## Original article

# Vistusertib (dual m-TORC1/2 inhibitor) in combination with paclitaxel in patients with high grade serous ovarian and squamous non-small cell lung cancer

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## Key message

This phase IB study established a dose and schedule of vistusertib (dual m-TORC1/2 inhibitor) in combination with weekly paclitaxel. Response rates in pre-treated populations of patients in the expansion cohorts of HGSOC and squamous NSCLC were 52% and 35%, respectively. Progression-free survival in both cohorts was 5.8 months. Randomised trials of this combination are warranted.

## Abstract

### Background

We have previously shown that raised p-S6K levels correlate with resistance to chemotherapy in ovarian cancer. We hypothesised that inhibiting p-S6K signalling with the dual m-TORC1/2 inhibitor in patients receiving weekly paclitaxel could improve outcomes in such patients.

### Patients and Methods

In dose escalation, weekly paclitaxel (80 mg/m$^2$) was given 6/7 weeks in combination with two intermittent schedules of vistusertib (dosing starting on the day of paclitaxel): schedule A, vistusertib dosed bd for 3 consecutive days per week (3/7days) and schedule B, vistusertib dosed bd for 2 consecutive days per week (2/7days). After establishing a recommended phase II dose (RP2D), expansion cohorts in high-grade serous ovarian cancer (HGSOC) and squamous non-small cell lung cancer (sqNSCLC) were explored in 25 and 40 patients, respectively.

### Results

The dose escalation arms comprised 22 patients with advanced solid tumours. The dose-limiting toxicities were fatigue and mucositis in schedule A and rash in schedule B. Based on toxicity, pharmacokinetic (PK) and pharmacodynamic (PD) evaluations, the RP2D was established as 80 mg/m$^2$ paclitaxel with 50 mg vistusertib bd 3/7 days for 6/7 weeks. In the HGSOC expansion RECIST and GCIG CA125 response rates were 13/25 (52%) and 16/25 (64%), respectively with median progression-free survival (mPFS) of 5.8 months (95% CI: 3.28 - 18.54). The RP2D was not well tolerated in the SqNSCLC expansion, but toxicities were manageable after the daily vistusertib dose was reduced to 25 mg bd for the following 23 patients. The RECIST response rate in this group was 8/23 (35%) and the mPFS was 5.8 months (95% CI: 2.76 - 21.25).

## Discussion

In this phase I trial we report a highly active and well tolerated combination of vistusertib, administered as an intermittent schedule with weekly paclitaxel, in patients with HGSOC and SqNSCLC.

**Clinical trial registration:** ClinicialTrials.gov identifier: CNCT02193633

**Key words:** Phase 1; m-TORC1/m-TORC2 inhibitor; combination therapy; ovarian cancer; squamous non-small cell lung cancer

## Introduction

We have previously studied cancer cells isolated from serous effusions and shown raised p-S6K to be associated with chemo-resistance and poor clinical outcome in ovarian and lung cancers, respectively [1, 2]. This led us to hypothesise that inhibition of m-TOR signalling, in combination with chemotherapy, could improve treatment outcomes in these tumour types. Analogues of rapamycin such as everolimus have been recognised to inhibit only m-TORC1 and not m-TORC2 in the m-TOR complex [3]. The dual m-TORC1/2 inhibitor vistusertib (AZD2014) has a short half-life, giving greater flexibility for intermittent dosing schedules [4, 5]. Weekly paclitaxel was chosen as the chemotherapy backbone as it is often used to treat advanced ovarian cancer. Pre-clinical studies of vistusertib and paclitaxel revealed an additive effect on growth *in vitro* and *in vivo*, with the combination showing increased apoptosis and metabolic effects consistent with the mechanism of action of vistusertib [6].

Here, we report the results of the TAX-TORC study, a phase Ib dose-escalation study, with a pre-planned dose-expansion cohort in HGSOC and an additional expansion cohort in sqNSCLC (supplementary Figure S1).

## Methods - Patients

### Conduct of the study

The academic sponsors of this study were The Institute of Cancer Research and The Royal Marsden (CCR3667) and the trial was reviewed by a central research ethics committee (REC ref: 13/LO/0066). The study was funded by AstraZeneca. Nine Experimental Cancer Medicine Centres across the UK participated in this study. All patients were treated after obtaining written, informed consent. Cancer Research UK trial number: CRUKD/12/013.

*Inclusion/exclusion criteria:* Inclusion criteria in the dose escalation arm included an ECOG performance status of 0 or 1. Haematological and biochemistry criteria were standard for phase I studies and details are available in the supplementary data.

### Treatment

80 mg/m$^2$ paclitaxel was administered once weekly for 6/7 weeks in a 7-week cycle. In the first week of the dose escalation cohorts, patients received only paclitaxel on C1D1, then vistusertib on C1D3 to allow for PK and PD sampling. Patients then received weekly paclitaxel (on days 8, 15, 22, 29, 36) with vistusertib, also starting on days 8, 15, 22, 29, 36, given orally twice daily either for three consecutive days per week (schedule A: 3/7days, 6/7 weeks) or two consecutive days per week (schedule B: 2/7days, 6/7 weeks). In the dose expansion, schedule A was taken forward with patients dosing with vistusertib weekly on days 1 - 3 for 6 weeks of a 7-week cycle.

### Evaluation of toxicity

NCI-CTCAE V4·0 was used to assess toxicity.

### Evaluation of response

RECIST v1.1 was used to assess tumour response supported by GCIG CA125 response in HGSOC patients. Response was assessed at the end of every 7-week cycle.

## Methods – Materials and methods

### Pharmacokinetic and pharmacodynamic evaluation

PK sampling was carried out for all patients in the dose escalation arm for 24 hours on C1D1 (paclitaxel alone), C1D3 (vistusertib alone), and on C1D1 (combination of paclitaxel and vistusertib). PD sampling was carried out for all patients in the dose escalation arm. Sampling for PD assays was carried out on the same days as PK sampling. Phosphorylation of AKTSer473 (Ser$^{473}$ p-AKT) was quantified in platelet-rich plasma (PRP) (for detailed methods see supplementary data) [7].

### Sequencing

DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) tumour blocks. In addition, circulating free DNA (cfDNA) when collected at baseline, at the end of cycle 1 and, where possible, at progression, was extracted from 4 - 8 mL of plasma. Sequencing libraries were constructed using a customised Generead DNAseq Mix-n-Match v2 panel (Qiagen) covering 4841 amplicons (310,077 bp) across 67 genes. Libraries were run using the MiSeq Sequencer (Illumina), sequence alignment and mutation calling were performed.

## Methods – Statistical analysis

The data cut-off for this paper was 01 October 2017. Demographics were analysed by descriptive statistics. Safety was assessed in all enrolled patients. Patients considered not assessable for response had no post-baseline CT scan. The number of patients required for the dose escalation phase was dependent on toxicities observed as the trial progressed. No formal power calculations were done.

Progression-free survival was estimated by the Kaplan-Meier method, beginning on the day of the first dose (C1D1) and continuing until disease progression. Patients who came off study for reasons other than disease progression (clinical or RECIST) were censored. This study is registered with ClinicalTrials.gov, identifier: NCT02193633.

## Results

### Dose escalation cohort

*Toxicity*

Twenty-two patients were recruited to the dose escalation cohort. The most common tumours were ovarian and lung cancer (supplementary Table S1). In the dose escalation phase, vistusertib was tested at 25 mg, 50 mg and 75 mg bd 3/7days, 6/7 weeks (schedule A) with no dose limiting toxicities (DLTs) in the 25 mg or 50 mg groups. Two of the three

patients in the 75 mg group experienced DLTs of fatigue and mucositis. Vistusertib was then tested at 50 mg and 75 mg bd 2/7 days (schedule B) with no DLTs. However, two of the three patients taking 100 mg bd 2/7 days experienced DLTs of rash (supplementary Figure S2). The maximally tolerated dose (MTD) of schedule A was thus 80 mg/m$^2$ weekly paclitaxel with 50 mg vistusertib bd 3/7 days for 6/7 weeks in a 7-week cycle, with dosing starting concurrently on day 1 of each week. The MTD of schedule B was 80 mg/m$^2$ weekly paclitaxel with 75 mg vistusertib bd 2/7 days for 6/7 weeks in a 7-week cycle, with dosing starting concurrently on day 1 of each week. The most common toxicities across both schedules were predominantly grade 1 - 2 fatigue, nausea, anaemia, and diarrhoea (Table 1), which are similar to that seen with weekly paclitaxel administration.

*Pharmacokinetics*

In all schedules tested, the PK of paclitaxel when administered alone or in combination with vistusertib was similar (Table 2). The PK of vistusertib alone or vistusertib in combination with paclitaxel in both schedules was comparable with previous single agent studies [5] (data not shown). The areas under the curve (AUC) *versus* dose of vistusertib was approximately dose proportional (supplementary Figure S3). Altogether, these suggest that there is no drug-drug PK interaction on drug exposure for either paclitaxel or vistusertib in combination compared with either agent administered alone.

