0)	1 (2.0)	7 (14.3)	1 (2.0)	4 (8.2)	0	17 (11.5)	2 (1.4)
Anorexia	3 (6.0)	0	9 (18.4)	0	3 (6.1)	0	15 (10.1)	0
Dysgeusia	1 (2.0)	0	5 (10.2)	0	8 (16.3)	0	14 (9.5)	0
Peripheral sensory neuropathy	6 (12.0)	0	2 (4.1)	0	5 (10.2)	0	11 (7.4)	1 (0.7)
Stomatitis	6 (12.0)	0	3 (6.1)	0	3 (6.1)	0	12 (8.1)	0
Arthralgia	2 (4.0)	0	4 (8.2)	0	5 (10.2)	0	11 (7.4)	0
Mucosal inflammation	3 (6.0)	0	2 (4.1)	0	5 (10.2)	1 (2.0)	10 (6.8)	1 (0.7)
Abdominal pain	1 (2.0)	1 (2.0)	1 (2.0)	0	5 (10.2)	0	7 (4.7)	1 (0.7)
Dyspepsia	5 (10.0)	0	0	0	2 (4.1)	0	7 (4.7)	0
Hyperglycemia	1 (2.0)	1 (2.0)	5 (10.0)	3 (6.1)	1 (2.0)	0	7 (4.7)	4 (2.7)
Nail discoloration	0	0	2 (4.1)	0	5 (10.2)	0	7 (4.7)	0

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Bcr-Abl tyrosine kinase inhibitor saracatinib (AZD0530) in platinum-resistant ovarian cancer, which was also supported by preclinical work, similarly did not show an improvement in activity of weekly paclitaxel [38]. The results of these two negative randomized trials of weekly paclitaxel in combination with novel (non-antiangiogenic) agents indicate the importance of gaining a better understanding of the signaling pathways involved in taxane resistance, and the identification of subgroups that may derive benefit. A limitation of this linsitinib-paclitaxel trial is that the protocol did not mandate tissue sample collection for biomarker analyses, and hence, the numbers of tissue samples collected were too low for meaningful interpretation. Analyses of circulating biomarkers, such as IGF2 and IGF1, may have provided further information; however, IGF1 levels were measured and were not associated with efficacy.

Despite the negative results of this first randomized trial targeting IGF1R with chemotherapy in ovarian cancer, there is a rationale for further investigation of IGF signaling in this disease, including targeting other molecules in the pathway and different combinations. Preclinical studies suggest that targeting IGF2 may be a preferable strategy compared with targeting IGF1R alone in taxane-resistant ovarian cancer [14]. In addition, in BRCA1-deficient ovarian cancer models, cells with impaired homologous recombination demonstrate over-activation of the IGF1R pathway and are more sensitive to IGF1R inhibition compared with homologous recombination-proficient cells. Furthermore, IGF-1R inhibition appeared to sensitize cells to poly-ADP ribose polymerase inhibitors, [39] suggesting that targeting of both IGF-1R and PARP could be an effective combination strategy increasing the population of patient who may benefit from these approaches.

In conclusion, this is the first randomized trial of an IGF1R inhibitor in ovarian cancer. This study investigated two dosing regimens of linsitinib in combination with weekly paclitaxel in patients with platinum-resistant/refractory ovarian cancer and found no benefit of either an intermittent or continuous linsitinib dosing schedule, compared with weekly paclitaxel alone, in terms of PFS, ORR, or OS. Further work on the significance of the IGF-signaling pathway in platinum-resistant ovarian cancer is needed.

Conflict of interest statement

N.C. reports other relevant financial activity outside the submitted work from Roche, AstraZeneca, Amgen, MSD, Clovis, Pharma Mar, Pfizer, and Tesaro. H.H. received personal fees outside the submitted work from AstraZeneca and Roche. S.P. had unspecified financial activity outside the submitted work. J.V., S.G., J.S., M.S., and S.P., are Astellas employees. G.Y. was an Astellas employee at the time of this study. A.O., S.K., C.S., R.W.N., and S.B. have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ygyno.2018.01.019>.

