FULL PAPER

BJC

British Journal of Cancer (2018) 118, 462–470 | doi: 10.1038/bjc.2017.428

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Keywords: copanlisib; gemcitabine; cisplatin; biliary tract cancer; phase Ib trial

Phase I dose-escalation study of copanlisib in combination with gemcitabine or cisplatin plus gemcitabine in patients with advanced cancer

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Background: Copanlisib is a pan-class I phosphatidylinositol 3-kinase (PI3K) inhibitor with predominant PI3K-α/δ activity that has demonstrated clinical activity and manageable safety when administered as monotherapy in a phase II study. Combination therapy may overcome compensatory signalling that could occur with PI3K pathway inhibition, resulting in enhanced inhibitory activity, and preclinical studies of copanlisib with gemcitabine have demonstrated potent anti-tumour activity *in vivo*.

Methods: A phase I, open-label, dose-escalation study to evaluate the safety, tolerability and recommended phase II dose (RP2D) of copanlisib with gemcitabine or with cisplatin plus gemcitabine (CisGem) in patients with advanced malignancies, including an expansion cohort in patients with biliary tract cancer (BTC) at the RP2D of copanlisib plus CisGem. Copanlisib and gemcitabine were administered on days 1, 8 and 15 of a 28-day cycle; maximum tolerated dose (MTD) and RP2D of copanlisib were determined. Copanlisib plus CisGem was administered on days 1 and 8 of a 21-day cycle; pharmacokinetics and biomarkers were assessed.

Results: Fifty patients received treatment as follows: dose-escalation cohorts, $n = 16$; copanlisib plus CisGem cohort, $n = 14$; and BTC expansion cohort, $n = 20$. Copanlisib 0.8 mg kg^{-1} plus gemcitabine was the MTD and RP2D for both combinations. Common treatment-emergent adverse events included nausea (86%), hyperglycaemia (80%) and decreased platelet count (80%). Copanlisib exposure displayed a dose-proportional increase. No differences were observed upon co-administration of CisGem. Response rates were as follows: copanlisib plus gemcitabine, 6.3% (one partial response in a patient with peritoneal carcinoma); copanlisib plus CisGem, 12% (one complete response and three partial responses all in patients with BTC (response rate 17.4% in patients with BTC)). Mutations were detected in *PIK3CA* (1 out of 43), *KRAS* (10 out of 43) and *BRAF* (2 out of 22), with phosphate and tensin homologue protein loss in 41% (12 out of 29).

Conclusions: Copanlisib plus CisGem demonstrated a manageable safety profile, favourable pharmacokinetics, and potentially promising clinical response.

Cellular metabolism, growth and differentiation are dependent on signalling through the phosphatidylinositol 3-kinase (PI3K)/α-serine/threonine-protein kinase (AKT)/mammalian target of rapamycin (mTOR) pathway, and dysregulation of this pathway has been shown to drive tumourigenesis (Yuan and Cantley, 2008; Ihle and Powis, 2009). Upregulation of the PI3K/AKT/mTOR

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Received 8 August 2017; revised 2 November 2017; accepted 7 November 2017; published online 18 January 2018

pathway attributed to mutations in *PIK3CA* has been identified in many cancer types, including biliary tract cancer (BTC) (Deshpande *et al,* 2011; Singh *et al,* 2015; Li *et al,* 2016).

Tumour suppressor gene phosphate and tensin homologue (PTEN) is involved in cell cycle regulation and is mutated in many cancers. PTEN mutations lead to activation of the PI3K/AKT pathway, and loss of PTEN function results in increased phosphatidylinositol (3,4,5)-trisphosphate levels and subsequent AKT phosphorylation and modulation of its downstream molecular oncogenic process (Wang *et al,* 2015). The PI3K/AKT/mTOR pathway is implicated in chemotherapy resistance (Lee *et al,* 2015). Overcoming this tumour survival mechanism by blocking intracellular signalling through the PI3K/AKT/mTOR pathway is a potential therapeutic target.

Copanlisib (Bayer AG, Leverkusen, Germany) is an intravenous, potent, highly selective and reversible pan-class I PI3K inhibitor with predominant activity against PI3K-δ and PI3K-α isoforms (Liu *et al,* 2013; Haike *et al,* 2014). In preclinical studies, copanlisib demonstrated anti-tumour activity in *PIK3CA*-mutated cells (Liu *et al,* 2013). The first-in-human study of copanlisib monotherapy determined the maximum tolerated dose (MTD) to be $0.8\,mg\,kg^{-1}$, with promising efficacy in patients with solid tumours and haematological malignancies (Patnaik *et al,* 2016), including clinically meaningful responses in patients with relapsed or refractory indolent or aggressive malignant lymphoma (Dreyling *et al,* 2017a, b).

