

Title: A Randomised, Multi-centre Phase II Trial of Weekly Paclitaxel and Vistusertib in Platinum-Resistant Ovarian High-Grade Serous Carcinoma: OCTOPUS Arm 1

Authors

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38 **Key Points**

39 **Question:** Does the addition of the dual mTORC inhibitor vistusertib (V) add benefit to weekly paclitaxel
40 (wP) in platinum resistant ovarian high grade serous carcinoma (PR-HGSC)?

41 **Findings:** In this randomised phase II study, V did not increase progression free survival (PFS), overall
42 survival or response rate when added to wP in patients with PR-HGSC.

43 **Meaning:** The addition of vistusertib to paclitaxel did not improve clinical outcomes in PR-HGSC.

44

45 **Abstract**

46 **Importance:** Patients with platinum-resistant or refractory ovarian high grade serous carcinoma (PR-HGSC)
47 have a poor prognosis and few therapeutic options. Preclinical studies support targeting PI3K/AKT/mTOR
48 signalling in this setting and a phase I study of the dual mTORC1/mTORC2 inhibitor vistusertib with weekly
49 paclitaxel (wP) showed activity.

50 **Objective:** To evaluate whether the addition of vistusertib to weekly paclitaxel improves clinical outcomes
51 in PR-HGSC.

52 **Design and Setting:** A randomised phase II, double-blind, placebo-controlled multi-centre study that
53 recruited patients from UK cancer centres between January 2016 and March 2018.

54 **Participants:** Patients with PR-HGSC of ovarian, fallopian tube or primary peritoneal origin and with
55 measurable or evaluable disease (RECISTv.1.1 and/or GCIG CA125 criteria) were eligible. There were no
56 restrictions on number of lines of prior therapy.

57 **Intervention:** Patients were randomised (1:1) to weekly paclitaxel (wP) (80mg/m² D1, 8, 15 of 28 day cycle)
58 plus oral vistusertib (V) (50mg BD) or placebo (P).

59 **Main Outcome(s) and Measure(s):** The primary endpoint was progression-free survival (PFS) in the
60 intention-to-treat (ITT) population. Secondary endpoints included Response Rate (RR), Overall Survival
61 (OS) and Quality of Life (QoL).

62 **Results:** 140 patients (median age 63 range: 36-86; 18% platinum-refractory; 54% ≥3 prior therapies) were
63 randomised. There was no difference in PFS (median 4.5 vs 4.1m (HR 0.84; 80% CI (0.67, 1.07); 1-sided
64 p=0.18), OS (median 9.7 vs 11.1m (HR 1.21; 80% CI (0.91, 1.60)) or RR (odds ratio 0.86; 80% CI (0.55,
65 1.36)). Grade 3/4 adverse events were 41.2% (wP+V) vs 36.7% (wP+P) and there was no difference in QoL.

66 **Conclusions and Relevance:** We report the results of the first randomised trial of weekly paclitaxel and dual
67 mTORC1/2 inhibition in PR-HGSC. V did not improve clinical activity of wP.

68

69 Trial Registration: ISRCTN16426935.

70

71 **Introduction**

72 Despite improvements in first line treatment for advanced ovarian cancer, approximately 85% of patients
73 recur and eventually develop fatal chemotherapy resistance. For patients relapse during ('platinum-
74 refractory') or within six months of completing ('platinum-resistant') prior platinum-based therapy, treatment
75 options are limited. Weekly paclitaxel has activity in platinum-resistant ovarian cancer, with objective
76 response rates of approximately 30%. However, the median PFS is generally 3-4 months and overall survival
77 approximately 12 months¹.

78

79 One potential mechanism of resistance to both platinum and taxane chemotherapy is activation of the
80 PI3K/AKT/mTOR signalling pathway²⁻⁴. Ovarian high grade serous carcinoma (HGSC) sensitivity to
81 paclitaxel may reflect PI3K pathway activity⁵, potentially driven by *MYC* amplification⁶. However, response
82 rates in single arm studies of first generation mTOR inhibitors were poor⁷, which prompted the development
83 of dual mTORC1/2 inhibitors, such as vistusertib. Following encouraging pre-clinical data in platinum-
84 resistant ovarian cancer xenografts⁵, a phase I study of weekly paclitaxel with vistusertib was conducted.
85 ORR was 52% (13/25) and median PFS 5.8 months (95% CI: 3.3–18.5) in the HGSC cohort⁸. Here, we
86 evaluated the clinical efficacy of the vistusertib/paclitaxel combination in OCTOPUS - a multi-centre,
87 randomised, placebo-controlled, phase II trial in platinum-resistant ovarian HGSC (PR-HGSC).

88 **Methods**

89 *Study design*

90 OCTOPUS was a phase II, randomised, double-blind, placebo-controlled, multicentre study evaluating the
91 efficacy and safety of vistusertib in combination with paclitaxel compared to paclitaxel alone in patients with
92 PR-HGSC. The study was conducted in accordance with the Research Governance Framework for Health
93 and Community Care (Second edition; 2006) and the Medicines for Human Use (Clinical Trials)
94 Regulations, 2004 SI 2004:1031 and Declaration of Helsinki Principles and was co-sponsored by University
95 of Glasgow and NHS Greater Glasgow and Clyde. Ethical approval was obtained from London–Brighton
96 and Sussex Research Ethics Committee (reference 15/LO/1302) and all patients provided written informed
97 consent.

98 *Patient population*

100 Patients (≥ 18 years) with histologically confirmed HGSC of ovarian, fallopian tube or primary peritoneal
101 origin with relapse in the platinum-resistant or refractory timeframe, were enrolled. Platinum
102 resistant/refractory status was defined as either radiological progression (based on RECISTv.1.1) or CA125
103 rise (according to GCIG CA125 criteria) plus symptoms indicative of progression either during (platinum-
104 refractory) or within six months of completing (platinum-resistant) prior platinum therapy. Measurable or
105 evaluable disease according to RECISTv.1.1 and/or GCIG CA125 criteria was required⁹. Patients who
106 received weekly paclitaxel in combination with platinum during first line treatment were eligible if the
107 interval since the last dose of weekly paclitaxel was >6 months at the time of randomisation. Patients who
108 received prior weekly paclitaxel for platinum-resistant/refractory disease were excluded. A biopsy was
109 mandatory at study entry if deemed technically feasible. There were no restrictions on number of lines of
110 prior therapy and the most recent chemotherapy did not have to be platinum-based. See supplementary
111 methods for full inclusion/exclusion criteria.

112 *Study Design, Treatment and Conduct*

113 140 participants were randomised (1:1) to receive:

- 115 • wP+V: paclitaxel 80mg/m² IV D1, 8, 15 plus vistusertib 50mg bd days 1-3, 8-10 and 15-17 of a 28
116 day cycle (experimental arm)
- 117 • wP+P: paclitaxel 80mg/m² IV D1, 8, 15 plus placebo bd days 1-3, 8-10 and 15-17 of a 28 day cycle
118 (control arm)

119
120 After informed consent and eligibility confirmation, participants were allocated to treatment using
121 minimisation with a random element, and the following stratification factors: treatment centre, measurable
122 disease (yes vs. no), platinum refractory vs. resistant, and taxane-free interval (<6 months vs. ≥ 6 months vs.
123 no prior taxane). The inclusion of taxane-free interval as a stratification factor was based upon our previous
124 randomised phase II trial of weekly paclitaxel in this patient population¹.

125

126 Further details of study conduct are detailed in the Supplementary methods.

127

128 *Assessments*

129 Imaging assessments (CT chest, abdomen and pelvis or MRI) were performed at baseline and then every 8
130 weeks until progression. CA125 was measured every 4 weeks. Post progression follow-up was performed
131 every 3 months for overall survival (OS). Adverse events were graded according to National Cancer Institute
132 Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.3. Quality of life (QoL) was assessed
133 using EQ-5D at baseline, prior to each cycle of treatment, end of treatment and follow up.