References

- [1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2015, *CA Cancer J. Clin.* 65 (2015) 5–29.
- [2] D.K. Armstrong, Relapsed ovarian cancer: challenges and management strategies for a chronic disease, *Oncologist* 7 (Suppl. 5) (2002) 20–28.
- [3] J. Pfisterer, J.A. Ledermann, Management of platinum-sensitive recurrent ovarian cancer, *Semin. Oncol.* 33 (2006) S12–6.
- [4] R.D. Baird, D.S. Tan, S.B. Kaye, Weekly paclitaxel in the treatment of recurrent ovarian cancer, *Nat. Rev. Clin. Oncol.* 7 (2010) 575–582.
- [5] S. Banerjee, S.B. Kaye, New strategies in the treatment of ovarian cancer: current clinical perspectives and future potential, *Clin. Cancer Res.* 19 (2013) 961–968.
- [6] E. Pujade-Lauraine, F. Hilpert, B. Weber, A. Reuss, A. Poveda, G. Kristensen, et al., Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: the AURELIA open-label randomized phase III trial, *J. Clin. Oncol.* 32 (2014) 1302–1308.
- [7] A.A. Samani, S. Yakar, D. LeRoith, P. Brodt, The role of the IGF system in cancer growth and metastasis: overview and recent insights, *Endocr. Rev.* 28 (2007) 20–47.
- [8] S.A. Rosenzweig, H.S. Atreya, Defining the pathway to insulin-like growth factor system targeting in cancer, *Biochem. Pharmacol.* 80 (2010) 1115–1124.
- [9] A.S. Parker, J.C. Cheville, C.A. Janney, J.R. Cerhan, High expression levels of insulin-like growth factor-1 receptor predict poor survival among women with clear-cell renal cell carcinomas, *Hum. Pathol.* 33 (2002) 801–805.
- [10] D. Spentzos, S.A. Cannistra, F. Grall, D.A. Levine, K. Pillay, T.A. Libermann, et al., IGF axis gene expression patterns are prognostic of survival in epithelial ovarian cancer, *Endocr. Relat. Cancer* 14 (2007) 781–790.
- [11] C.S. Mitsiades, N.S. Mitsiades, C.J. McMullan, V. Poulaki, R. Shringarpure, M. Akiyama, et al., Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors, *Cancer Cell* 5 (2004) 221–230.
- [12] J.L. Gooch, C.L. Van Den Berg, D. Yee, Insulin-like growth factor (IGF)-I rescues breast cancer cells from chemotherapy-induced cell death—proliferative and anti-apoptotic effects, *Breast Cancer Res. Treat.* 56 (1999) 1–10.
- [13] N. Eckstein, K. Servan, B. Hildebrandt, A. Politz, G. von Jonquieres, S. Wolf-Kummeth, et al., Hyperactivation of the insulin-like growth factor receptor 1 signaling pathway is an essential event for cisplatin resistance of ovarian cancer cells, *Cancer Res.* 69 (2009) 2996–3003.
- [14] J. Brouwer-Visser, J. Lee, K. McCullagh, M.J. Cossio, Y. Wang, G.S. Huang, Insulin-like growth factor 2 silencing restores taxol sensitivity in drug resistant ovarian cancer, *PLoS One* 9 (2014), e100165.
- [15] J. Du, H.R. Shi, F. Ren, J.L. Wang, Q.H. Wu, X. Li, et al., Inhibition of the IGF signaling pathway reverses cisplatin resistance in ovarian cancer cells, *BMC Cancer* 17 (2017) 851.
- [16] G.S. Huang, J. Brouwer-Visser, M.J. Ramirez, C.H. Kim, T.M. Hebert, J. Lin, et al., Insulin-like growth factor 2 expression modulates Taxol resistance and is a candidate biomarker for reduced disease-free survival in ovarian cancer, *Clin. Cancer Res.* 16 (2010) 2999–3010.
- [17] R.K. Singh, S.M. Gaikwad, A. Jinager, S. Chaudhury, A. Maheshwari, P. Ray, IGF-1R inhibition potentiates cytotoxic effects of chemotherapeutic agents in early stages of chemoresistant ovarian cancer cells, *Cancer Lett.* 354 (2014) 254–262.