Inhibition of the PI3K/AKT/mTOR pathway releases negative feedback that can result in activation of compensatory signalling pathways; therefore, combination therapy regimens have been suggested to overcome this (Lee *et al,* 2015).

Cisplatin and gemcitabine are the current standard of care in many advanced cancer types, including pancreatic, bladder and BTC (cholangiocarcinoma and gallbladder cancer). Most BTCs are advanced or metastatic at diagnosis and median overall survival is typically <1 year (Ebata *et al,* 2017); therefore, combining DNA-targeting therapies with copanlisib may be an attractive treatment strategy. In preclinical studies, gemcitabine combined with copanlisib demonstrated anti-tumour activity in a mutant BTC model in nude mice (Hägebarth *et al,* 2012).

Here we report the results of a phase I study to determine the safety, tolerability and recommended phase II dose (RP2D) of copanlisib in combination with gemcitabine or with cisplatin plus gemcitabine (CisGem) in patients with advanced solid malignancies, including BTC (www.clinicaltrials.gov, Identifier: NCT01460537).

METHODS

Study design. This was a phase I, multicentre, open-label, non-randomised, dose-escalation study. Patients were initially enrolled for treatment with copanlisib plus gemcitabine alone to evaluate safety and tolerability. Intravenous gemcitabine ($1000\,mg\,m^{-2}$) and copanlisib (1 h) at a starting dose of $0.6\,mg\,kg^{-1}$ were administered on days 1, 8 and 15 of a 28-day treatment cycle. Copanlisib dose escalation was performed following a standard $3+3$ design, planned to a maximum dose of $0.8\,mg\,kg^{-1}$ in a second cohort. Details of dose-limiting toxicities (DLTs) and permitted dose reductions are provided in the Supplementary Materials section, available online at *British Journal of Cancer*.

Once the MTD was determined for copanlisib with gemcitabine, this copanlisib dose was administered in combination with intravenous CisGem at fixed doses of $25\,mg\,m^{-2}$ and $1000\,mg\,m^{-2}$, respectively, on days 1 and 8 of a 21-day treatment cycle. If gemcitabine (or cisplatin) was discontinued for toxicity, copanlisib could be continued as monotherapy or in a doublet at the discretion of the investigator if a clinical benefit was observed.

Upon determination of the RP2D of copanlisib plus CisGem, enrolment in an expansion cohort of up to 20 patients with BTC was planned.

Study population. Patients were eligible if they were aged $\geqslant 18$ years with histologically or cytologically confirmed, advanced or refractory solid tumours, and if gemcitabine and cisplatin were medically appropriate. Patients were eligible for the expansion cohort if they had a histologically confirmed diagnosis of BTC (including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma or gallbladder cancer). Patients were required to have at least one measurable lesion or evaluable disease, as determined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, and an Eastern Cooperative Oncology Group performance status of 0 or 1. Further inclusion and exclusion criteria are included in the Supplementary Methods section.

This study was approved by independent ethics committees and institutional review boards for each study site and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. All patients provided written, informed consent before participation.

Objectives and assessments. The primary objectives were to determine the safety, tolerability and RP2D of copanlisib plus gemcitabine and of copanlisib plus CisGem, and to characterise the pharmacokinetics (PK) of copanlisib, gemcitabine and cisplatin when administered concomitantly in patients with advanced malignancies. Secondary objectives included assessment of clinical response and biomarkers that may predict response to the combination drugs.

Adverse events (AEs) and serious AEs were observed from enrolment until 30 days after the last dose of study treatment. Laboratory toxicities and AEs were graded by the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0.

Tumour assessment by computed tomography scan or magnetic resonance imaging was performed at screening and within 7 days of the end of every second cycle using RECIST version 1.1.

PIK3CA and *KRAS* mutations were assayed in circulating tumour DNA isolated from pre-treatment plasma samples (collected during the screening period) using beads, emulsions, amplification and magnetics technology (Sysmex Inostics GmbH, Hamburg, Germany) (Diehl *et al,* 2006). Next-generation sequencing of DNA isolated from formalin-fixed, paraffin-embedded pre-treatment tumour samples (either archival or at screening) was performed using the FoundationOne next-generation sequencing panel (Foundation Medicine, Cambridge, MA, USA). Tumour PTEN protein levels were analysed by immunohistochemistry in pre-treatment tumour tissue samples.