*Pharmacodynamics*

At the recommended phase II dose level of 50 mg bd of vistusertib and 80 mg/m$^2$ of paclitaxel, there was a statistically non-significant increase in levels of Ser$^{473}$ p-AKT 4 hours following 80 mg/m$^2$ paclitaxel (1.4 fold; $P = 0.14$). Vistusertib (50 mg bd 3/7) in addition to paclitaxel produced a reduction in Ser$^{473}$ p-AKT at 4 hours post-vistusertib to 53% of pre-dose levels ($P = 0.0495$). This was 62% lower than the corresponding time-point following paclitaxel alone, suggesting that at the RP2D of the combination, there is a significant reduction in p-AKT levels in normal tissue compared to baseline (Figure 1).

*Recommended phase II dose*

In combination with weekly paclitaxel administered at 80 mg/m$^2$ once weekly, the MTD of vistusertib was 50 mg bd (3/7days) (schedule A) or 75 mg bd (2/7days) (schedule B). Both doses had acceptable PK and PD profiles and would be acceptable as per the pharmacological audit trail [8]. Weekly vistusertib 50 mg bd 3 days on/4 days off combined with weekly paclitaxel 80 mg/m$^2$ was taken forward as the RP2D based on reduced occurrence of grade 3 fatigue in this cohort.

*Ovarian cancer expansion*

Twenty-seven patients with relapsed/refractory HGSOC were treated at the recommended phase II dose. Two patients were replaced as per protocol and were not considered for assessment of response. The median number of previous treatments was three: the majority (26/27; 96%) of patients having received paclitaxel and 3/27 patients (11%) having previously received weekly paclitaxel (supplementary Table S2). The RECIST and CA125 response rates were 13/25 (52%) and 16/25 (64%), respectively (Figure 2A). The mPFS was 5·8 months (95% CI: 3.3 - 18.5).

DNA sequencing (targeted panel of 67 genes) of FFPE tissue revealed the most common mutation was TP53 detected in 23/25 (92%) patients. There was no correlation between specific mutations and response (Figure 2B).

*Squamous lung cancer expansion*

Following two partial responses in patients with sqNSCLC in the dose escalation cohort, we conducted a dose expansion in a cohort of 40 patients, starting at the RP2D of 80 mg/m$^2$ paclitaxel and 50 mg vistusertib bd 3/7 days. This schedule was poorly tolerated with fatigue, diarrhoea and pneumonia being seen more frequently than in the dose escalation cohort (supplementary Table S3A). The safety review committee reviewed the data of the first 17

patients and decided to reduce the dose of vistusertib to 25 mg bd 3/7 days for the remaining 23 patients due to be treated in this cohort. This dose was known to be pharmacodynamically active [5] and was better tolerated (supplementary Table S3B). The RECIST response rate in patients with sqNSCLC in the 25 mg cohort was 8/23 (35%) (Figure 3A), with an mPFS of 5.8 months (95% CI 2.8-21.3). Two patients with *PIK3CA* mutations had partial responses but there were no clear patterns linking mutations to response (Figure 3B).

## Discussion

We report the first study of the combination of weekly paclitaxel with the dual m-TORC1/2 inhibitor, vistusertib, establishing a safe dose and schedule and preliminary evidence of efficacy in HGSOC and SqNSCLC. We chose to investigate the m-TORC1/2 inhibitor in the context of weekly paclitaxel as this regimen is often used in the setting of platinum-resistant ovarian cancer [9] and taxanes are commonly used in the treatment of platinum-resistant NSCLC (with comparable efficacy between weekly paclitaxel and docetaxel and better tolerability profile) [10, 11].

Toxicities of fatigue, nausea, anaemia and diarrhoea in this dose escalation cohort were not dissimilar to previous studies combining m-TOR inhibitors such as everolimus [12], ridaforolimus [13] or the m-TORC1/2 inhibitor, MLN028 [14], with weekly paclitaxel regimens. Hyperglycaemia, which has been commonly reported with m-TOR inhibitors, occurred at a very low incidence in our study (all grades: $N = 8$ (11%), grade 3/4: $N = 1$(1%)). It was noted that many previous studies were in breast cancer where weekly paclitaxel is often used as standard-of-care. Of interest, in our study patients with heavily pre-treated HGSOC tolerated vistusertib at 50 mg bd 3 days per week in combination with weekly paclitaxel. However, patients with sqNSCLC needed a dose reduction of vistusertib to 25 mg bd 3 days per week. Patients with sqNSCLC often have risk factors and co-morbidities that correlate with poor tolerance of chemotherapy such as hypoxia, a history of smoking and pulmonary fibrosis

[15]. We have previously reported on the increased risk of infections of patients treated with PI3K pathway inhibitors used as part of combination therapy [16]. In our experience, this is the first time that it has been necessary to recommend two separate doses for different tumour types within the same study.

The pharmacokinetic profile of vistusertib was not significantly different from previous reports in single agent studies [5], and was no different when administered alone or in combination with paclitaxel. The pharmacodynamic profile of vistusertib in PRP showed administration of vistusertib led to abrogation of AKTSer473 phosphorylation, providing proof-of-principle of the desired biological effect of inhibiting the PI3K–Akt-m-TOR pathway.

The clinical outcomes of patients receiving the combination of weekly paclitaxel and vistusertib in this non-randomised phase I expansion were encouraging for the patient groups explored. In the ovarian cohort the median lines of previous treatment were three: 12% of patients were platinum-refractory, 48% had progressed within 6 months of the last platinum treatment, and 96% had progressed within a year of their last platinum treatment. In this cohort the RECIST and CA125 response rate was 52% and 64%, respectively, with a progression-free interval of 5.8 months, which is better than historic data reported for the use of weekly paclitaxel therapy [9]. The control chemotherapy arm of a contemporary phase III study studying the addition of bevacizumab to chemotherapy in the setting of 2$^{nd}$ or 3$^{rd}$ line chemotherapy in a platinum-resistant disease state achieved a response rate of 12% and progression-free survival was 3.9 months [17]. The results of the TAX-TORC study have led to a randomised phase II study of weekly paclitaxel *versus* paclitaxel and vistusertib, which is ongoing (ISRCTN16426935) [18].

The standard-of-care of sqNSCLC changed with the introduction of immune checkpoint inhibitors with response rates of ~15% in patient cohorts not selected for PD-L1 expression confirmed by randomised control trials [19]. In the TAX-TORC study, at the tolerated doses

of paclitaxel (80 mg/m$^2$/week) and vistusertib (25 mg bd 3/7 days), the response rate and progression-free survival was 35% and 5.8 months, respectively. These data exceed traditional outcomes for the sqNSCLC population beyond first-line therapy and demonstrate potential for benefit and warrant further evaluation. A possible use of this regimen could be in the setting of patients with sqNSCLC who do not have expression of PD-L1 [20].

We attempted to identify biomarkers of response to the combination by studying a panel of 67 genes that were known to be commonly mutated in HGSOC [21] and sqNSCLC [22]. The mutations found in our study were in keeping with those described elsewhere in these tumour types; however, there were no significant differences in mutation profiles of responders and non-responders in this small dataset.

**Conclusion**

We report a phase I study combining weekly paclitaxel and a dual m-TORC1/2 inhibitor, vistusertib, with expansions in high-grade serous ovarian cancer and squamous non-small cell lung cancer, which are both areas of unmet need. The trial showed tolerable schedules in expansion cohorts of over 20 patients. The response rates and progression-free survival in these non-randomised phase I expansions show promise and randomised phase II studies are recommended to study these combinations further.

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## Disclosure

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## Table legends

**Table 1.** *Toxicity in the dose escalation arm*

All drug related events (possibly, probably and, definitely related) seen in more than 20% of patients in the dose escalation cohorts. A total of 22 patients were treated in the dose escalation. One patient was treated with vistusertib on schedule B at 50 mg instead of 100 mg due to urgent reporting of two dose-limiting toxicities. The patient did not have grade 3 or 4 toxicity or a DLT, was evaluable, but has not been represented in the table for simplicity.

**Table 2.** *Pharmacokinetic profile of vistusertib*

The area under the curve (AUC), maximal concentration (Cmax) and half-life (HL) of vistusertib on C1D3 (administered as a single agent) and C1D8 (administered in combination with paclitaxel) across the different dose levels in the dose escalation cohort.

## Figure legends

**Figure 1**

*Pharmacodynamic profile of vistusertib at 50 mg bd 3/7*

Phosphorylation of AKT (Ser473) in platelet-rich plasma was quantified using MSD electrochemiluminescent immunoassays and normalised to corresponding total AKT values. Baseline values were established prior to the start of treatment. On C1D1 only paclitaxel (80 $mg/m^2$) was administered and a non-significant rise in p-ATK at 4 hours following treatment was noted. On C1D4 a single dose of vistusertib was administered and non-significant reduction of p-AKT was seen. On C1D8 the combination of paclitaxel and vistusertib was administered, which caused a significant reduction of p-AKT compared to baseline. Points represent individual patients, orange line represents mean of up to $N = 6$ patients. Four samples were excluded because of haemolysis which interfered with the assay (*$P<0.05$; paired t test).

**Figure 2**

*Clinical outcomes of patients in the ovarian cancer expansion treated at the R2PD for ovarian cancer*

A) Waterfall plot of 23/25 patients with ovarian cancer treated at the RP2D for ovarian cancer that were evaluable for response; two patients clinically progressed with bowel obstruction in the first cycle and did not have a repeat CT scan to assess response. Nineteen of 25 (76%) patients showed a reduction in size of their tumour, with 13/25 (52%) achieving a partial response. B) Mutations in tumour tissue or plasma of patients compared with clinical response. C) Spider plots representing percentage change in measured sum of tumour dimensions of individual patients over time (each cycle is 7 weeks).