134

135 *Statistical Considerations*

136 The primary end point was progression-free survival (PFS) in the intention-to-treat (ITT) population, defined
137 as the time from randomisation until the first appearance of confirmed progressive disease as defined by
138 combined RECIST v1.1/GCIG CA125 criteria⁹ or death from any cause. Patients still alive and without
139 progression at the time of analysis were censored at the date last known to be alive and progression-free.
140 Secondary endpoints were Response Rate (RR) (best recorded response according to combined RECISTv1.1
141 and GCIG CA125 criteria), OS, defined as the time from randomisation until death from any cause, safety
142 and tolerability (according to NCI CTCAE Version 4) and Quality of Life as measured by EQ-5D.

143

144 The trial followed a three-outcome design¹⁰: a PFS difference in favour of wP+V that was statistically
145 significant at 10% was a clear signal that a subsequent phase III study is warranted. A result statistically
146 significant at the 20% level (but not 10%) would require supportive data with improved response rate before
147 a subsequent phase III would be considered.

148

149 A total of 122 PFS events were required to detect a 50% improvement in median PFS from 3.7 months with
150 wP+P to 5.55 months with wP+V with 90% power, at the 20% one-sided level of statistical significance (or
151 equivalently with 80% power at the 10% level of statistical significance). This required 140 patients (70
152 patients per arm) recruited over 16.5 months with eight months subsequent follow-up. This incorporated an
153 interim analysis for futility, after 40 PFS events had been observed, using a Lan-DeMets spending function¹¹
154 and Pocock type boundary¹².

155

156 All analyses were pre-planned, unless specified. Efficacy analyses were undertaken on the ITT population,
157 including all patients randomised into the study. Safety and tolerability analyses were undertaken on the
158 safety population, defined as participant who received at least one dose of study treatment. The Kaplan-
159 Meier method was used to calculate both progression-free and overall survival. Results were compared using
160 the log-rank test, adjusted for stratification factors, and hazard ratios were estimated using the adjusted Cox
161 model. All statistical analyses were performed using SPSS (v27), whilst data were visualised using Prism
162 (v.9.4.1, GraphPad, CA).

163

164 QoL was analysed using mixed effects and repeated measures models, adjusting for minimisation factors, as
165 well as time-point and interaction terms (a change from the SAP). Both complete case analyses (CC;
166 ignoring missing data), and analyses accounting for missing values using multiple imputation (MI) with 40
167 imputations were undertaken¹³.

168

169 **Translational analyses**

170 Assessment of PI3-kinase pathway activity as a predictive biomarker of vistusertib efficacy was a pre-
171 planned translational endpoint, although the methods for assessing pathway activity were not pre-specified.
172 PTEN immunohistochemistry (IHC) was quantified using Histo-score (H-score) derived using QuPath. We
173 calculated the median tumour and non-tumour H-Score for each sample and defined a sample as PTEN high
174 if median tumour H-score $\geq$ corresponding median non-tumour H-score as previously¹⁴; conversely, a sample
175 was classified as PTEN low when median tumour H-score $<$ corresponding median non-tumour H-score. We
176 also used DNA ploidy, somatic copy number and copy number signatures as correlates of PI3-kinase
177 pathway activation. Full details of the translational samples and analysis are given in Supplementary
178 Methods.

179 **Results**

180 *Patient Demographics*

181 One hundred and forty patients were randomised (n=70 vistusertib, n=70 placebo) from 20 UK sites between
182 Jan-2016 and Mar-2018 and constitute the ITT population (Figure 1). Baseline characteristics are
183 summarised in Table 1. Seventy-five (53.6%) patients had received ≥ 3 prior treatment lines and 25 (17.9%)
184 were platinum-refractory. Approximately 20% (29 patients) had received prior weekly paclitaxel in
185 combination with carboplatin.

187 *Efficacy*

188 Median PFS was 4.5 vs 4.1 months, and the adjusted hazard ratio (HR) was 0.84 with an 80% CI of 0.67,
189 1.07 (one-sided P=0.18) for the wP+V arm relative to wP+P arm (Figure 2A). Median OS was 9.7 vs 11.1
190 months (adjusted HR 1.21; 80% CI (0.91, 1.60)) (Figure 2B). Testing of the proportional hazards assumption
191 confirmed that the above HR from the Cox proportional hazards model are appropriate. Median follow up for
192 PFS and OS across the whole population was 13.5 and 32.1 months, respectively.

194 RECISTv1.1 response rates were 29% vs 30% and GCIG combined RECISTv1.1/CA125 criteria response
195 rates were 53% and 54% for wP+V and wP+P, respectively. There was no difference in response rate by
196 combined GCIG RECIST1.1/CA125 criteria (adjusted odds ratio 0.86; 80% CI (0.55, 1.36)). Although PFS
197 was significant at the 20% level, there was no improvement in response rate and therefore the primary
198 endpoint was not met as per statistical considerations. There was no difference in PFS or OS according to
199 measurable disease status, platinum status (refractory/resistant) or taxane-free interval (Figure S1).

201 *Tolerability, treatment delivery and quality of life*

202 The safety population comprised 136 participants (n=68 in each arm). Table S1 summarises adverse events
203 that occurred during combination therapy at grade 2 or above in $>10\%$ of patients in either arm or that are
204 different significantly between the arms. Grade 3/4 adverse events were reported in 41.2% (wP+V) vs 36.7%
205 (wP+P). The most frequent grade 3/4 toxicities were lymphopenia (13% wP+V vs 9% wP+P) and fatigue
206 (9% vs 4%). There was significantly more gastro-oesophageal reflux (grade 1/2 10% v 0%), rash (grade 2/3
207 9% v 0%) and lymphopenia (grade 2/3/4 47 v 31%) with wP+V compared to wP+P. There were no grade 4
208 or 5 events.

210 Dose intensity of weekly paclitaxel was similar between the wP+V and wP+P arms (Table S2). Dose
211 reductions, missed doses (Table S2) and dose discontinuation due to toxicity (5% vs 0%) were greater for
212 vistusertib than placebo.

214 One hundred and twenty-six patients were included in the QoL analysis. Overall QoL completion at time-
215 points used in the analysis (up to and including week 28) was 79.3%. There were no statistically significant

216 differences (at either 10% or 20% level) between the two treatment arms for EQ5D index or EQ5D VAS
217 ($p>0.25$ in all analyses), (see Figure S2).

218

219 *Biomarker Analysis*

220 Digital quantification of IHC PTEN status showed a high correlation between QuPath and pathologist scores
221 ($r=0.94$, $p<0.0001$ for tumour: $r=0.70$, $p=0.009$ for non-tumour – Figure S3, S4). Expression of PTEN was
222 more variable in tumour (H-score range 23-258) than non-tumour cells (range 44-177). Using previously
223 described criteria, 13 (19.1%) cases were defined as PTEN low and 55 (80.9%) as PTEN high. There was a
224 significant interaction between the randomisation arm and PTEN status ($p=0.015$) for PFS. Patients with
225 PTEN-low tumours showed a longer PFS compared with PTEN-high patients in the experimental arm
226 (median 9.4 (95%CI 2.8-16) vs 4.1 (95%CI 1.1-7) months, respectively) but not in the control arm (4.8
227 (95%CI 2.5-7.0) vs 4.2 (95%CI 2.5-5.9) months, respectively) (Figure 3A). No difference in OS was
228 recorded, although there was a trend toward a longer OS for PTEN-low patients in the experimental arm.