Further details of assessments and PK evaluation are provided in the Supplementary Methods section.

Statistical analyses. Pharmacokinetic concentration calculated for each of the sampling time points included arithmetic mean, standard deviation and coefficient of variation, geometric mean, geometric standard deviation and coefficient of variation, minimum, median, maximum value and the number of measurements. Means at any time were calculated only if at least two out of three of the individual data were measured and were above the lowest limit of quantification (LLOQ). For the calculation of the mean value, a data point below the LLOQ was substituted by one-half of this limit. PK characteristics, except for time to reach maximum plasma concentration after single-dose administration (t_{max}) and time of last observed plasma concentration value above the LLOQ (t_{last}), were summarised using the statistics mentioned previously. T_{max} and t_{last} were described using minimum, maximum, median and frequency counts.

Pharmacokinetic parameters of primary interest for copanlisib, copanlisib metabolite M-1, gemcitabine, gemcitabine metabolite 2',2'-difluorodeoxyuridine and total and free platinum were maximum observed plasma concentration after single-dose administration (C_{max}) and area under the plasma concentration *vs* time curve (AUC) from time 0 to the last observed plasma concentration value above the LLOQ ($AUC_{(0-tlast)}$). The metabolite ratio of copanlisib metabolite M-1 to copanlisib was calculated for $AUC_{(0-tlast)}$ and AUC from 0 to 25 h after the start of the infusion ($AUC_{(0-25)}$) using molar concentrations. Dose-normalised PK characteristics were calculated for C_{max}, $AUC_{(0-tlast)}$ and $AUC_{(0-25)}$.

For immunohistochemistry results, *H*-score values were calculated to determine the average intensity of positive staining given weight by the percentage of cells showing positive staining ($3\times$ (% of tumour cells staining positive at intensity $3+$) $+2\times$ (% of tumour cells staining positive at intensity $2+$) $+1\times$ (% of tumour cells staining positive at intensity $1+$)).

RESULTS

Baseline patient demographics and disease characteristics. Fifty patients were assigned to treatment, a small majority of whom were female (56%). Median age at screening was 63 years (range 33–79) (Table 1). Most patients had intrahepatic bile duct cancer (16 patients, 32%) and gallbladder cancer (seven patients, 14%), primarily because of the BTC expansion cohort (23 patients in total). The majority (39 patients, 78%) had received at least one prior systemic anti-cancer therapy (median number of regimens, 3 (range 1–11)); 11 patients with BTC had not received any prior systemic anti-cancer therapy.

Sixteen patients were treated in the copanlisib plus gemcitabine dose-escalation cohorts; eight patients received the starting dose of 0.6 mg kg^{-1} and eight patients received the maximum dose of 0.8 mg kg^{-1}. The remaining 34 patients received copanlisib 0.8 mg kg^{-1} plus CisGem, 20 of whom comprised the BTC expansion cohort.

Dose escalation and safety. The mean copanlisib dose was 46.0 mg in patients receiving copanlisib 0.6 mg kg^{-1} and the mean copanlisib dose ranged from 52.5 to 57.4 mg for the three cohorts receiving copanlisib 0.8 mg kg^{-1}. Median duration of copanlisib treatment overall was 6.1 weeks (range 0.1–90.1) or two cycles (range 1–30). One patient in the BTC expansion cohort achieved 30 cycles.

Of the eight patients in the copanlisib 0.6 mg kg^{-1} plus gemcitabine dose-escalation cohort, six were evaluable for DLT assessment (one withdrawing because of clinical progression and one because of non-drug-related grade 2 fatigue and thrombocytopenia in cycle 1). One DLT was reported (grade 3 posterior reversible encephalopathy syndrome attributed to copanlisib and gemcitabine) and copanlisib dose escalation continued to 0.8 mg kg^{-1}.