**Figure 3**

*Clinical outcomes of patients in the squamous NSCLC expansion treated at the R2PD for squamous NSCLC*

A) Waterfall plot of 21/23 patients with sqNSCLC treated at RP2D of the combination; two patients clinically progressed within their first cycle and repeat radiological evaluation was not done. Eighteen of the 23 (78%) patients showed reduction in the size of their tumour with 8/23 (35%) achieving a partial response. B) Mutations in tumour tissue or plasma of patients compared with clinical response. C) Spider plots representing percentage change in measured sum of tumour dimensions of individual patients over the time (each cycle is 7 weeks).

| Adverse Event | Escalation 3d on, 4d off | | | | | | Escalation 2d on, 5d off | | | | Total (*N*=21) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 25 mg (*N*=3) | | 50 mg (*N*=6) | | 75 mg (*N*=3) | | 75 mg (*N*=6) | | 100 mg (*N*=3) | | |
| | Grade 1-2 | Grade 3-4 | Grade 1-2 | Grade 3-4 | Grade 1-2 | Grade 3-4 | Grade 1-2 | Grade 3-4 | Grade 1-2 | Grade 3-4 | |
| Fatigue | 3 | 0 | 5 | 0 | 0 | 3 | 1 | 2 | 1 | 1 | 16 |
| Nausea | 3 | 0 | 4 | 0 | 0 | 0 | 4 | 0 | 2 | 0 | 13 |
| Anaemia | 2 | 0 | 4 | 0 | 2 | 0 | 2 | 0 | 2 | 0 | 12 |
| Diarrhoea | 1 | 0 | 3 | 1 | 1 | 1 | 3 | 0 | 1 | 0 | 11 |
| Peripheral sensory neuropathy | 1 | 0 | 2 | 0 | 1 | 0 | 3 | 0 | 2 | 0 | 9 |
| Skin rash | 1 | 0 | 1 | 0 | 1 | 0 | 3 | 0 | 1 | 2 | 9 |
| Alopecia | 1 | 0 | 4 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 8 |
| Dysgeusia | 0 | 0 | 3 | 0 | 1 | 0 | 4 | 0 | 0 | 0 | 8 |
| Mucositis | 1 | 0 | 2 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 7 |
| Neutropenia | 0 | 0 | 2 | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 7 |
| Dyspepsia/gastric reflux | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 5 |
| Hypophosphataemia | 1 | 0 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 5 |
| Pain | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 4 |
| Paronychia | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 5 |

**Table 1**

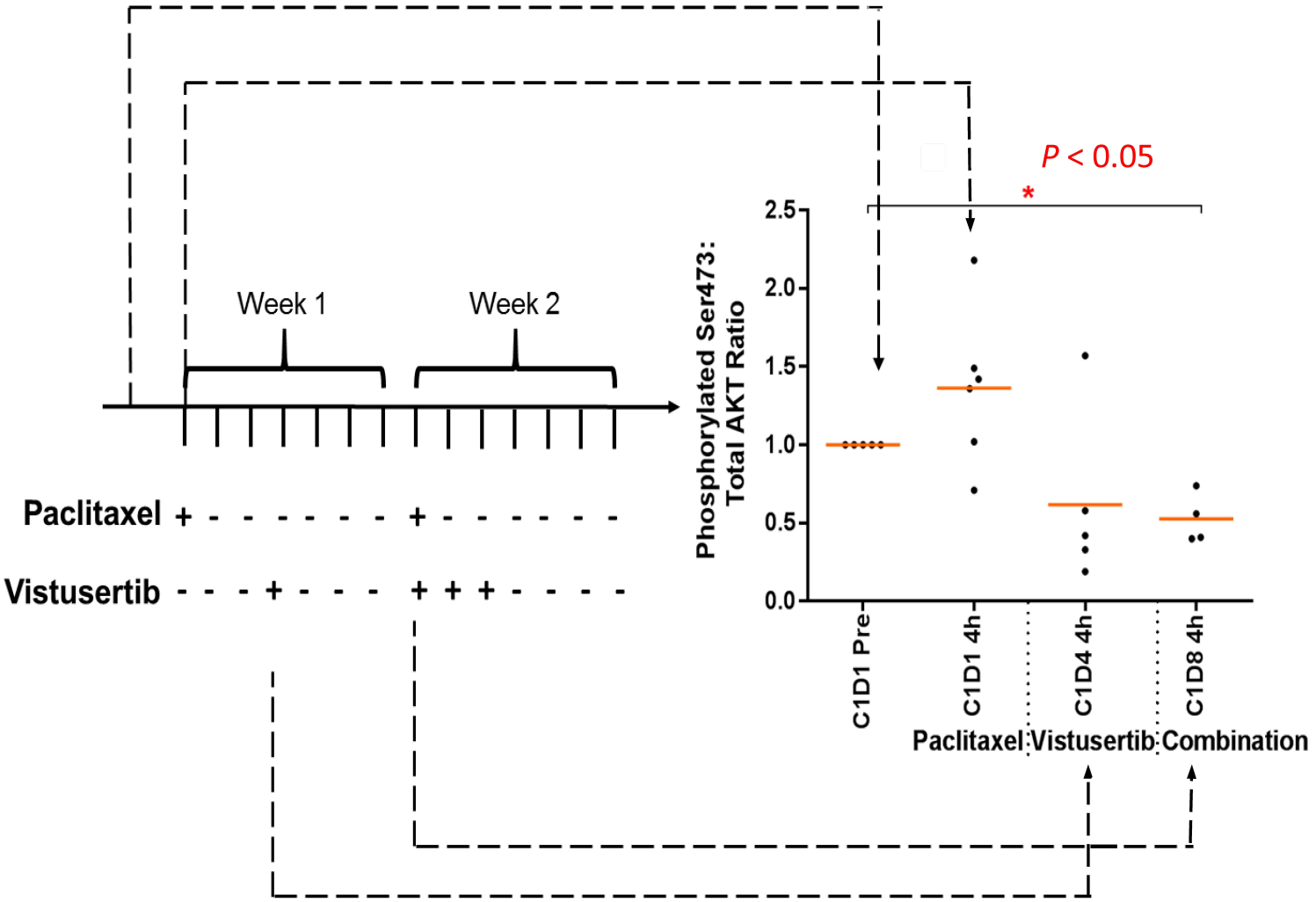
*Toxicity in the dose escalation arm*

All drug related events (possibly, probably and, definitely related) seen in more than 20% of patients in the dose escalation cohorts.

A total of 22 patients were treated in the dose escalation. One patient was treated with vistusertib on schedule B at 50 mg instead of 100 mg due to urgent reporting of two dose-limiting toxicities. The patient did not have grade 3 or 4 toxicity or a DLT, was evaluable, but has not been represented in the table for simplicity.

| Variable | Day | AZD2014/Paclitaxel | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 25 mg/80 mg | | 50 mg/80 mg | | 75 mg/80 mg | | 100 mg/80 mg | |
| | | Geometric Mean | N | Geometric Mean | N | Geometric Mean | N | Geometric Mean | N |
| AUClast (h*ng/mL) | 3 | 2090 (1290-3462) | 3 | 2602 (708-11486) | 7 | 7543 (4192-16542) | 9 | 7556 (4188-12884) | 3 |
| | 8 | 1054 (181-2785) | 3 | 2026 (800-6137) | 7 | 5209 (1576-13363) | 8 | 7347 (4875-13997) | 3 |
| Cmax (ng/mL) | 3 | 579 (478-785) | 3 | 840 (462-3580) | 7 | 1840 (983-2870) | 9 | 1960 (1180-2670) | 3 |
| | 8 | 248 (80-507) | 3 | 500 (244-764) | 7 | 1122 (442-1920) | 8 | 1830 (1490-2420) | 3 |
| HL Lambda_z (h) | 3 | 3.3 (2.3-4.2) | 3 | 1.8 (0.8-3.2) | 6 | 2.7 (1.2-5.9) | 8 | 3.0 (2.5-3.3) | 3 |
| | 8 | 3.5 (1.9-6.1) | 3 | 2.2 (1.7-2.9) | 6 | 3.1 (1.7-9.7) | 8 | 2.8 (1.2-5.3) | 3 |

**Table 2.** *Pharmacokinetic profile of vistusertib*
The area under the curve (AUC), maximal concentration (Cmax) and half-life (HL) of vistusertib on C1D3 (administered as a single agent) and C1D8 (administered in combination with paclitaxel) across the different dose levels in the dose escalation cohort.