229

230 We used shallow WGS to analyse genome-wide absolute CN in 78 samples from 66 patients (with 12
231 matched diagnosis/study-entry pairs). There were no differences in ploidy, rates of focal somatic CN
232 alterations or CN signature exposure¹⁵ between diagnosis and study-entry (Figure S5), allowing us to
233 combine diagnosis and study-entry samples as a single cohort for survival analyses. No CN signature had a
234 statistically significant predictive value although a trend toward an interaction between the treatment and
235 exposure to normalized signature 4 ($p=0.071$) was recorded for PFS. Therefore, we divided our cohort in two
236 groups according to a pre-defined cut-off, the mean normalized signature 4 exposure. Forty (60.6%) patients
237 had high exposure (defined as $\geq$ mean) and 26 (39.4%) low (defined as $<$ mean). High exposure to normalized
238 signature 4 was associated with a worse outcome in the control arm with a PFS of 2.3 (95%CI 0.2-4.4) vs 4.6
239 (95%CI 3.1 vs 6.2) months respectively, but a numerically longer PFS in the experimental arm (Figure 3B).
240 No difference in OS was observed.

241 Discussion

242 OCTOPUS is the first randomised trial to evaluate whether adding a dual mTORC inhibitor to weekly
243 paclitaxel might improve PFS and OS in PR-HGSC. It is also the first platinum-resistant/refractory study
244 exclusively in the HGSC histological subtype and to permit prior weekly paclitaxel treatment. Although the
245 safety profile was manageable, the study failed to reach its primary endpoint. The observed outcomes in the
246 control arm (ORR 30%, median PFS 4.4 months; median OS 11.1 months) were in keeping with previous
247 studies of weekly paclitaxel in this patient population¹ and there was a small improvement in PFS (HR=0.84)
248 in the experimental arm not accompanied by improved response rate or OS.

250 Recurrent ovarian cancer that is resistant or refractory to platinum-based chemotherapy has a dismal
251 prognosis and there have been strikingly few positive randomised clinical trials in this patient population^{16,17}.
252 Treatment still relies upon single agent chemotherapy¹⁸ and previous attempts to improve activity of weekly
253 paclitaxel with targeted drugs have been largely negative in trials that have recruited all histological subtypes
254 of ovarian cancer^{1,19-22}. The key exception is bevacizumab¹⁸. However, the activity of the weekly
255 paclitaxel/bevacizumab combination was reported in an exploratory, sub-group analysis and use is restricted
256 to patients who are bevacizumab-naïve and have received no more than two prior lines of therapy²³⁻²⁷. This
257 study is the first arm of OCTOPUS, a novel phase II umbrella framework that aims to test the addition of
258 new target drugs to weekly paclitaxel, comparing these combinations to a rolling control arm with paclitaxel
259 alone. OCTOPUS provides a flexible and rapid platform to move promising combinations from phase I into
260 a randomised assessment with this first arm emphasizing the importance of randomization and embedding
261 biomarker research within clinical trials with a prospective collection of archival and study entry samples.

263 Abnormalities in the PI3K/AKT pathway are frequent in HGSC, most commonly due to amplifications or
264 activating mutations in *PIK3CA*, loss of *PTEN* through deletion or genomic rearrangements, and
265 amplification of *AKT1/2*^{28,29}, and may be key drivers of drug resistance³⁰. However, translating this
266 knowledge into effective therapies has proven extremely challenging³¹ and predictive biomarkers of response
267 in HGSC and other cancers remain elusive³². Assessment of single nucleotide variants in individual genes in
268 the pathway is not predictive⁸ and new data have added complexity, focusing on the importance of
269 PI3K/AKT/mTORC pathway signalling in the tumour microenvironment, including the differentiation and
270 activity of both innate and adaptive immune cell populations^{33,34}. Thus, newer biomarkers will need to assess
271 both tumour and non-tumour cells and provide readouts of overall pathway activity.

273 We utilised two potential predictive biomarkers. Digital image analysis allows automated segmentation of
274 tumour vs non-tumour cells and produces reproducible and quantitative assessment of protein expression.
275 Given the key role of PTEN in regulating PI3K/AKT/mTORC signalling and the frequency PTEN loss in
276 HGSC¹⁴, we assessed PTEN expression by IHC using validated protocols.

278 Using a pre-defined classification, our results indicate that low tumour cell PTEN expression is associated
279 with improved PFS in the experimental arm but, importantly, not in the control arm. There are conflicting
280 data on the prognostic importance of PTEN loss^{14,35} and our analysis of archival material appears to confirm
281 that PTEN loss is not simply a prognostic biomarker¹⁴. This is one of the first uses of digital pathological
282 quantification in ovarian cancer trials, but any predictive effect of PTEN loss will need prospective
283 confirmation in a separate study cohort, such as the ongoing DICE trial (weekly paclitaxel +/- TAK-228;
284 Clinicaltrials.gov NCT03648489).

285

286 HGSC is the archetypal C class tumour³⁶, marked by extensive copy number abnormalities. However, using
287 genome-wide copy number information to inform precision medicine strategies is technically challenging.
288 We recently described copy number signatures, patterns of copy number alterations in HGSC that reflect
289 underlying mutational processes¹⁵. CN signature 4, marked by high segment copy number, was significantly
290 associated with whole genome duplication and mutations in the PI3K/AKT pathway¹⁵. There is a link
291 between CN signature 4 and high ploidy as well as poor prognosis both in early and late stage HGSC³⁷. We
292 explored the putative predictive value of CN signature 4 exposure in this study, showing a numerical
293 improvement in PFS in the experimental arm and a worse prognosis in the control arm for patients with high
294 exposure.

295

296 *Strengths and limitations*

297 There are several shortcomings in this study. The first and foremost is that it failed to reach its primary
298 endpoint. Secondly, by restricting enrolment to patients with HGSC histology only, we excluded cases with
299 endometrioid histology, where PTEN loss is frequent³⁸, whilst our translational biomarker assays, although
300 pre-defined at the start of the translational studies, were not pre-planned and were used neither as
301 stratification factors nor to select patients for study entry. In addition, these analyses are based on a low
302 number of available samples. We used simple cut-offs to define PTEN high vs low and CN signature 4 high
303 vs low, an approach which is applied to other markers such as assays of homologous recombination³⁹ but is
304 pragmatic rather than biological. Indeed, protein expression and CN signature exposure are continuous
305 variables and application of cut-offs should ideally be based on biological parameters. A further challenge
306 with CN signature analysis is that the data are compositional (ie sum to 1 in each sample) and interdependent
307 meaning that any increase in one signature must, by definition, be accompanied by a decrease in at least one
308 other.

309

310 Despite these limitations, the first arm of OCTOPUS has demonstrated that a platform trial design may have
311 utility in PR-HGSC for drug screening, taking into account the low number of patients and the rapid
312 recruitment. However, the non-negligible level of uncertainty inherent in this trial design, in particular the
313 wide type I and II error allowance, means that confirmatory phase III trials will always be necessary in the
314 case of positive results. We also showed that it is possible to make study-entry biopsies mandatory in these

315 studies when feasible without compromising recruitment – 140 patients were recruited in approximately 24
316 months, with study-entry biopsies obtained in 64% of participants.

317

318 *Conclusion*

319 In summary, the first randomised trial of a dual mTORC1/2 inhibitor in ovarian cancer showed that the
320 addition of vistusertib to weekly paclitaxel is safe and achievable in PR-HGSC but does not improve PFS or
321 OS in an unselected population. Potential predictive biomarkers will need to be evaluated in separate study
322 cohorts.