- [18] M.J. Mulvihill, A. Cooke, M. Rosenfeld-Franklin, E. Buck, K. Foreman, D. Landfair, et al., Discovery of OSI-906: a selective and orally efficacious dual inhibitor of the IGF-1 receptor and insulin receptor, *Future Med. Chem.* 1 (2009) 1153–1171.
- [19] P.D. Leiphakpam, E. Agarwal, M. Mathiesen, K.L. Haferbier, M.G. Brattain, S. Chowdhury, In vivo analysis of insulin-like growth factor type 1 receptor humanized monoclonal antibody MK-0646 and small molecule kinase inhibitor OSI-906 in colorectal cancer, *Oncol. Rep.* 31 (2014) 87–94.
- [20] R.L. Jones, E.S. Kim, P. Nava-Parada, S. Alam, F.M. Johnson, A.W. Stephens, et al., Phase I study of intermittent oral dosing of the insulin-like growth factor-1 and insulin receptors inhibitor OSI-906 in patients with advanced solid tumors, *Clin. Cancer Res.* 21 (2015) 693–700.
- [21] I. Puzanov, C.R. Lindsay, L. Goff, J. Sosman, J. Gilbert, J. Berlin, et al., A phase I study of continuous oral dosing of OSI-906, a dual inhibitor of insulin-like growth factor-1 and insulin receptors, in patients with advanced solid tumors, *Clin. Cancer Res.* 21 (2015) 701–711.
- [22] M. Fassnacht, A. Berruti, E. Baudin, M.J. Demeure, J. Gilbert, H. Haak, et al., Linsitinib (OSI-906) versus placebo for patients with locally advanced or metastatic adrenocortical carcinoma: a double-blind, randomised, phase 3 study, *Lancet Oncol.* 16 (2015) 426–435.
- [23] V.M. Macaulay, M.R. Middleton, S.G. Eckhardt, C.M. Rudin, R.A. Juegens, R. Gedrich, et al., Phase I dose-escalation study of Linsitinib (OSI-906) and Erlotinib in patients with advanced solid tumors, *Clin. Cancer Res.* 22 (2016) 2897–2907.
- [24] N.B. Leighl, N.A. Rizvi, L.G. de Lima Jr., W. Arpornwirat, C.M. Rudin, A.A. Chiappori, et al., Phase 2 study of Erlotinib in combination with linsitinib (OSI-906) or placebo in chemotherapy-naïve patients with non-small-cell lung cancer and activating epidermal growth factor receptor mutations, *Clin. Lung Cancer* 18 (2017) 34–42 (e2).
- [25] J.A.L. Liefers-Visser, R.A.M. Meijering, A.K.L. Reyners, A.G.J. van der Zee, S. de Jong, IGF system targeted therapy: therapeutic opportunities for ovarian cancer, *Cancer Treat. Rev.* 60 (2017) 90–99.
- [26] E.R. King, Z. Zu, Y.T. Tsang, M.T. Deavers, A. Malpica, S.C. Mok, et al., The insulin-like growth factor 1 pathway is a potential therapeutic target for low-grade serous ovarian carcinoma, *Gynecol. Oncol.* 123 (2011) 13–18.
- [27] J. Tang, J. Li, G. Zeng, Y. Tang, W. Tian, J. He, et al., Antisense oligonucleotide suppression of human IGF-1R inhibits the growth and survival of in vitro cultured epithelial ovarian cancer cells, *J. Ovarian Res.* 6 (2013) 71.
- [28] M. Shao, S. Hollar, D. Chambliss, J. Schmitt, R. Emerson, B. Chelladurai, et al., Targeting the insulin growth factor and the vascular endothelial growth factor pathways in ovarian cancer, *Mol. Cancer Ther.* 11 (2012) 1576–1586.
- [29] W. Harb, C. Sessa, H. Hirte, S. Kaye, S. Banerjee, A. Christinat, et al., A phase I study evaluating the combination of OSI-906, a dual inhibitor of insulin growth factor-1 receptor (IGF-1R) and insulin receptor (IR) with weekly paclitaxel (PAC) in patients with advanced solid tumors, *J. Clin. Oncol.* 29 (2011) (suppl; abstr 3099).