Week 1
Week 2
Paclitaxel + - - - - - - + - - - - - -
Vistusertib - - - + - - - + + + - - - -
P < 0.05
*
Phosphorylated Ser473: Total AKT Ratio
2.5
2.0
1.5
1.0
0.5
0.0
C1D1 Pre
C1D1 4h
C1D4 4h
C1D8 4h
Paclitaxel
Vistusertib
Combination

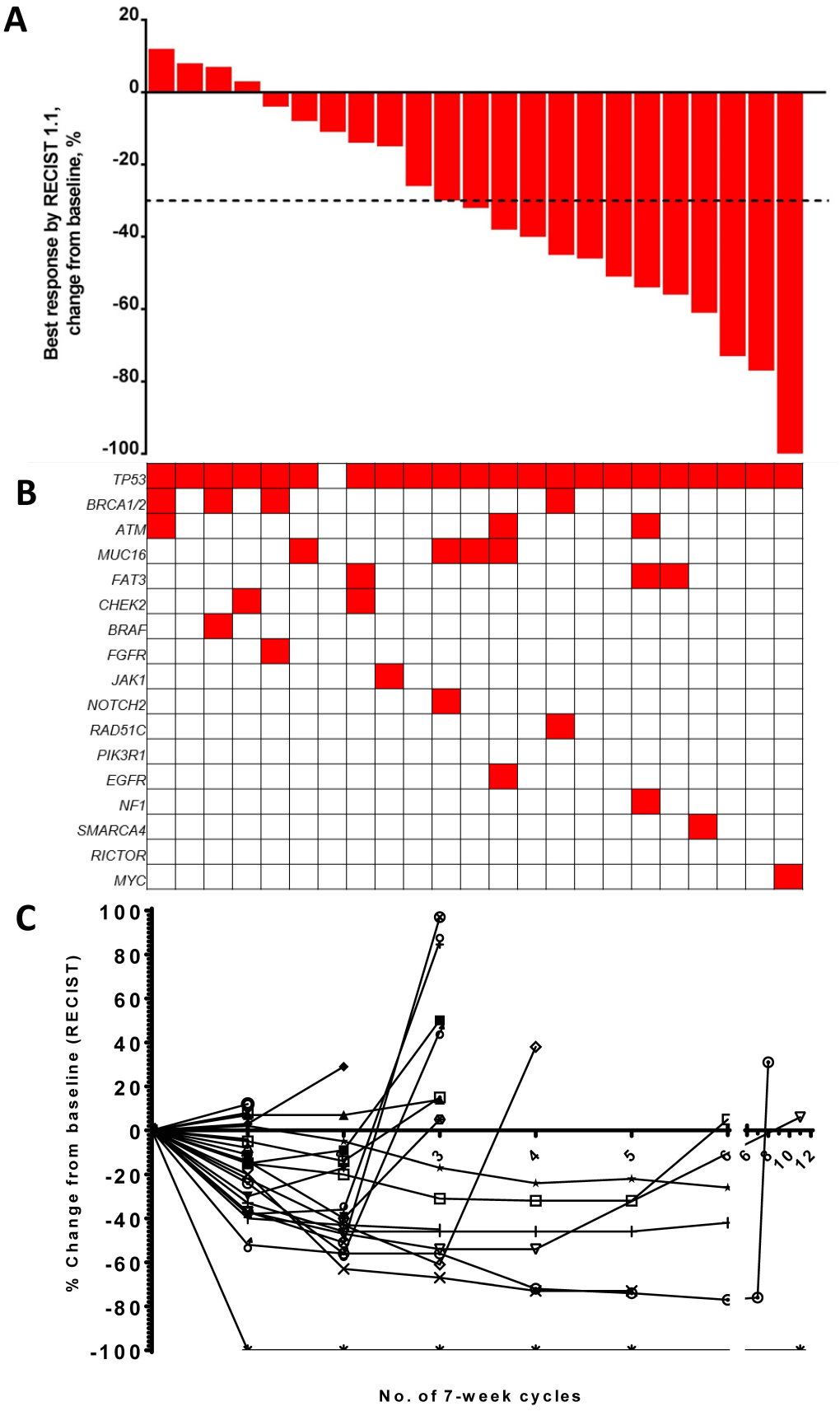

A
20
0
-20
-40
-60
-80
-100
Best response by RECIST 1.1, change from baseline, %
B
TP53
BRCA1/2
ATM
MUC16
FAT3
CHEK2
BRAF
FGFR
JAK1
NOTCH2
RAD51C
PIK3R1
EGFR
NF1
SMARCA4
RICTOR
MYC
C
100
80
60
40
20
0
-20
-40
-60
-80
-100
% Change from baseline (RECIST)
3
4
5
6
8
10
12
No. of 7-week cycles

**Figure 2**

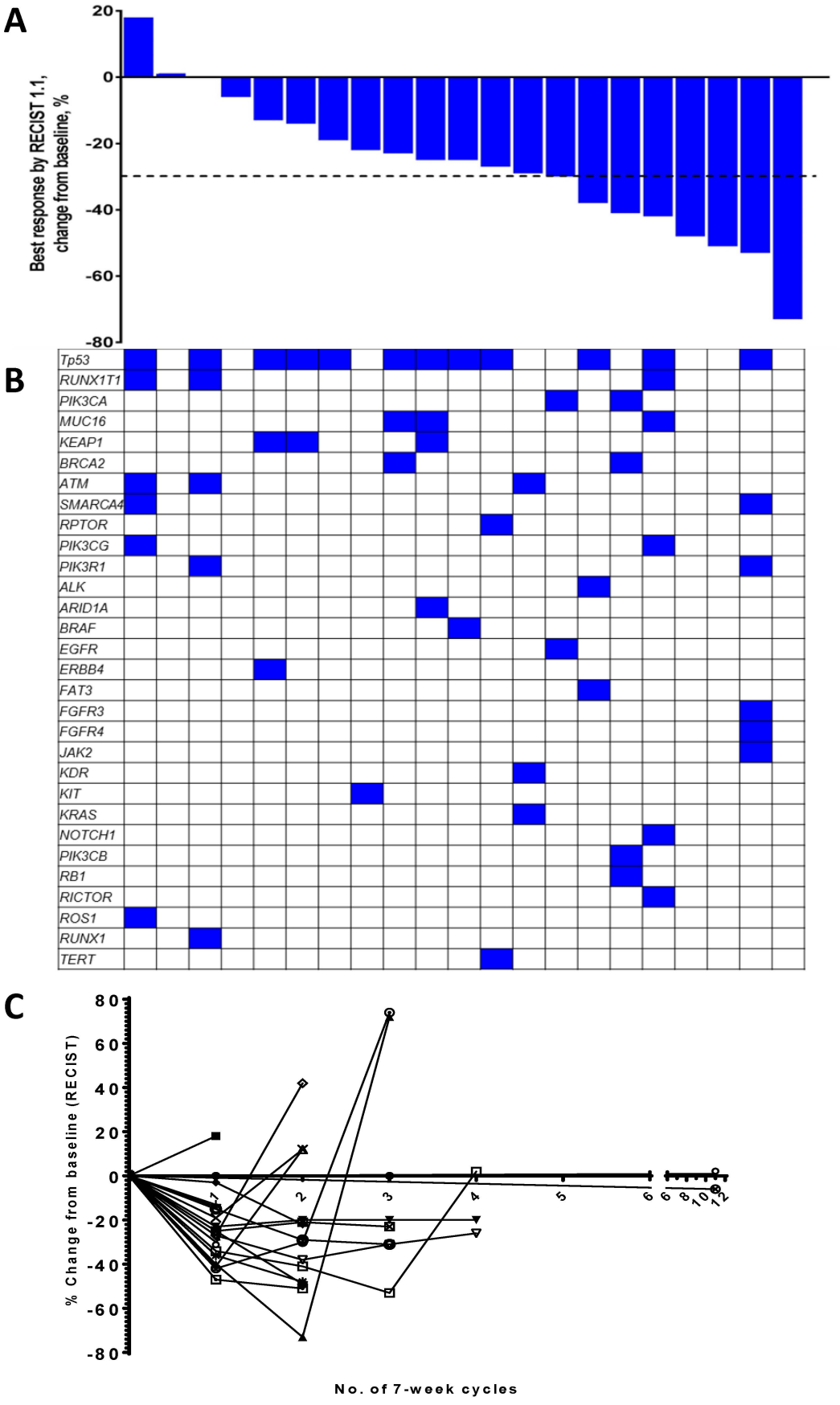

A
Best response by RECIST 1.1, change from baseline, %
20
0
-20
-40
-60
-80
B
Tp53
RUNX1T1
PIK3CA
MUC16
KEAP1
BRCA2
ATM
SMARCA4
RPTOR
PIK3CG
PIK3R1
ALK
ARID1A
BRAF
EGFR
ERBB4
FAT3
FGFR3
FGFR4
JAK2
KDR
KIT
KRAS
NOTCH1
PIK3CB
RB1
RICTOR
ROS1
RUNX1
TERT
C
% Change from baseline (RECIST)
80
60
40
20
0
-20
-40
-60
-80
1
2
3
4
5
6
6
8
10
12
No. of 7-week cycles

Figure 3

| Characteristics | Total on Dose Escalation |
|---|---|
| | ***N* (%)** |
| **Total number of patients** | 22 |
| **Primary tumour** | |
| Ovary | 8 (36) |
| Lung adenocarcinoma | 4 (18) |
| Lung squamous | 2 (9) |
| Breast | 1 (5) |
| Endometrial | 4 (18) |
| Gastroesophageal | 1 (5) |
| Cervix | 2 (9) |