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355

356

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464

Tables

Table 1. Baseline characteristics

		wP+V (n=70)	wP+P (n=70)	Total (n=140)
RECIST Measurable disease	Yes	62 (88.6%)	61 (87.1%)	123 (87.9%)
	No	8 (11.4%)	9 (12.9%)	17 (12.1%)
Platinum status	Refractory (progressed on platinum)	12 (17.1%)	13 (18.6%)	25 (17.9%)
	Resistant (progressed within 6 months of completing platinum)	58 (82.9%)	57 (81.4%)	115 (82.1%)
Lines of previous treatment	1	9 (12.9%)	4 (5.7%)	13 (9.3%)
	2	25 (35.7%)	27 (38.6%)	52 (37.1%)
	3	21 (30%)	22 (31.4%)	43 (30.7%)
	>3	15 (21.4%)	17 (24.3%)	32 (22.9%)
Taxane-free interval	<6 months	6 (8.6%)	4 (5.7%)	10 (7.1%)
	≥6 months	58 (82.9%)	64 (91.4%)	122 (87.1%)
	No Prior Taxane	6 (8.6%)	2 (2.9%)	8 (5.7%)
Age	Median (IQR)	65.0 (58–70)	61.0 (58–67)	63.0 (58–69)

Figures

Figure 1. CONSORT diagram

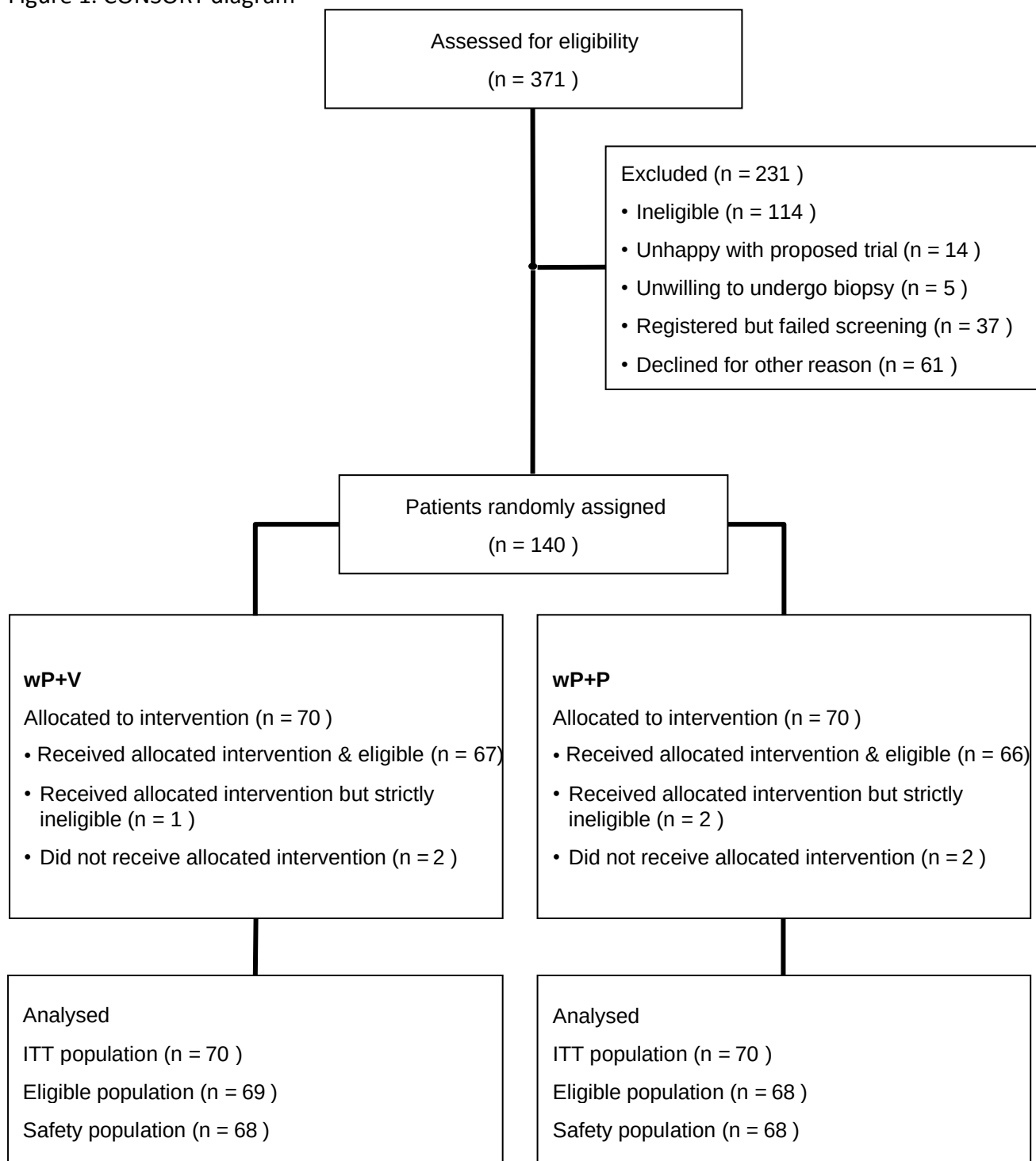
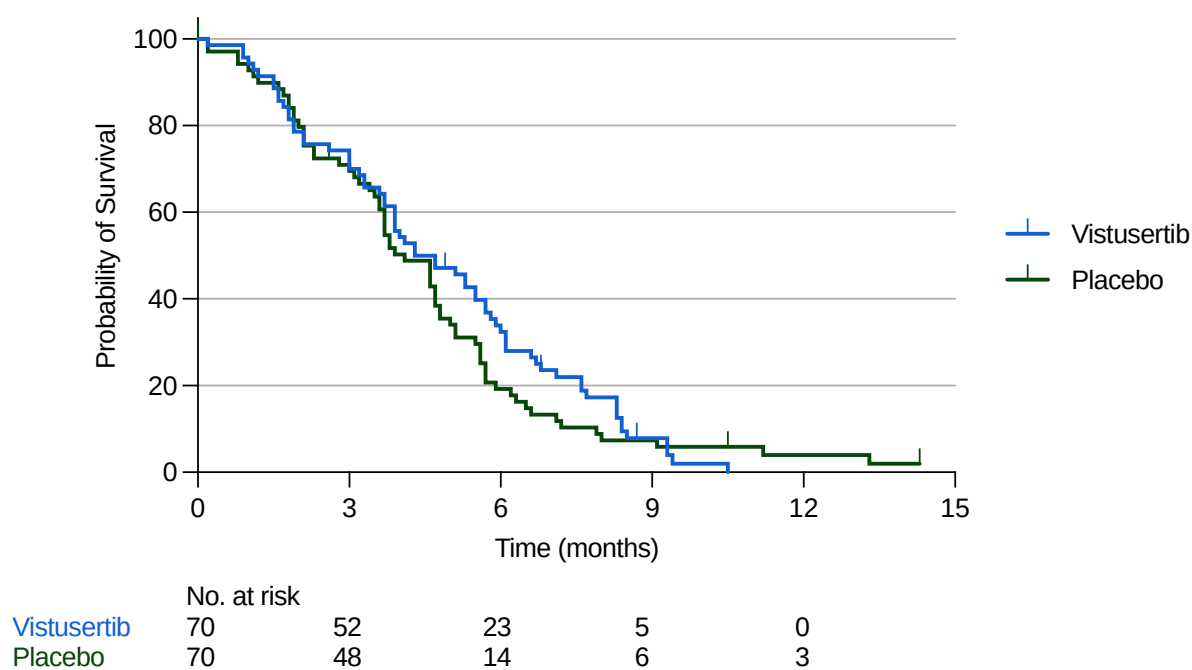


Figure 2. Progression-free survival (A) overall survival (B) for the whole patient population
A