Table 1. Baseline patient demographics and disease characteristics

	Dose-escalation cohorts		Initial copanlisib + CisGem combination cohort	BTC expansion cohort	
	Copanlisib 0.6 mg kg^{-1} + gemcitabine ($n=8$)	Copanlisib 0.8 mg kg^{-1} + gemcitabine ($n=8$)	Copanlisib 0.8 mg kg^{-1} + gemcitabine + cisplatin ($n=14$)	Copanlisib 0.8 mg kg^{-1} + gemcitabine + cisplatin ($n=20$)	Total ($N=50$)
Median age, years (range)	63.0 (36–73)	64.5 (58–70)	59.5 (33–79)	63.5 (37–76)	63.0 (33–79)
Females, *n* (%)	4 (50.0)	4 (50.0)	9 (64.3)	11 (55.0)	28 (56.0)
ECOG performance status[a], *n* (%)					
0	3 (37.5)	1 (12.5)	5 (35.7)	8 (40.0)	17 (34.0)
1	5 (62.5)	7 (87.5)	8 (57.1)	12 (60.0)	32 (64.0)
Cancer diagnosis, *n* (%)					
Breast cancer	1 (12.5)	2 (25.0)	2 (14.3)	0	5 (10.0)
Gallbladder cancer	1 (12.5)	0	1 (7.1)	5 (25.0)	7 (14.0)
Intrahepatic bile duct cancer	0	0	1 (7.1)	15 (75.0)	16 (32.0)
Non-small-cell lung cancer	0	2 (25.0)	2 (14.3)	0	4 (8.0)
Pancreatic adenocarcinoma	1 (12.5)	3 (37.5)	1 (7.1)	0	5 (10.0)
Small-cell lung cancer	1 (12.5)	0	0	0	1 (2.0)
Other	4 (50.0)	1 (12.5)	7 (50.0)	0	12 (24.0)
Cancer stage at study entry[a,b], *n* (%)					
II	0	0	0	1 (5.0)	1 (2.0)
III	0	3 (37.5)	0	4 (20.0)	7 (14.0)
IV[c]	6 (75.0)	5 (62.5)	13 (92.9)	15 (75.0)	39 (78.0)
Prior systemic therapy (chemotherapy, immunotherapy or hormone therapy), *n* (%)	8 (100)	8 (100)	12 (85.7)	11 (55.0)	39 (78.0)
1 prior regimen	1 (12.5)	1 (12.5)	4 (28.6)	1 (12.5)	7 (14.0)
2 prior regimens	1 (12.5)	2 (25.0)	1 (12.5)	4 (28.6)	8 (16.0)
⩾3 prior regimens	6 (75.0)	5 (62.5)	7 (50.0)	6 (40.0)	24 (48.0)
Prior radiotherapy, *n* (%)	4 (50.0)	5 (62.5)	2 (14.3)	8 (40.0)	19 (38.0)
Prior local therapy, *n* (%)	0	0	0	2 (10.0)	2 (4.0)

Abbreviations: BTC = biliary tract cancer; CisGem = cisplatin plus gemcitabine; ECOG = Eastern Cooperative Oncology Group.
[a]Data are missing for one patient who received copanlisib 0.6 mg kg^{-1} + CisGem.
[b]Data are missing for one patient who received copanlisib 0.8 mg kg^{-1} + CisGem.
[c]One patient with small-cell lung cancer had extensive-stage disease, but a cancer stage was not provided.

Of the eight patients in the copanlisib 0.8 mg kg^{-1} plus gemcitabine dose-escalation cohort, two were withdrawn during cycle 1 because of grade 2 abdominal pain assessed as copanlisib-related and grade 3 fatigue assessed as related to both copanlisib and gemcitabine, the latter fulfilling the DLT criteria. Copanlisib 0.8 mg kg^{-1} + gemcitabine 1000 mg m^{-2} weekly in a 3 weeks on/1 week off schedule was the MTD and RP2D of this combination.

Fourteen patients were enrolled in the initial copanlisib 0.8 mg kg^{-1} plus CisGem cohort. One patient did not receive any copanlisib because of serious AEs (grade 3 abdominal pain and grade 3 non-cardiac chest pain) following the first administration of CisGem, and treatment was permanently discontinued. Two patients withdrew because of grade ⩾3 AEs assessed as related to copanlisib (grade 3 hypertension and grade 3 fatigue). In the subsequent expansion cohort of 20 patients with BTC, three withdrew because of grade ⩾3 AEs assessed as related at least to copanlisib (grade 4 sinus tachycardia, grade 3 non-cardiac chest pain and grade 4 decreased neutrophil count). Based on the evaluation of both cohorts, the RP2D of copanlisib plus CisGem was copanlisib 0.8 mg kg^{-1} + cisplatin 25 mg m^{-2} + gemcitabine 1000 mg m^{-2} weekly in a 2 weeks on/1 week off schedule.

All patients experienced at least one treatment-emergent AE (TEAE) (Supplementary Table S1), the most common being nausea (86%), hyperglycaemia (80%), decreased platelet count (80%), decreased neutrophil count (78%) and fatigue (76%).