**Supplementary Table 1. Demographic profile of patients treated in the dose escalation cohort**

| Characteristics | Subtypes | OVARIAN Expansion (50 mg AZD2014/80 mg Paclitaxel) | LUNG Expansion (25 mg AZD2014/80 mg Paclitaxel) |
|---|---|---|---|
| | | *N* (%) | *N* (%) |
| Primary tumour | High grade serous ovarian | 27 (100) | - |
| | Lung squamous | - | 24 (100) |
| Gender | Male | - | 11 (46) |
| | Female | 27 (100) | 13 (54) |
| Age (years) | 18 - 64 | 11 (41) | 11 (44) |
| | ≥65 | 16 (59) | 13 (56) |
| ECOG performance status | 0 | 7 (26) | 4 (17) |
| | 1 | 20 (74) | 20 (83) |
| Median previous lines of therapy | | 3 (range: 1 to 12) | 2 (range: 1 to 3) |
| Previously treated with taxane (incl. docetaxel or paclitaxel) | | 26 (96)<br>(Previous weekly taxol = 3) | 6 (25) |
| RECIST response | | 13 PR | 8 PR (33) |
| Median number of weeks on treatment | | 21 | 18 |
| Ongoing on 1-Oct-2017 | | 1 | 1 |
| Platinum sensitivity | Platinum refractory | 6 (22) | 1 (4) |
| | Platinum resistance (< 6 months) | 11 (41) | 15 (63) |
| | Platinum intermediate sensitive (6 - 12 months) | 9 (33) | 4 (17) |
| | Platinum sensitive (> 12 months) | 1 (4) | 4 (17) |

**Supplementary Table 2. Demographic profile of patients treated in the dose expansion cohort**

| Lung Expansion 50 mg Vistusertib (*N*=17) | | | | |
|---|---|---|---|---|
| **Adverse Event, *N* (%)** | **Gr.1 - Gr.2** | **Gr.3 - Gr.4** | **Total** | **%** |
| Fatigue | 6 | 4 | 10 | 59 |
| Skin rash | 8 | 1 | 9 | 53 |
| Anorexia/Appetite loss/Weight loss | 8 | 0 | 8 | 47 |
| Diarrhoea | 5 | 2 | 7 | 41 |
| Neuropathy | 6 | 0 | 6 | 35 |
| Thrush (oral) | 6 | 0 | 6 | 35 |
| Anaemia | 4 | 1 | 5 | 29 |
| Bronchitis/Bronchial or respiratory infection | 4 | 1 | 5 | 29 |
| Dyspnea/Shortness of breath | 5 | 0 | 5 | 29 |
| Mucositis/Oral mucositis/Mouth ulcers | 5 | 0 | 5 | 29 |
| Nausea | 4 | 1 | 5 | 29 |
| Vomiting | 5 | 0 | 5 | 29 |
| Pneumonitis/Pneumonia | 3 | 1 | 4 | 24 |
| Cough | 3 | 0 | 3 | 18 |
| Dry skin/Eczema | 3 | 0 | 3 | 18 |
| Dyspepsia/Indigestion/GI tox | 3 | 0 | 3 | 18 |
| Hyperglycaemia | 2 | 1 | 3 | 18 |
| Leucopenia | 2 | 1 | 3 | 18 |
| Neutropenia | 2 | 1 | 3 | 18 |
| Aches and pain | 2 | 0 | 2 | 12 |
| Alopecia | 2 | 0 | 2 | 12 |
| Dry mouth | 2 | 0 | 2 | 12 |
| Dysgeusia | 2 | 0 | 2 | 12 |
| Elevated ALT | 2 | 0 | 2 | 12 |
| Elevated AST | 2 | 0 | 2 | 12 |
| Malaise/Sweats | 2 | 0 | 2 | 12 |
| Paronychia/Nail changes | 2 | 0 | 2 | 12 |
| Sore throat | 2 | 0 | 2 | 12 |
| Cellulitis | 0 | 1 | 1 | 6 |
| Drug reaction | 1 | 0 | 1 | 6 |
| Epistaxis | 1 | 0 | 1 | 6 |
| Glucose intolerance | 1 | 0 | 1 | 6 |
| Headache | 1 | 0 | 1 | 6 |
| Hyperbilirubinaemia | 1 | 0 | 1 | 6 |
| Myalgia | 1 | 0 | 1 | 6 |
| Red skin (face) | 1 | 0 | 1 | 6 |
| Trigeminal nerve disorder | 1 | 0 | 1 | 6 |
| UTI | 0 | 1 | 1 | 6 |
| **Total** | **110** | **16** | **126** | |

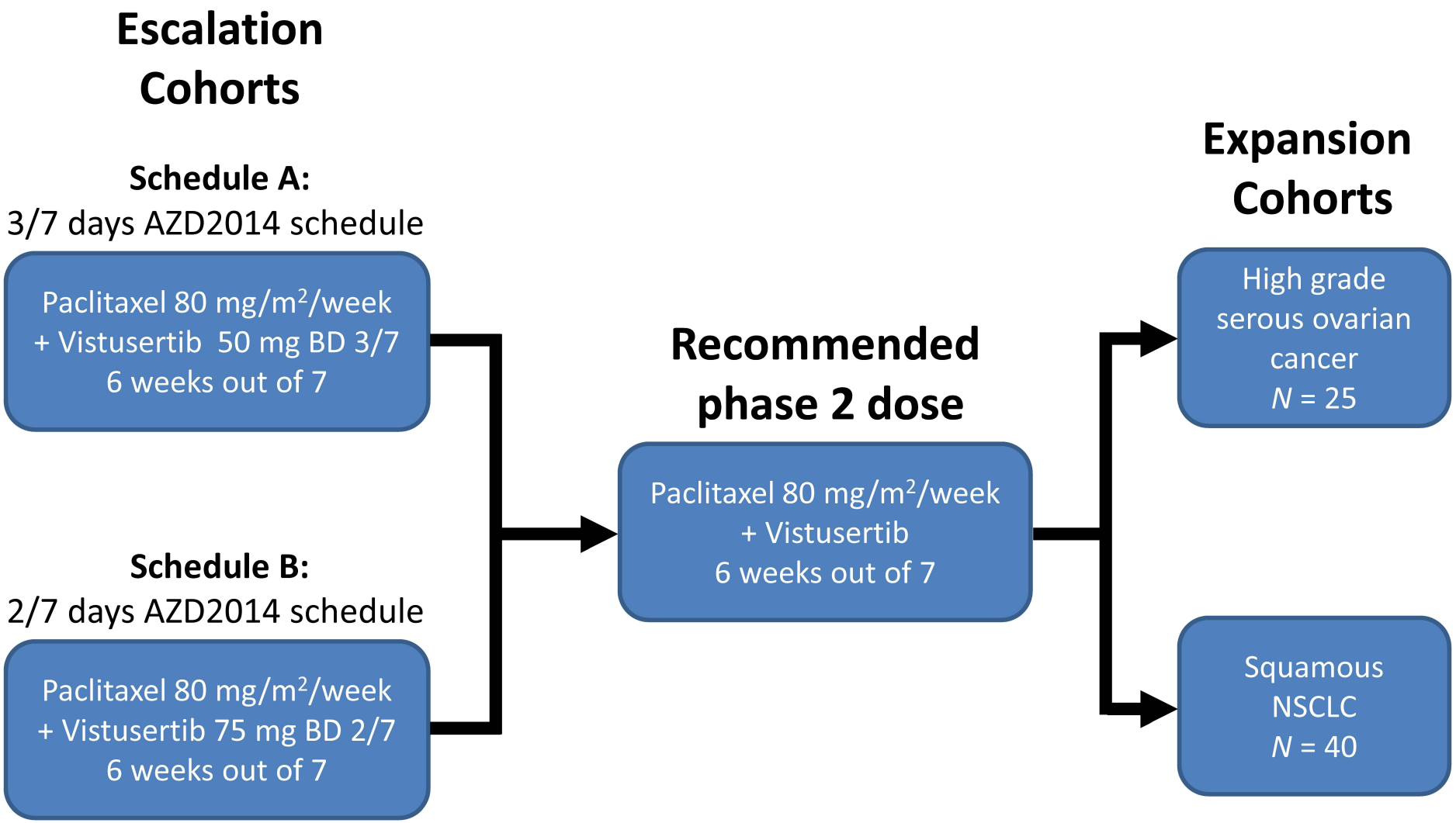
**Supplementary Table 3A. Toxicity profile of patients treated on the squamous non-small cell lung cancer expansion cohort**

Patients administered with 80 mg/m$^2$ paclitaxel with 50 mg vistusertib bd 3/7 days for 6/7 weeks, dosing starting concurrently on day 1 of each week