B

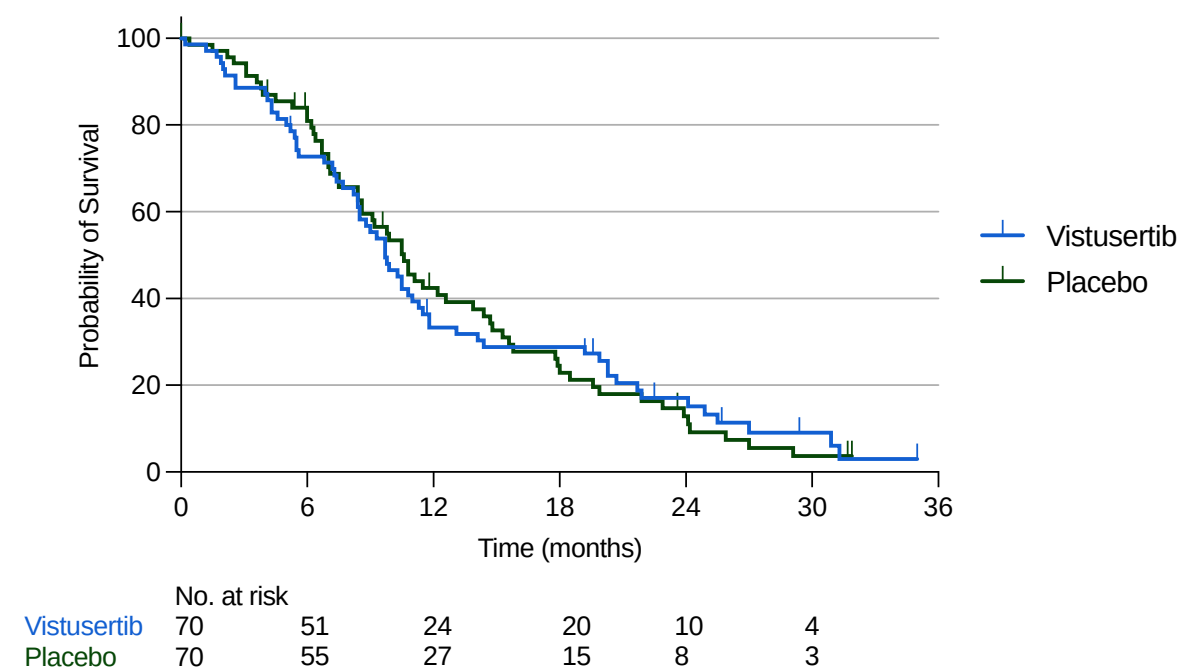
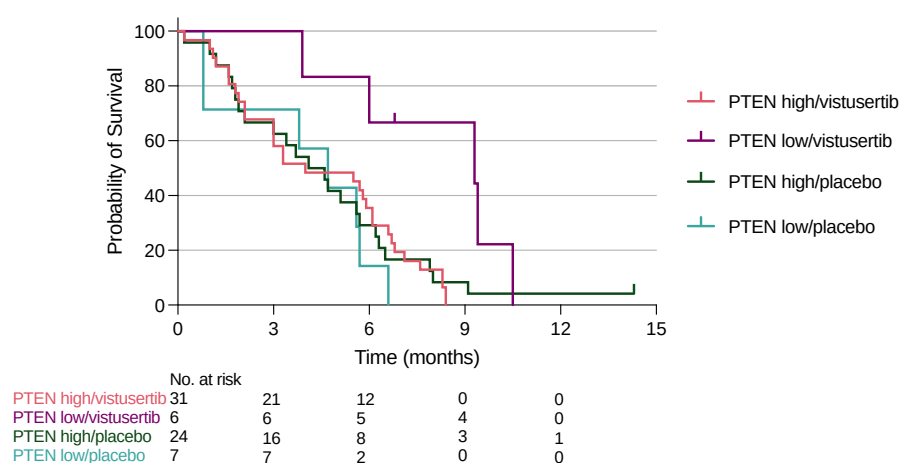


Figure 3. Progression-free survival according to PTEN IHC status (A) and Copy Number 4 (CN4) exposure (B)

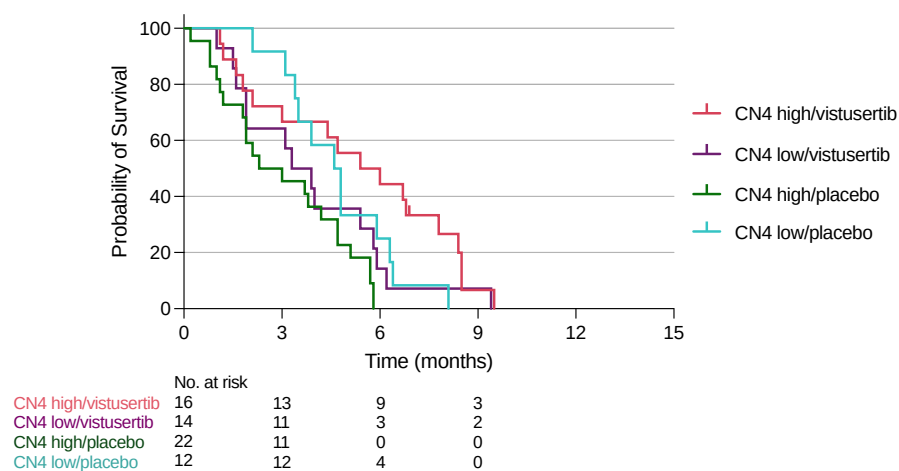
A. Control arm (paclitaxel plus placebo); median PFS 4.2m (PTEN high) vs 4.8m (PTEN low); Experimental arm (paclitaxel plus vistusertib): median PFS 4.1m (PTEN high) vs 9.4m (PTEN low);

B. Control arm (paclitaxel plus placebo); median PFS 2.3m (CN4 high) vs 4.6m (CN4 low); Experimental arm (paclitaxel plus vistusertib): median PFS 5.4m (CN4 high) vs 3.3m (PTEN low);

A



B



Supplementary materials:

Table S1. Adverse events that occurred during combination therapy at grade 2 or above in >10% of patients in either arm or that are different significantly between the arms.

Table S2. Treatment delivery of weekly paclitaxel and vistusertib/placebo.

Figure S1. Forest plot for clinical minimisation factors with p-values for interaction test.

Figure S2. EQ5D scores by time-point for vistusertib (A) and placebo (B).

Figure S3 Hierarchical pathway on QuPath for PTEN Scoring.

Figure S4. Quantitative PTEN IHC.

Figure S5. Copy number analysis.

Supplementary methods.

Table S1. Adverse events that occurred during combination therapy at grade 2 or above in >10% of patients in either arm or that are different significantly between the arms. *; statistically significant difference between arms ($p < 0.05$).

		wP+V (n=68)		wP+P (n=68)		Total (n=136)	
		N	%	N	%	N	%
Alopecia	Grade 0	26	38.2	31	45.6	57	41.9
	Grade 1	9	13.2	9	13.2	18	13.2
	Grade 2	31	45.6	26	38.2	57	41.9
	Grade 3	1	1.5	1	1.5	2	1.5
	Grade 4	1	1.5	1	1.5	2	1.5
Diarrhoea	Grade 0	37	54.4	47	69.1	84	61.8
	Grade 1	19	27.9	15	22.1	34	25.0
	Grade 2	10	14.7	5	7.4	15	11.0
	Grade 3	2	2.9	1	1.5	3	2.2
Dyspnea	Grade 0	57	83.8	64	94.1	121	89.0
	Grade 1	8	11.8	4	5.9	12	8.8
	Grade 2	3	4.4	0	0.0	3	2.2
Fatigue	Grade 0	19	27.9	21	30.9	40	29.4
	Grade 1	25	36.8	26	38.2	51	37.5
	Grade 2	18	26.5	18	26.5	36	26.5
	Grade 3	6	8.8	3	4.4	9	6.6
Gastroesophageal reflux disease*	Grade 0	61	89.7	68	100.0	129	94.9
	Grade 1	4	5.9	0	0.0	4	2.9
	Grade 2	3	4.4	0	0.0	3	2.2
Nausea	Grade 0	34	50.0	36	52.9	70	51.5
	Grade 1	22	32.4	25	36.8	47	34.6
	Grade 2	11	16.2	7	10.3	18	13.2
	Grade 3	1	1.5	0	0.0	1	0.7
Rash maculo-papular*	Grade 0	52	76.5	61	89.7	113	83.1
	Grade 1	10	14.7	7	10.3	17	12.5
	Grade 2	5	7.4	0	0.0	5	3.7
	Grade 3	1	1.5	0	0.0	1	0.7
Vomiting	Grade 0	55	80.9	53	77.9	108	79.4
	Grade 1	6	8.8	7	10.3	13	9.6
	Grade 2	7	10.3	6	8.8	13	9.6
	Grade 3	0	0.0	2	2.9	2	1.5
Haemoglobin	Grade 0/1	38	55.9	38	55.9	76	55.9
	Grade 2	29	42.6	30	44.1	59	43.4
	Grade 3	1	1.5	0	0.0	1	0.7
Lymphocytes*	Grade 0/1	36	52.9	47	69.1	83	61.0
	Grade 2	23	33.8	15	22.1	38	27.9
	Grade 3	8	11.8	6	8.8	14	10.3
	Grade 4	1	1.5	0	0.0	1	0.7
Neutrophils	Grade 0/1	51	75.0	55	80.9	106	77.9
	Grade 2	12	17.6	12	17.6	24	17.6
	Grade 3	4	5.9	1	1.5	5	3.7
	Grade 4	1	1.5	0	0.0	1	0.7
WBC	Grade 0/1	48	70.6	53	77.9	101	74.3
	Grade 2	15	22.1	15	22.1	30	22.1
	Grade 3	5	7.4	0	0.0	5	3.7
Albumin	Grade 0/1	52	76.5	53	77.9	105	77.2
	Grade 2	16	23.5	14	20.6	30	22.1
	Grade 3	0	0.0	1	1.5	1	0.7
Bilirubin	Grade 0/1	60	88.2	60	88.2	120	88.2
	Grade 2	4	5.9	2	2.9	6	4.4
	Grade 3	4	5.9	6	8.8	10	7.4
Magnesium (low)	Grade 0/1	57	83.8	59	86.8	116	85.3
	Grade 2	9	13.2	5	7.4	14	10.3
	Grade 3	2	2.9	4	5.9	6	4.4
Phosphate	Grade 0/1	50	73.5	56	82.4	106	77.9
	Grade 2	16	23.5	11	16.2	27	19.9
	Grade 3	2	2.9	1	1.5	3	2.2