Overall, 48 patients (96%) experienced at least one grade ⩾3 TEAE, most commonly decreased neutrophil count (62%), hypertension (42%), decreased platelet count (38%), hyperglycaemia (24%) and fatigue (22%). Two patients experienced a grade 5 TEAE (death): respiratory failure attributed to disease progression and heart failure not attributed to disease progression in a patient with a history of myocardial infarctions. Three additional patients died more than 30 days after discontinuation of study treatment, with the primary cause being AEs associated with clinical disease progression in two patients and disease progression in one patient. No deaths were considered related to study treatment.

Forty-seven patients (94%) experienced at least one copanlisib-related TEAE, most commonly hyperglycaemia (76%), nausea (74%), fatigue (66%), decreased neutrophil count (62%), decreased platelet count (58%), anorexia (56%), hypertension (46%), anaemia (42%) and vomiting (36%). Of grade ⩾3 copanlisib-related AEs, decreased neutrophil count (48%), hypertension (38%) and decreased platelet count (28%) were the most frequently observed (Table 2). Infusion-related increases in plasma glucose and plasma insulin (any grade) were observed in nearly all patients, and 14 patients received insulin as remedial drug therapy. The majority of infusion-related increases in plasma glucose and plasma insulin were self-limited and resolved within 24–48 h of copanlisib administration. Ten patients (20%) experienced a TEAE of grade 3 hyperglycaemia and two (4%) experienced grade 4 (Table 2).

Overall, 26 patients (52%) experienced a serious AE, most commonly lung infection (six patients, 12%), abdominal pain (four patients, 8%) and dehydration (three patients, 6%). Grade 4 serious TEAEs included sepsis (two patients, 4%) and lung infection, hyponatremia, anaemia, decreased neutrophil count, decreased platelet count, thromboembolic event, atrial fibrillation and sinus tachycardia (one patient each). Eleven patients (22%) experienced at least one serious copanlisib-related AE, including one patient (2%) with grade 4 sinus tachycardia and grade 4 sepsis.

Dose modifications (interruption or dose reduction) for copanlisib, gemcitabine or cisplatin due to TEAEs were necessary in 45 patients (90%); 26 (52%) had a dose modification because of copanlisib-related TEAEs. Overall, nine patients (18%) permanently discontinued copanlisib because of TEAEs, with fatigue (three patients) being the most common.

Pharmacokinetic evaluation. Forty-seven patients (94%) were valid for PK analysis.

Following infusion of copanlisib on cycle 1, day 1 at both doses in combination with gemcitabine or copanlisib 0.8 mg kg^{-1} in combination with CisGem, geometric mean C_{max} of copanlisib in plasma was between 249 and 414 μg l^{-1} at a median time to reach t_{max} of about 1 h (Figure 1A). Copanlisib demonstrated dose-proportional increase in plasma exposure (C_{max} and AUC) over the dose range of 0.6–0.8 mg kg^{-1} (Figure 2). Geometric mean terminal half-life for copanlisib was between 25 and 26 h. Interpatient variability of copanlisib exposure (coefficient of variation) ranged from 34 to 88% (Supplementary Table S2).

No substantial differences in plasma exposure (C_{max} and $AUC_{(0–25)}$) of copanlisib were observed upon co-administration of CisGem, and PK parameters did not differ considerably over the different dosing regimens (Supplementary Table S2).

Copanlisib metabolite M-1 was measured only at the 0.8 mg kg^{-1} dose. Geometric mean C_{max} for M-1 ranged between 5.2 and 9.3 μg l^{-1} at a median t_{max} of 2–4 h (Figure 1B and Supplementary Table S3). Estimated geometric mean terminal half-life was comparable with that of copanlisib. Interpatient variability of metabolite M-1 exposure was high, with coefficient of variation ranging from 66 to 115% (Supplementary Table S3). The metabolite ratio of copanlisib metabolite M-1 to copanlisib in plasma for $AUC_{(0–25)}$ ranged from 1.5 to 19%. The amount of copanlisib and metabolite M-1 excreted into urine during the 25 h urine collection interval was low, with about 4.9% and 0.9%, respectively, of dose excreted.

The PK of total and free platinum, gemcitabine and its metabolite 2',2'-difluorodeoxyuridine were not influenced by co-administration with copanlisib (further details provided in the Supplementary Methods section).

Copanlisib is a low renally excreted drug and a strong inhibitor of multidrug and toxin extrusion protein 2K (MATE2-K) *in vitro*. Patients treated with copanlisib plus cisplatin, a known substrate of MATE2-K, showed no signs of increased nephrotoxicity caused by intrarenal accumulation of cisplatin, suggesting a low potential effect of copanlisib on MATE2-K (data not shown). In addition, the plasma exposure of cisplatin measured in combination with copanlisib was in the range of previous historical PK observed for cisplatin (Himmelstein *et al*, 1981).