- [30] J. Kaern, M. Baekelandt, C.G. Trope, A phase II study of weekly paclitaxel in platinum and paclitaxel-resistant ovarian cancer patients, *Eur. J. Gynaecol. Oncol.* 23 (2002) 383–389.
- [31] M.A. Osman, M.S. Elkady, K.E. Nasr, Weekly paclitaxel versus three-weekly paclitaxel in recurrent platinum-resistant epithelial ovarian and peritoneal cancers: a phase III study, *Clin. Med. Insights Oncol.* 10 (2016) 35–41.
- [32] B.J. Monk, A. Poveda, I. Vergote, F. Raspagliesi, K. Fujiwara, D.S. Bae, et al., Anti-angiopoietin therapy with trebananib for recurrent ovarian cancer (TRINOVA-1): a randomised, multicentre, double-blind, placebo-controlled phase 3 trial, *Lancet Oncol.* 15 (2014) 799–808.
- [33] S. Pignata, D. Lorusso, G. Scambia, D. Sambataro, S. Tamberi, S. Cinieri, et al., Pazopanib plus weekly paclitaxel versus weekly paclitaxel alone for platinum-resistant or platinum-refractory advanced ovarian cancer (MITO 11): a randomised, open-label, phase 2 trial, *Lancet Oncol.* 16 (2015) 561–568.
- [34] A.M. Poveda, F. Selle, F. Hilpert, A. Reuss, A. Savarese, I. Vergote, et al., Bevacizumab combined with weekly paclitaxel, Pegylated liposomal doxorubicin, or Topotecan in platinum-resistant recurrent ovarian cancer: analysis by chemotherapy cohort of the randomized phase III AURELIA trial, *J. Clin. Oncol.* 33 (2015) 3836–3838.
- [35] Avastin [package insert]., Genentech, Inc, South San Francisco, CA, 2017.
- [36] P.J. Beltran, F.J. Calzone, P. Mitchell, Y.A. Chung, E. Cajulis, G. Moody, et al., Ganitumab (AMG 479) inhibits IGF-II-dependent ovarian cancer growth and potentiates platinum-based chemotherapy, *Clin. Cancer Res.* 20 (2014) 2947–2958.
- [37] I. Brana, R. Berger, T. Golan, P. Haluska, J. Edenfield, J. Fiorica, et al., A parallel-arm phase I trial of the humanised anti-IGF-1R antibody dalotuzumab in combination with the AKT inhibitor MK-2206, the mTOR inhibitor ridaforolimus, or the NOTCH inhibitor MK-0752, in patients with advanced solid tumours, *Br. J. Cancer* 111 (2014) 1932–1944.
- [38] I.A. McNeish, J.A. Ledermann, L. Webber, L. James, S.B. Kaye, M. Hall, et al., A randomised, placebo-controlled trial of weekly paclitaxel and saracatinib (AZD0530) in platinum-resistant ovarian, fallopian tube or primary peritoneal cancer, *Ann. Oncol.* 25 (2014) 1988–1995.
- [39] J. Fogh, W.C. Wright, J.D. Loveless, Absence of HeLa cell contamination in 169 cell lines derived from human tumors, *J. Natl. Cancer Inst.* 58 (1977) 209–214.