Table S2. Treatment delivery of weekly paclitaxel and vistusertib/placebo.

Treatment			wP+V (n=68)	wP+P (n=68)	Total (n=136)
Paclitaxel	Dose intensity	Median (Standard deviation)	97.7 (12.1)	97.9 (13.9)	97.8 (13.0)
	Number of dose reductions	0	50 (73.5%)	54 (79.4%)	104 (76.5%)
		1	14 (20.6%)	6 (8.8%)	20 (14.7%)
		2	3 (4.4%)	4 (5.9%)	7 (5.1%)
		3	1 (1.5%)	4 (5.9%)	5 (3.7%)
	Cycle delays / omissions	0	16 (23.5%)	16 (23.5%)	32 (23.5%)
		1	20 (29.4%)	17 (25.0%)	37 (27.2%)
		2	11 (16.2%)	17 (25.0%)	28 (20.6%)
		3	8 (11.8%)	11 (16.2%)	19 (14.0%)
		4+	13 (19.1%)	7 (10.3%)	20 (14.7%)
Vistusertib / placebo	Dose intensity	Median (Standard deviation)	99.7 (14.0)	100.0 (12.4)	99.9 (13.2)
	Number of dose reductions	0	41 (60.3%)	58 (85.3%)	99 (72.8%)
		1	14 (20.6%)	6 (8.8%)	20 (14.7)
		2	9 (13.2%)	4 (5.9%)	13 (9.6%)
		3	4 (5.9%)	0 (0.0%)	4 (2.9%)
	Missed daily doses	Yes	43 (63.2%)	24 (35.3%)	67 (49.3%)

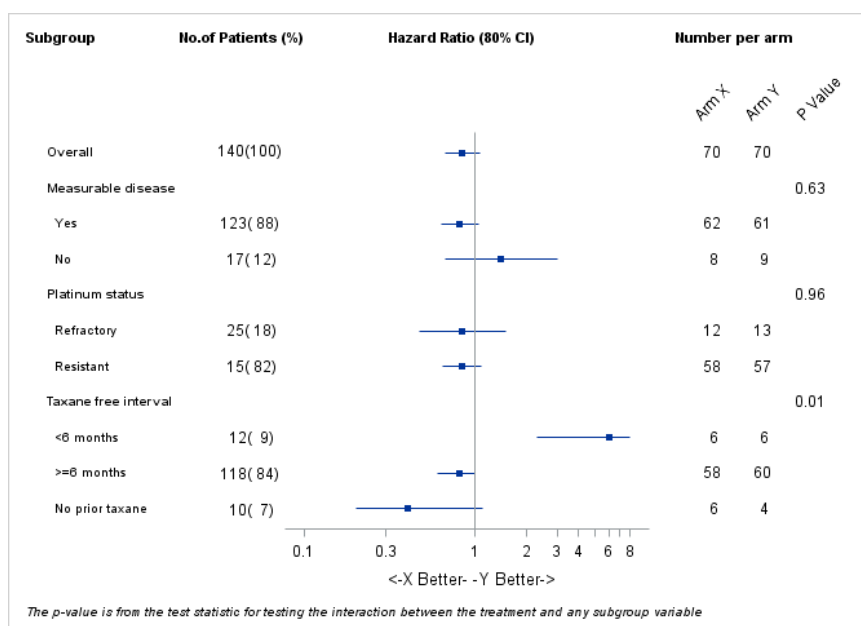
Figure S1. Forest plot for clinical minimisation factors with p-values for interaction test.

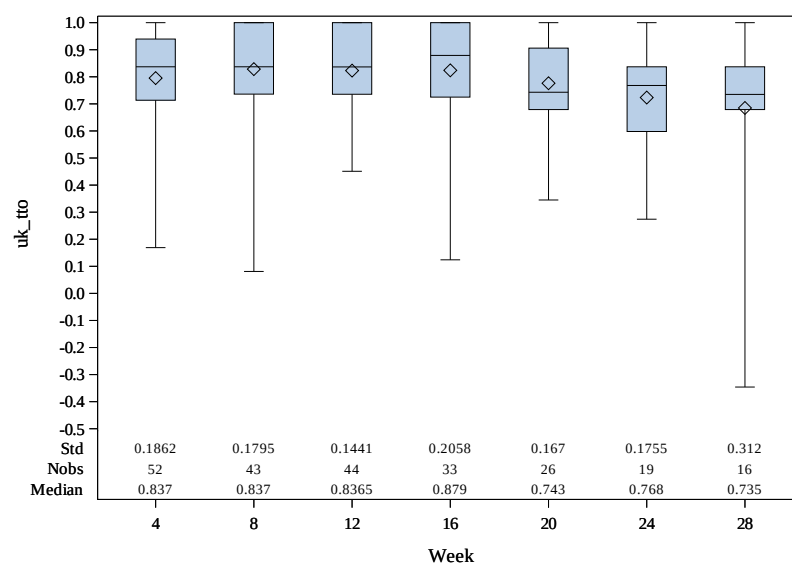
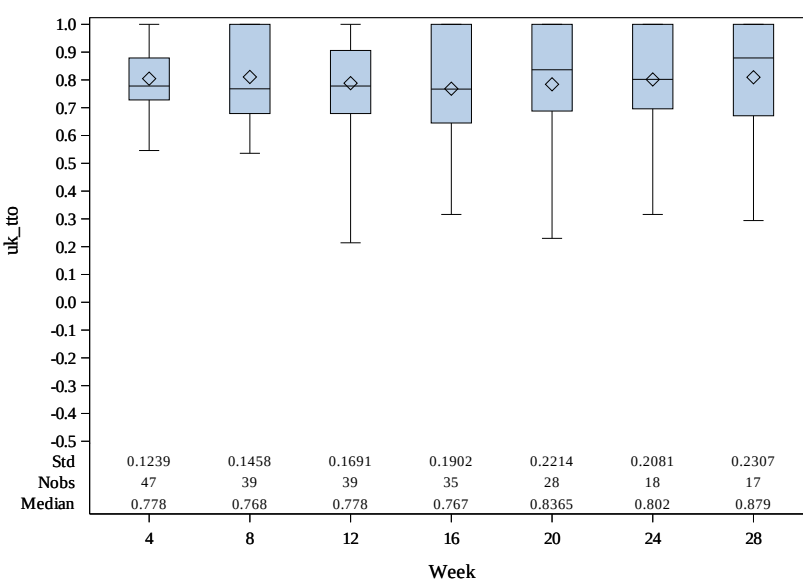
Figure S2. EQ5D scores by time-point for vistusertib (A) and placebo (B).**A****B**

Figure S3 Hierarchical pathway on QuPath for PTEN Scoring.