Efficacy analysis. All 50 patients were evaluable for efficacy and 40 (80%) underwent imaging for post-baseline RECIST assessment and were evaluable for best overall tumour response. One patient (2%) achieved a complete response, four (8%) achieved a partial response and 19 (38%) had stable disease, resulting in an objective response rate of 10% overall.

Of the 16 patients who received copanlisib plus gemcitabine, the response rate was 6.3% (1 out of 16 patients). One patient achieved a partial response (copanlisib dose of 0.6 mg kg^{-1}; primary peritoneal carcinoma), discontinuing the study because of disease progression after eight cycles.

The response rate in patients who received copanlisib plus CisGem was 12% (4 out of 34 patients). One patient achieved a complete response (gallbladder cancer) and three patients achieved a partial response (intrahepatic biliary cancer) (Figure 3). Two patients who achieved a partial response were in the BTC expansion cohort, giving a response rate of 10% in that cohort (2 out of 20 patients).

Of the 23 patients with BTC overall, the response rate was 17% (4 out of 23 patients). All four responders had not received any prior anti-cancer therapy, giving a response rate of 36% in the 11 patients with BTC who had not received prior anti-cancer therapy. No response was observed in the 12 patients who had received prior CisGem or gemcitabine-containing chemotherapy.

Further efficacy details are provided in the Supplementary Methods section.

Biomarkers. Mutation data from circulating tumour DNA were generated from 43 out of 50 patients, and 22 out of 43 patients had tumour next-generation sequencing data. *PIK3CA* alterations were detected in 2% of patients (1 out of 43; a gene amplification), *KRAS* mutations in 23% (10 out of 43) and *BRAF* mutations in 9% (2 out of 22). Complete tumour PTEN

Table 2. Summary of copanlisib-related grade 3, 4 or 5 adverse events occurring in ⩾5% of patients overall

		Dose-escalation cohorts		Initial copanlisib + CisGem combination cohort	BTC expansion cohort	
n **(%)**	**Grade**	**Copanlisib 0.6 mg kg^{-1} + gemcitabine (*n* = 8)**	**Copanlisib 0.8 mg kg^{-1} + gemcitabine (*n* = 8)**	**Copanlisib 0.8 mg kg^{-1} + gemcitabine + cisplatin (*n* = 14)**	**Copanlisib 0.8 mg kg^{-1} + gemcitabine + cisplatin (*n* = 20)**	**Total (*N* = 50)**
Hypertension	3	0	3 (37.5)	5 (35.7)	11 (55.0)	19 (38.0)
Decreased neutrophil count	3	2 (25.0)	2 (25.0)	4 (28.6)	6 (30.0)	14 (28.0)
	4	0	1 (12.5)	1 (7.1)	8 (40.0)	10 (20.0)
Fatigue	3	0	2 (25.0)	4 (28.6)	4 (20.0)	10 (20.0)
Hyperglycaemia	3	1 (12.5)	2 (25.0)	1 (7.1)	6 (30.0)	10 (20.0)
	4	0	1 (12.5)	0	1 (5.0)	2 (4.0)
Decreased platelet count	3	1 (12.5)	3 (37.5)	2 (14.3)	3 (15.0)	9 (18.0)
	4	0	0	0	5 (25.0)	5 (10.0)
Decreased lymphocyte count	3	0	0	1 (7.1)	6 (30.0)	7 (14.0)
Decreased white blood cell	3	0	1 (12.5)	2 (14.3)	4 (20.0)	7 (14.0)
	4	0	0	0	1 (5.0)	1 (2.0)
Nausea	3	1 (12.5)	1 (12.5)	1 (7.1)	2 (10.0)	5 (10.0)

Abbreviations: BTC = biliary tract cancer; CisGem = cisplatin plus gemcitabine.
All adverse events were defined according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0.