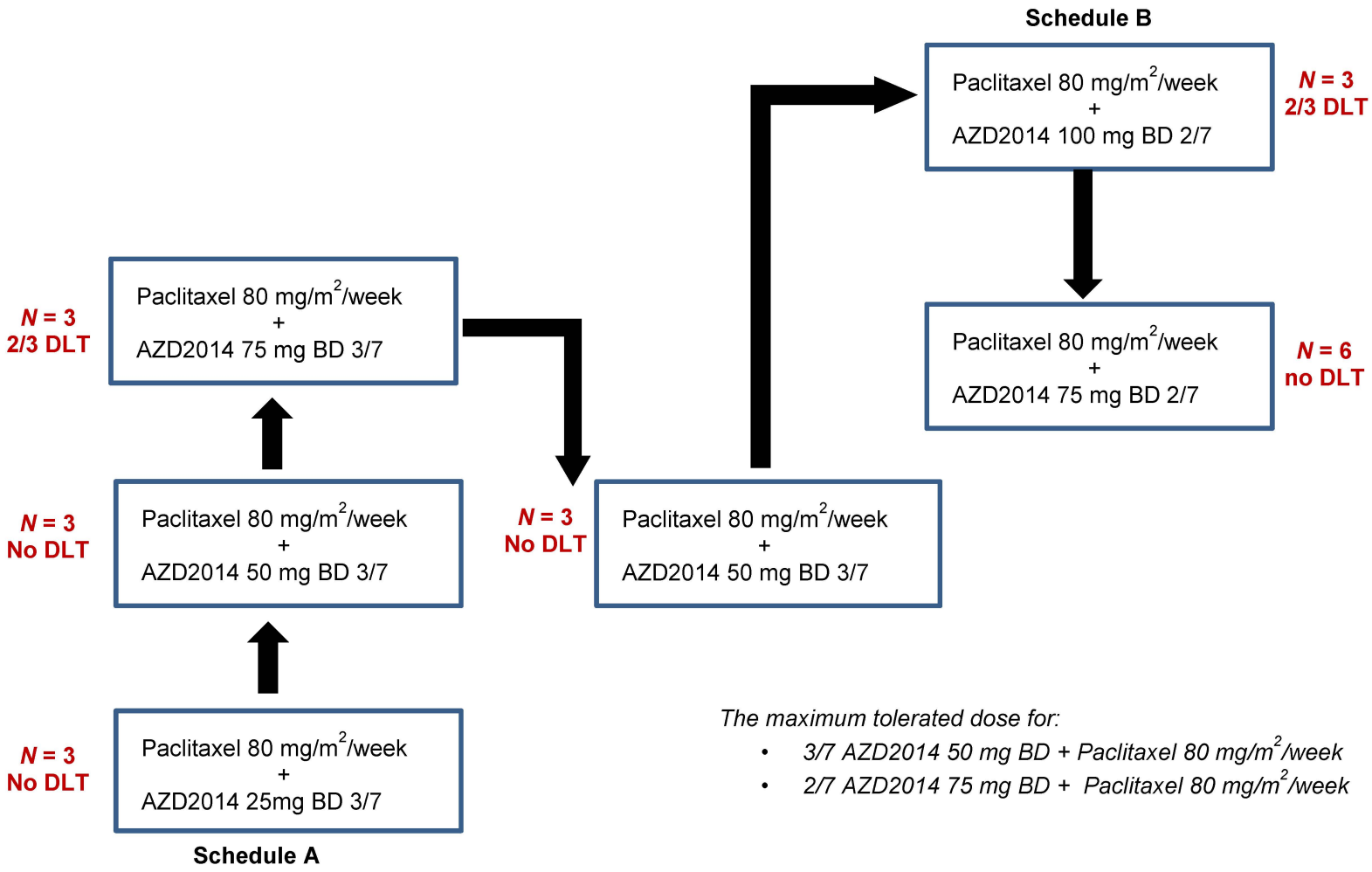
| Lung Expansion 25 mg Vistusertib (*N*=24) | | | | |
|---|---|---|---|---|
| **Adverse Event, *N* (%)** | **Gr.1 - Gr.2** | **Gr.3 - Gr.4** | **Total** | **%** |
| Anaemia | 14 | 0 | 14 | 58 |
| Fatigue | 13 | 1 | 14 | 58 |
| Alopecia | 13 | 0 | 13 | 54 |
| Neuropathy | 11 | 0 | 11 | 46 |
| Nausea | 10 | 0 | 10 | 42 |
| Bronchitis/Bronchial or Respiratory infection | 4 | 4 | 8 | 33 |
| Diarrhoea | 8 | 0 | 8 | 33 |
| Skin rash | 7 | 0 | 7 | 29 |
| Dyspepsia/Indigestion | 5 | 0 | 5 | 21 |
| Loss of appetite/Anorexia/Weight loss | 4 | 1 | 5 | 21 |
| Mucositis/Mouth ulcers/Stomatitis | 5 | 0 | 5 | 21 |
| Arthalgia/Myalgia | 4 | 0 | 4 | 17 |
| Leucopenia | 4 | 0 | 4 | 17 |
| Dry skin/Eczema | 3 | 0 | 3 | 13 |
| Dyspnoea | 3 | 0 | 3 | 13 |
| Lesion/Lump on skin | 3 | 0 | 3 | 13 |
| Nail loss/discoloration | 3 | 0 | 3 | 13 |
| Pneumonitis/Pneumonia | 3 | 0 | 3 | 13 |
| Vomiting | 3 | 0 | 3 | 13 |
| Abdominal cramps/pain | 2 | 0 | 2 | 8 |
| Cough | 2 | 0 | 2 | 8 |
| Elevated creatinine | 2 | 0 | 2 | 8 |
| Epistaxis | 2 | 0 | 2 | 8 |
| Hyperkalaemia | 2 | 0 | 2 | 8 |
| Hypophosphataemia | 1 | 1 | 2 | 8 |
| Leg oedema | 1 | 1 | 2 | 8 |
| Neutropenia | 1 | 1 | 2 | 8 |
| Tachycardia | 2 | 0 | 2 | 8 |
| Coryzal symptoms | 1 | 0 | 1 | 4 |
| Dehydration | 0 | 1 | 1 | 4 |
| Dry mouth | 1 | 0 | 1 | 4 |
| Early satiety | 1 | 0 | 1 | 4 |
| Haemoptysis | 1 | 0 | 1 | 4 |
| High magnesium | 1 | 0 | 1 | 4 |
| High urea | 1 | 0 | 1 | 4 |
| Hyponatraemia | 0 | 1 | 1 | 4 |
| Hypotensive | 0 | 1 | 1 | 4 |
| Increased glucose | 1 | 0 | 1 | 4 |
| Long nails | 1 | 0 | 1 | 4 |
| Low magnesium | 1 | 0 | 1 | 4 |
| Naval discoloration | 1 | 0 | 1 | 4 |
| Pruritis | 1 | 0 | 1 | 4 |
| Thrush | 1 | 0 | 1 | 4 |
| Wound breakdown | 1 | 0 | 1 | 4 |
| Total | 148 | 12 | 161 | |

## Supplementary Table 3B. Toxicity profile of patients treated on the squamous non-small cell lung cancer expansion cohort

Patients administered with 80 mg/m$^2$ paclitaxel with 25 mg vistusertib bd 3/7 days for 6/7 weeks, dosing starting concurrently on day 1 of each week treated at paclitaxel 80 mg/m$^2$/week and vistusertib 25 mg bd 3/7.

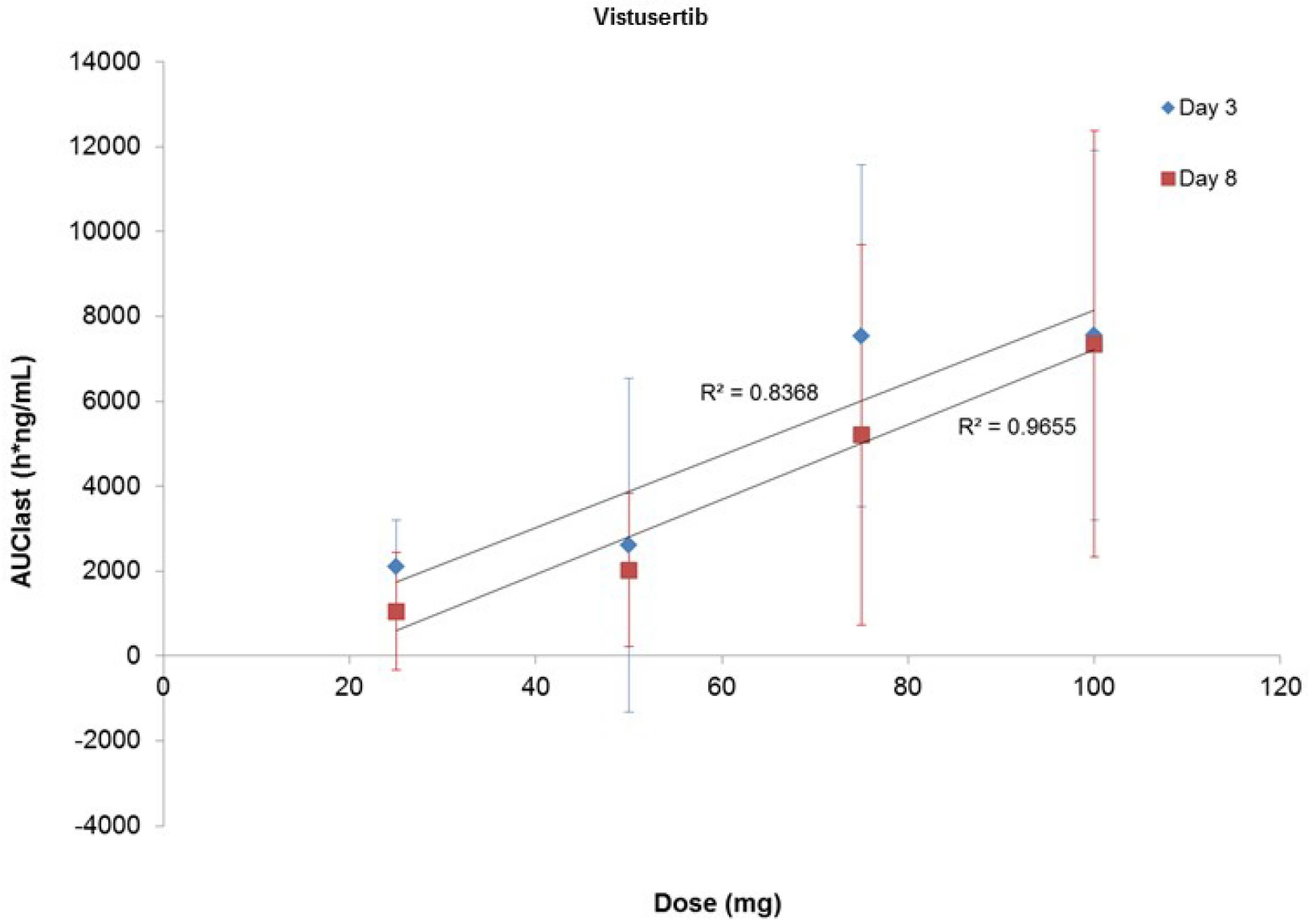



**Supplementary Figure 1. Design of the clinical trial**
The clinical trial aimed to evaluate two intermittent schedules of vistusertib in combination with a backbone of weekly paclitaxel treatment. This was followed by expansion cohorts in ovarian cancer and squamous non-small lung cancer.