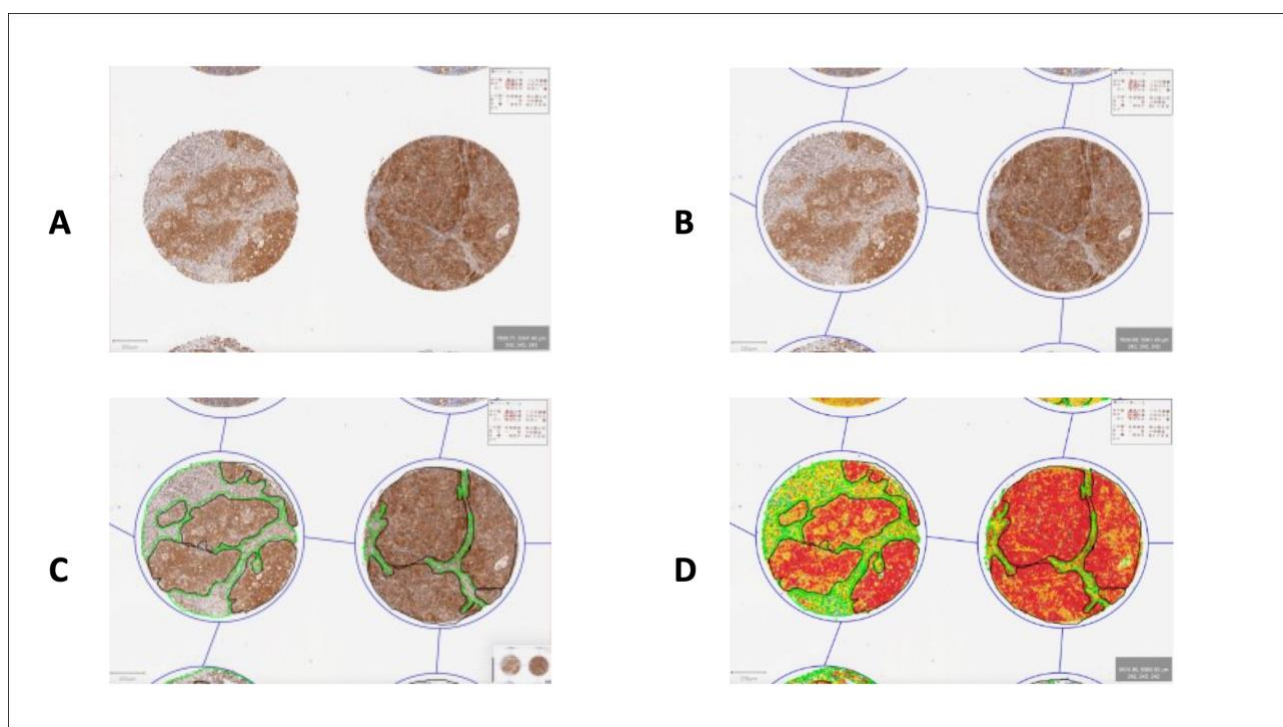


Figure S4. Quantitative PTEN IHC.

A heatmap with average H-score in tumour and non-tumour cells across the TMAs;

B correlation between QuPath and pathologist scores ($r=0.94$, $p<0.0001$ for tumour: $r=0.70$, $p=0.009$ for non-tumour).

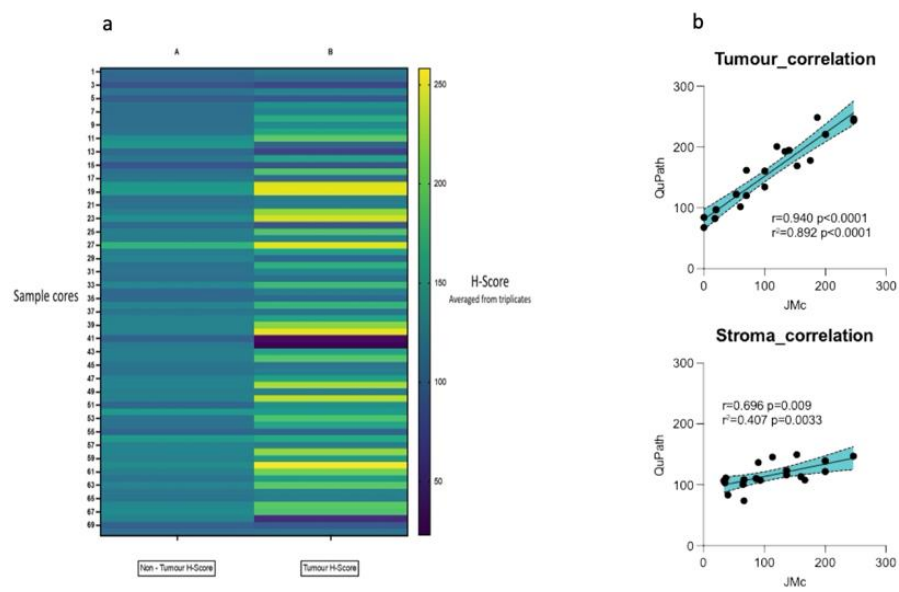
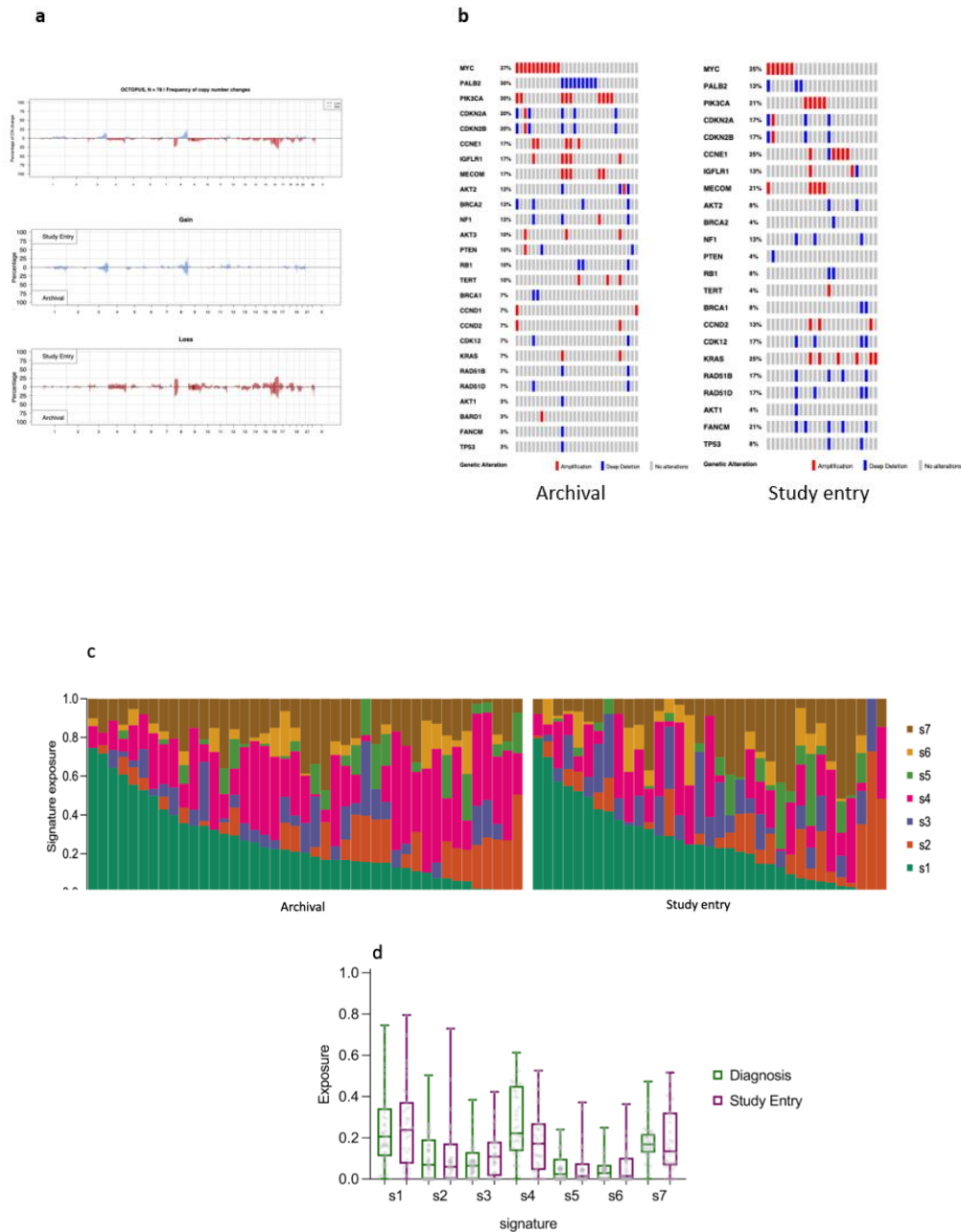