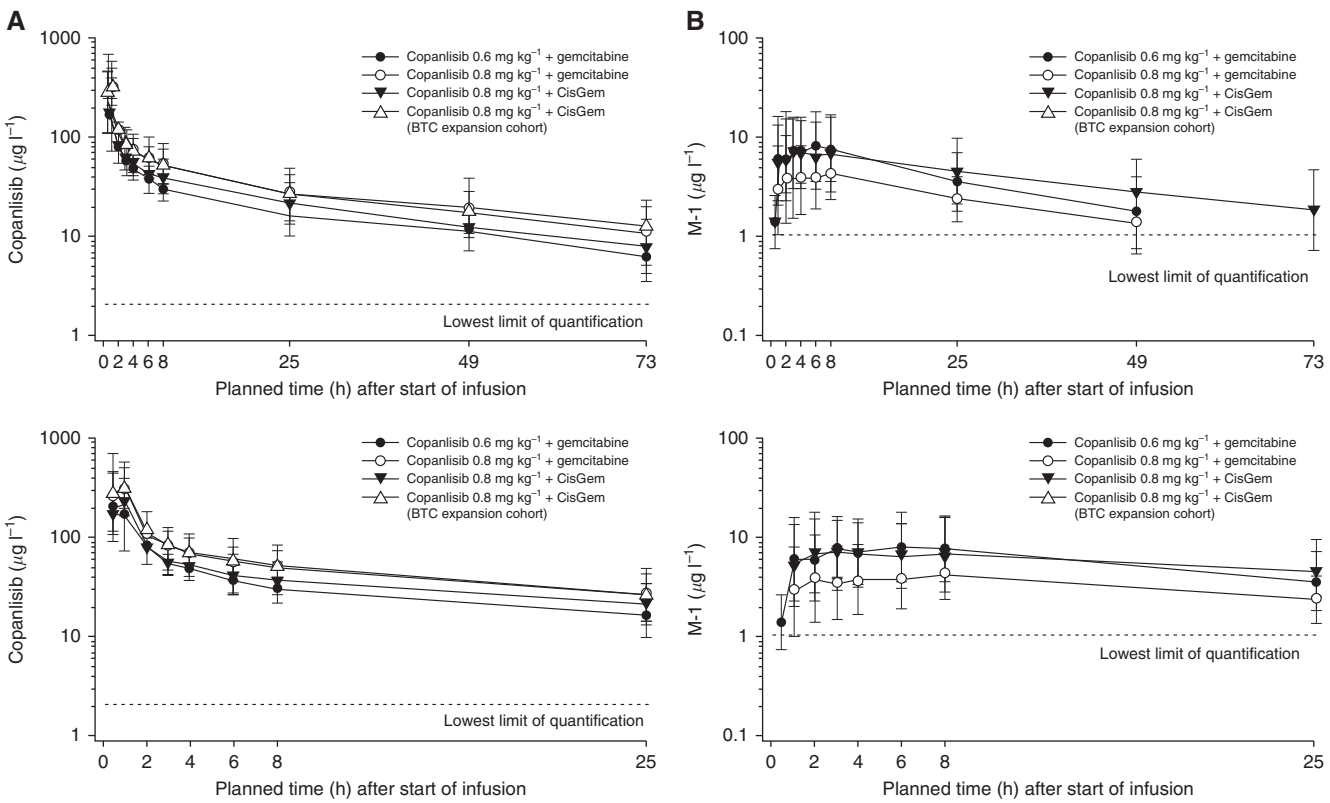

Figure 1. Plasma concentrations *vs* time profiles of copanlisib and its metabolite M-1. Geometric mean/s.d. concentrations *vs* time profiles of copanlisib (μg l^{-1}) (**A**) and its metabolite M-1 (μg l^{-1}) (**B**) in plasma after copanlisib dosing on cycle 1, day 1 (the lower figures of each panel show the first 25 h after the start of the infusion). BTC = biliary tract cancer; CisGem = cisplatin (25 mg m^{-2}) plus gemcitabine (1000 mg m^{-2}).