**Supplementary Figure 2. Schema of dose escalation**

In dose escalation, paclitaxel (80 mg/m$^2$) was administered weekly for 6 out of 7 weeks in a 7-week cycle in combination with intermittent dosing of vistusertib. Initially, 25 mg bd vistusertib was administered weekly for three consecutive days (3/7 days), with dosing starting on the same day as paclitaxel (schedule A). Schedule A was escalated through 50 mg bd (3/7 days) and 75 mg bd (3/7 days) and, after expanding the schedule to include 6 patients, the tolerable dose was defined as 50 mg bd (3/7 days) (0/6 DLTs). Following this, schedule B was explored 100 mg bd and then 75 mg bd vistusertib was administered weekly for two consecutive days (2/7 days), with dosing starting on the same day as paclitaxel. Vistusertib 100 mg bd, 2/7 days, was not tolerated whereas 75 mg bd 2/7 was found to be tolerable with 0/6 DLTs.




**Supplementary Figure 3. Pharmacokinetics of vistusertib**
Correlation between dose administered and the area under the curve (AUC) of vistusertib on day 3 (single agent vistusertib) and day 8 (co-administration of vistusertib and paclitaxel).

## Supplementary data

### Inclusion and exclusion criteria

### Inclusion Criteria:

1. Histologically- or cytologically-proven solid tumour refractory to conventional treatment, or for which no conventional therapy exists or is declined by the patient, or where treatment with paclitaxel is an appropriate treatment option. Patients will be enrolled into two cohorts on the expansion phase. One cohort of the patients must have recurrent high grade serous ovarian cancer. Patients with clear cell ovarian are excluded. The other cohort of patients at expansion must have squamous cell lung cancer.
2. Patients who have had conventional treatment and where paclitaxel is appropriate. In instances where paclitaxel is appropriate but the patient has not already received it the patient may be enrolled after discussion between the referring oncologist and Principal Investigator.
3. Life expectancy of at least 12 weeks
4. ECOG performance status of 0-1 (Appendix 1)
5. Females should be using adequate contraceptive measures (see restrictions below), should not be breast-feeding and must have a negative pregnancy test prior to start of dosing if of child-bearing potential or must have evidence of non-child-bearing potential by fulfilling one of the following criteria at screening:

   Post-menopausal defined as aged more than 50 years and amenorrhoeic for at least 12 months following cessation of all exogenous hormonal treatments

Documentation of irreversible surgical sterilisation by hysterectomy, bilateral oophorectomy or bilateral salpingectomy but not tubal ligation

6. Male patients should be willing to use barrier contraception i.e., condoms
7. Measurable or evaluable disease. Patients enrolled in the expansion phase should have measurable disease by RECIST v1.1 criteria (Appendix 3).
8. Haematological and biochemical indices within the ranges shown below. These measurements must be performed within one week (Day -7 to Day 1) before the patient goes in the trial.

| **Laboratory Test** | **Value required** |
|---|---|
| Haemoglobin (Hb) | ≥ 9.0 g/dL |
| Absolute neutrophil count | ≥ 1.5 x $10^9$/L |
| Platelet count | ≥ 100 x $10^9$/L |
| Serum bilirubin | ≤ 1.5 x upper limit of normal (ULN) |
| Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) | ≤ 2.5 x (ULN) if no demonstrable liver metastases or ≤ 5 times ULN in the presence of liver metastases |
| Alkaline phosphatase (ALP) | < 5 x ULN |
| Creatinine clearance<br>OR<br>Serum creatinine | ≥ 50 mL/min (uncorrected value)<br><br>≤ 1.5 x ULN |
| Fasting glucose | ≤ 125 mg/dL (7 mmol/L) |
| Erythrocyte-HbA1c | ≤ 59 mmol/mol |

9. 18 years or over

10. Written (signed and dated) informed consent and be capable of co-operating with treatment and follow-up

**Exclusion criteria:**

1. Radiotherapy (except for palliative reasons), chemotherapy, endocrine therapy, or immunotherapy during the previous 3 weeks (4 weeks for investigational medicinal products and 6 weeks for nitrosoureas and Mitomycin-C) before treatment.

   N.B. Exceptions to this are patients receiving weekly Taxol as standard of care who have not had a partial or complete response after 6 to 12 weekly doses. Those patients should discontinue their weekly Taxol treatment and may be enrolled to the dose expansion phase without a wash out period.
2. Ongoing toxic manifestations of previous treatments. Exceptions to this are alopecia or certain Grade 1 toxicities, which in the opinion of the Investigator and the DDU should not exclude the patient.
3. Known leptomeningeal involvement, brain metastases or spinal cord compression
4. Known hypersensitivity (>Grade 2) to taxanes, drugs containing Cremophor, AZD2014 or structurally/chemically similar drugs
5. Unresolved bowel obstruction
6. Current refractory nausea and vomiting, chronic gastrointestinal disease, inability to swallow formulated product or previous significant bowel resection that would preclude adequate absorption of AZD2014
7. Patients with Diabetes Type I or uncontrolled Type II (HbA1c >59 mmol/mol assessed locally) as judged by the investigator
8. Major surgery within 4 weeks prior to entry to the study (excluding placement of

   vascular access), or minor surgery within 2 weeks of entry into the study and

from which the patient has not yet recovered

9. Treatment with warfarin. Patients on warfarin for DVT/PE can be converted to LMWH.

10. Exposure to potent or moderate inhibitors or inducers of CYP3A4/5 if taken within the stated washout periods before the first dose of study treatment:
    - Inhibitors (competitive): ketoconazole, itraconazole, indinavir, saquinovir, nelfinavir, atazanavir, amprenavir, fosamprenavir, troleandomycin, telithromycin, fluconazole, nefazodone, cimetidine, aprepitant, miconazole, fluvoxamine, P-glycoprotein, grapefruit juice, or Seville oragnes (1 week minimum wash-out period), amiodarone (27 week minimum wash-out period)
    - Inhibitors (time dependent): erythromycin, clarithromycin, verapamil, ritonavir, diltiazem (2 week minimum wash-out period)
    - Inducers: phenytoin, rifampicin, St. John's Wort, carbamazepine, primidone, griseofulvin, carbamazepine, barbiturates, troglitazone, pioglitazone, oxcarbazepine, nevirapine, efavirenz, rifabutin (3 week minimum wash-out period) and phenobarbitone (5 week minimum washout period)

11. Exposure to potent or moderate inhibitors or inducers of CYP2C8 if taken within the stated washout periods before the first dose of study treatment:
    - Inhibitors: Gemfibrozil, trimethoprim, glitazones, montelukast, quercetin (1 week minimum wash-out period)
    - Inducers: Rifampicin (3 week minimum wash-out period)

12. At high medical risk because of non-malignant systemic disease including active uncontrolled infection e.g. interstitial lung disease, severe hepatic impairment, uncontrolled chronic renal disease

13. Known to be serologically positive for hepatitis B, hepatitis C or human immunodeficiency virus (HIV).
14. Patients who have experienced any of the following procedures or conditions currently or in the preceding 12 months:
    - coronary artery bypass graft
    - angioplasty
    - vascular stent
    - myocardial infarction (MI)
    - uncontrolled angina pectoris
    - congestive heart failure NYHA Grade 2
    - ventricular arrhythmias requiring continuous therapy
    - supraventricular arrhythmias including AF, which are uncontrolled
    - Torsades de Pointes
    - haemorrhagic or thrombotic stroke, including transient ischaemic attacks or any
    - other central nervous system bleeding
15. Resting ECG with measurable QTc interval of >470ms msec at 2 or more time points within a 24 hour period.
16. Concomitant medications known to prolong QT interval, or with factors that increase the risk of QTc prolongation or risk of arrhythmic events (such as heart failure, hypokalaemia, congenital long QT syndrome, family history of long QT syndrome), or unexplained sudden death under 40 years of age. Inability to discontinue medication with agents designated as having a risk of Torsades de Pointes due to QT prolongation (see Appendix 5)

17. Left ventricular (LV) dysfunction (LVEF outside institutional range of normal) by MUGA or echocardiogram.
18. Current malignancies of other types, with the exception of adequately treated cone-biopsied in situ carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin. Cancer survivors, who have undergone potentially curative therapy for a prior malignancy who have no evidence of that disease currently are eligible for the trial.
19. Prior bone marrow transplant or have had extensive radiotherapy to greater than 25% of bone marrow within eight weeks of starting trial
20. Patients participating in or planning to participate in another interventional clinical trial whilst on this study. Participation in an observational trial is acceptable.
21. Any other condition which in the Investigator's opinion would not make the patient a good candidate for the clinical trial.