Figure S5. Copy number analysis.**A** Global gains and losses in the whole cohort in the two cohorts;**B** Focal somatic copy number alterations in 18 key genes in archival (left) and study entry biopsies (right);**C** Copy number (CN) signature exposures in archival and study entry samples (each column represents one sample);**D** CN signature exposure in study entry and archival cohorts. There was no statistical difference in CN exposures across the two groups.

Supplementary Methods

Immunohistochemistry and PTEN expression

Triplicate 1mm cores were obtained from 70 archival samples (68 patients) and a TMA was constructed and stained for PTEN (Cell Signaling #9559, 1:250) after deparaffinization and rehydration. Antigen retrieval was performed by microwave, pH 6.0 citrate buffer.

Secondary antibody was vector rabbit ABC #PK6101. Negative control was performed using vector rabbit IgG serum. The PTEN antibody was validated using PC3 and 22RV1 xenografts. PTEN expression was initially quantified by two experts in pathology. Slides were scanned (Aperio ScanScope scanner) and Aperio.svs DAB images imported into QuPath (version v0.3.0) from a secure networked server. Following TMA de-arraying, cores were adjusted to 1.2mm diameter and tissue folds removed.

Cells were detected using the haematoxylin channel using default parameters. Cell type classification was conducted using training annotations (non-tumour and tumour) to train a random forest classifier to distinguish tumour and non-tumour compartments, under the direction of pathologists. We evaluated PTEN staining both in tumour and non-tumour cells and the intensity was described as negative (0), weak (1+), moderate (2+) and strong (3+) according to these predefined DAB thresholds (negative = <0.05 ; 1+ = 0.05, 2+ = 0.15, 3+ = 0.4). Histoscore (H-score) was used as an output and calculated as follows: $[(0 \times \% \text{ negative cells}) + (1 \times \% \text{ 1+ cells}) + (2 \times \% \text{ 2+ cells}) + (3 \times \% \text{ 3+ cells})]$, with a range of 0-300. We validated our PTEN scoring by randomly assigning 20% of the cores to be scored semi-quantitatively by an experienced pathologist. The triplicates of each TMA sample were concordant in terms of tumour cellularity ($p < 0.001$ using one-way ANOVA) and there was no difference in the intensity of PTEN staining if evaluated in the whole cells, in the cytoplasm or only in the nucleus of tumour and non-tumour cells. Whole cell scores were used to assign the final H-score for both tumour and non-tumour areas. In four cases, one triplicates was not assessable while for three samples only one core each was scorable. In two cases, more than one sample per patient was available and we randomly selected one of them. We calculated median tumour and non-tumour H-Score for each sample and compared median non tumour H-score with the median tumour H-score. We pre-defined PTEN high as median

tumour H-score $\geq$ corresponding median non tumour H-score. PTEN low was pre-defined as median tumour H-score $<$ corresponding median non-tumour H-score ¹⁻³.

Quality check of samples, DNA extraction and sequencing

From each FFPE block, an H&E slide was prepared, and all samples underwent pathological review (two researchers independently performed a blinded review of each representative H&E and then all the cases were reviewed by a pathologist with expertise in Gynaecological malignancies). All samples confirmed as ovarian high grade serous carcinoma (HGSC) and with a tumour cellularity $>20\%$ were marked up and sections were cut in the Experimental Cancer Medicine Centre (ECMC)/Imperial Cancer Biomarker Resource Centre (ICBRC) laboratory.

DNA was thereafter extracted from 5–10 consecutive 10 μm sections using QIAmp DNA FFPE Tissue Kit (Qiagen, UK) according to the manufacturer's protocol. 50-200ng was sheared with a Covaris LE220 focused ultrasonicator (Covaris, Woburn, MA) to produce 100-200bp fragments. Quantity was assessed using Qbit and they were immediately frozen in an ultra-low temperature freezer with a remote alarm system. Thereafter libraries were prepared in batches.

Shallow Whole Genome Sequencing

sWGS was executed using a HiSeq4000 system (Illumina Cambridge, UK), with paired-end 150 bp protocols. The input DNA was 250 ng according to the manufacturer's instructions. Assuming a mean ploidy of 3 and 30kb bin size, we aimed to get 10 million reads per sample. This should allow detection of a CN change of 1 (tumour purity 0.6) – 2 (tumour purity 0.3) at 80% power, $\alpha=0.01$ https://gmacintyre.shinyapps.io/sWGS_power/. Five 150PE HiSeq4000 lanes allowed us to sequence 146 DNA samples. The minimum number of reads per sample was set at 5–10 million (mean coverage of 0.1 $\times$).

Absolute copy number and copy number signature calling

sWGS reads were aligned to reference human genome hg19 using the BWA-MEM ⁴. Relative copy numbers (CN) were obtained for predefined 30kb bins using a modified version of the QDNASeq package ⁵. We obtained absolute CN using ACE ⁶. For each sample, we looked at

top 10 best fits for purity and ploidy from ACE and chose the best fit that closely matched the *TP53* Variant Allele Frequency, as determined using targeted next-generation sequencing (Illumina AmpliSeq). In most cases, the ACE top fit was found to be the closest to *TP53* VAF. We defined Gain as a total copy number ≥ 5 when the average genome ploidy ≤ 2.7 or a total copy number ≥ 9 with an average genome ploidy > 2.7 . We defined Loss as an average genome ploidy ≤ 2.7 and total copy number = 0 or an average genome ploidy > 2.7 and total copy number $< (\text{average genome ploidy} - 2.7)^{7,8}$.

CN signatures were called as previously described⁹ and compared between cohorts using Kruskal Wallis test with Dunn's multiple comparisons test on pairwise analysis of signatures-by-cohort.

Statistics and analyses

CN signature data are compositional (i.e. they sum to 1 in each sample). Thus, we selected one normalizing signature to perform a COX regression analysis. Signature 5 was chosen as it had the lowest variability across our two cohorts (archival and study entry). To avoid division errors, all signature exposures equal to 0 were converted to 0.01. The normalization of the remaining signature exposures was calculated using the log ratio of their exposure to the exposure of signature 5. A p value of 0.05 or lower was considered statistically significant. Unless otherwise specified, statistical analyses were performed using IBM SPSS Statistics (v27).

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