**Prohibited concomitant medications**

Potent and moderate inhibitors and inducers of CYP3A4/5 if taken within the stated washout periods:

*Inhibitors (competitive):* ketoconazole, itraconazole, indinavir, saquinovir, nelfinavir, atazanavir, amprenavir, fosamprenavir, troleandomycin, telithromycin, fluconazole, nefazodone, cimetidine, aprepitant, miconazole, fluvoxamine (1 week minimum wash-out period), amiodarone (27 week minimum wash-out period)

*Inhibitors (time dependent):* erythromycin, clarithromycin, verapamil, ritonavir, diltiazem (2 week minimum wash-out period)

*Inducers*: phenytoin, rifampicin, St. John's Wort, carbamazepine, dexamethasone, primidone, griseofulvin, carbamazepine, barbiturates, troglitazone, pioglitazone, oxcarbazepine, nevirapine, efavirenz, rifabutin (3 week minimum wash-out period) and phenobarbitone (5 week minimum washout period)

Potent and moderate inhibitors and inducers of CYP2C8 if taken within the stated washout periods:

*Inhibitors:* Gemfibrozil, trimethoprim, glitazones, montelukast, quercetin (1 week minimum wash-out period)

*Inducers:* Rifampicin (3 week minimum wash-out period)

## Methods

### PK assay

Outline

This method is applicable to the analysis of AZD2014 in human plasma treated with K2EDTA anticoagulant. AZD2014 and the internal standard (ISTD), 13C22H2AZ12729279 are extracted from human plasma by solid-phase extraction (SPE). After evaporation under nitrogen, the residue is reconstituted and analysed using liquid chromatography (LC) with tandem mass spectrometric detection (MS/MS). The standard curve range is from 20.0 to 20,000 ng/mL for AZD2014, using a plasma sample volume of 0.0250 mL.

Solid phase extraction

Condition the 96 Well Format SPE (Oasis HLB, 30 µm, 10 mg): Add 500 µL of methanol to the SPE cartridges and apply low pressure to let the solvent pass. Add 500 µL of 10 mM ammonium formate in water to the SPE cartridges and apply low pressure to let the solvent pass. Load the entire 500 µL of the samples onto the SPE cartridges and apply very low pressure to let samples pass. Wash the SPE cartridges with 500 µL of 10 mM ammonium formate in water [MA1] and apply low pressure to let the solvent pass. Wash the SPE cartridges with 500 µL of methanol:water:ammonium hydroxide (30:65:5, v:v:v) and apply low pressure to let the solvent pass. Dry the cartridges using maximum pressure for at least 20 seconds. Blot the tips dry on paper towelling. Place the cartridges on top of a clean pre-labelled Axygen 96-well plate. Add 300 µL of 2% formic acid in methanol to each SPE cartridge. Let the cartridges stand for 1 minute on top of the 96-well plate. Slowly elute the samples into the Axygen 96-well collection plate using minimal pressure. After

complete elution increase pressure to remove any remaining drips and dry the SPE packing material. Evaporate the extracts to dryness under a stream of nitrogen using the SPE Dry-96. Set the upper and lower gas temperature to 40°C. Set both upper and lower gas flow to approximately 50 L/min. The total evaporation time is approximately 20 minutes. Using an Eppendorf repeating pipette or Tomtec, reconstitute the samples by adding 500 µL of 10mM ammonium formate:methanol (3:2, v:v) [RC1] to each sample. Cover the 96-well plate with a dimpled sealing mat and vortex-mix at a low speed for approximately 1 minute. Perform a 20x dilution of each sample using a Tomtec. Transfer 25 µL of each sample from Step 5 to a clean pre-labeled Axygen 96-well plate. Dilute each sample by adding 475 µL of 10mM ammonium formate:methanol (3:2, v:v) [RC1]. Cover the 96-well plate with a dimpled sealing mat and vortex-mix at a low speed for approximately 1 minute. Keep sample extracts at refrigerated conditions if needed prior to injection.

Chromatographic Conditions

Column: Waters, Acquity UPLC® BEH C18, 2.1 x 50 mm, 1.7 µm particle size

Pre-filter: Waters, Acquity column inline filter

Column Temp.: 60°C

Mobile Phase: A: 10 mM ammonium formate in water B: methanol

Gradient Program: Initial Conditions: 0.600 mL/min; 50 % B

| Time (minutes) | Module | Function Value | (%) |
|---|---|---|---|
| 0.30 | Pumps | Pump B Conc. | 50 |
| 2.00 | Pumps | Pump B Conc. | 75 |

| | | | | |
|---|---|---|---|---|
| 2.10 | Pumps | Pump B Conc. | 98 | |
| 2.50 | Pumps | Pump B Conc. | | 98 |
| 2.60 | Pumps | Pump B Conc. | 50 | |
| 3.50 | System Controller | Stop | | |

Flow Rate: 0.600 mL/min

Back Pressure: 410 Bar (Typical)

Sample Tray Temp: Refrigerated temperature (2 to 8°C)

Injection Volume: 5-10 µL (Typical); Not to exceed 20.0 µL

Rinse Port Injector

Wash Solution: 10mM ammonium formate:methanol (3:2, v:v)

Rinse Pump Injector

Wash Solution: Methanol: DMSO (4:1, v:v)

Needle Stroke: 47 mm

Rinse Pump Setting: Rinse pump → Rinse port

Rinse Volume: 500 µL

Rinse Mode: Before and After Sampling

Rinse Dip Time: 3 seconds

Rinse Time: 1 second

Acquisition Time: Approximately 3.5 minutes

Cycle Time: Approximately 4.0 minutes (injection start to next injection start)

Mass Spectrometer Parameters

Mass Spectrometer: Sciex API 4000

Ionization: Positive Ion Electrospray (ESI+)

Mode: MRM

IonSpray Voltage: 4500V

TurboIon Spray Temp: 600°C

Curtain Gas Type: Nitrogen Setting: 30

CAD Gas Type: Nitrogen Setting: 6

Nebulizing Gas (Gas1) Type: Nitrogen Setting: 50

Auxiliary Gas (Gas 2) Type: Nitrogen Setting: 70

Needle Position: Y = 5 mm

X = 5 mm

**Pharmacodynamic biomarker analysis: peripheral blood mononuclear cells (PBMCS) and platelet rich plasma (PRP)**

Peripheral blood mononuclear cells (PBMCs) fixed with 4% formaldehyde were incubated for ten minutes with 4% fetal calf serum (FCS), 0.1% Triton X-100, PBS as a blocking agent followed by staining with excess anti-phospho 4EBP1 (Thr37/46) (236B4) Rabbit monoclonal antibody Alexa 488 conjugate (2846, Cell Signaling Technology, Massachusetts, USA). Stained samples were washed once in 4% FCS, 0.1% Triton X-100, PBS and once in 0.1% Triton X-100, PBS. Stained samples were suspended in an appropriate volume of 0.1% Triton X-100, PBS and analysed using a FACSCantoII. A rabbit IgG isotype control (4340, Cell Signaling Technology, Massachusetts, USA) was used to set the negative population and assess the percentage of monocytes positive for phosphorylated 4EBP1. During validation the inter-assay precision, ranged from 2.8 to 3.3%.

pAkt was assessed in platelet rich plasma (PRP) using a sandwich immunoassay as per manufacturer's instructions (K11100D, Meso Scale Discovery, Maryland, USA) which consisted of a multiplex assay with plates pre-coated with phospho(Ser473) Akt and total Akt capture antibodies. These plates were blocked for 1 hour, incubated with sample for 1 hour, and finally incubated with an anti-total Akt detection antibody conjugated to MSD-SULFO-TAG for 1 hour before being detected on a Meso Scale Discovery Sector Imager 6000 using electrochemiluminescence in order to determine the percentage of Akt phosphorylated. During validation the inter-assay precision ranged from 6.75 to 7.17%.

Inter-assay precision for both methods was determined as the co-efficient of variance (CV) of 3 independent measurements across 3 days.

## Genomic Sequencing

DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) tumour blocks using the FFPE Tissue DNA kit (Qiagen). DNA was quantified with the Quant-iT high-sensitivity PicoGreen double-stranded DNA Assay Kit (Invitrogen). Sequencing libraries were constructed from 40 ng of DNA using a customized Generead DNAseq Mix-n-Match v2 panel (Qiagen) covering 4841 amplicons (310,077 bp) across 67 genes. Libraries were run using the MiSeq Sequencer (Illumina) at a mean of 650X (tissue) and 1018X (cfDNA). FASTQ files were generated using the Illumina MiSeq Reporter v2.5.1.3. Sequence alignment and mutation calling were performed using BWA tools and the GATK variant annotator by the Qiagen GeneRead Targeted Exon Enrichment Panel Data Analysis Web Portal. In addition, circulating free DNA (cfDNA) when collected at baseline, at the end of cycle 1 and, where possible, at progression, was extracted from 4 - 8 mL of plasma using the QIAsymphony (Qiagen) and the Circulating DNA Kit (Qiagen) and quantified by Quant-iT High Sensitivity Picogreen Kit (Invitrogen).