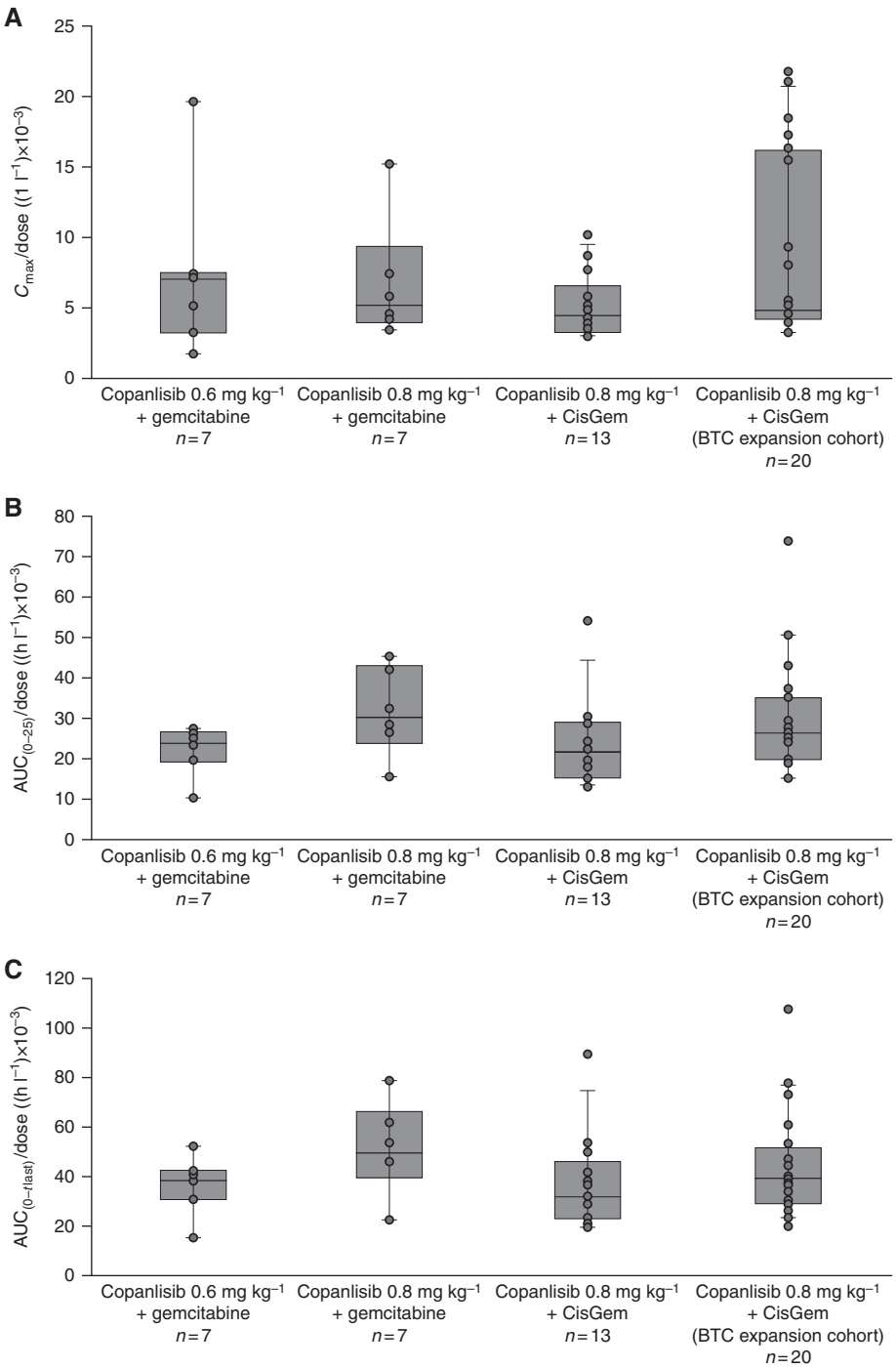

Figure 2. Dose-normalised plasma pharmacokinetic parameters of copanlisib. Boxplots of dose-normalised parameters C_{max}/dose (**A**), $AUC_{(0–25)}$/dose (**B**) and $AUC_{(0–tlast)}$/dose (**C**) of copanlisib in plasma following administration of copanlisib on cycle 1, day 1. AUC = area under the plasma concentration *vs* time curve; $AUC_{(0–25)}$ = AUC from 0 to 25 h after the start of the infusion; $AUC_{(0–tlast)}$ = area under the plasma concentration *vs* time curve from 0 to time of last observed plasma concentration value above the lower limit of quantification; BTC = biliary tract cancer; C_{max} = maximum observed plasma concentration after single-dose administration; CisGem = cisplatin (25 mg m^{-2}) plus gemcitabine (1000 mg m^{-2}).

protein loss was identified in 41% of patients (12 out of 29). Among patients with BTC, the mutation rates for *PIK3CA*, *KRAS* and *BRAF* were 0% (0 out of 22), 18% (4 out of 22) and 22% (2 out of 9), respectively, and PTEN protein loss was observed in 69% of patients (9 out of 13). No mutations in PTEN were identified by next-generation sequencing.

Two patients with objective tumour response had PTEN protein loss, one in conjunction with a *KRAS* mutation (patient with cholangiocarcinoma; BTC expansion cohort) and the other with a *BRAF* mutation (patient with cholangiocarcinoma; initial CisGem safety evaluation cohort) (Figure 3). No evidence of molecular aberration in these factors was observed in the other three patients with tumour response. No biomarker was clearly associated with outcome, although not all biomarkers could be assayed in all patients because of limitations of sample availability and appropriate informed consent.

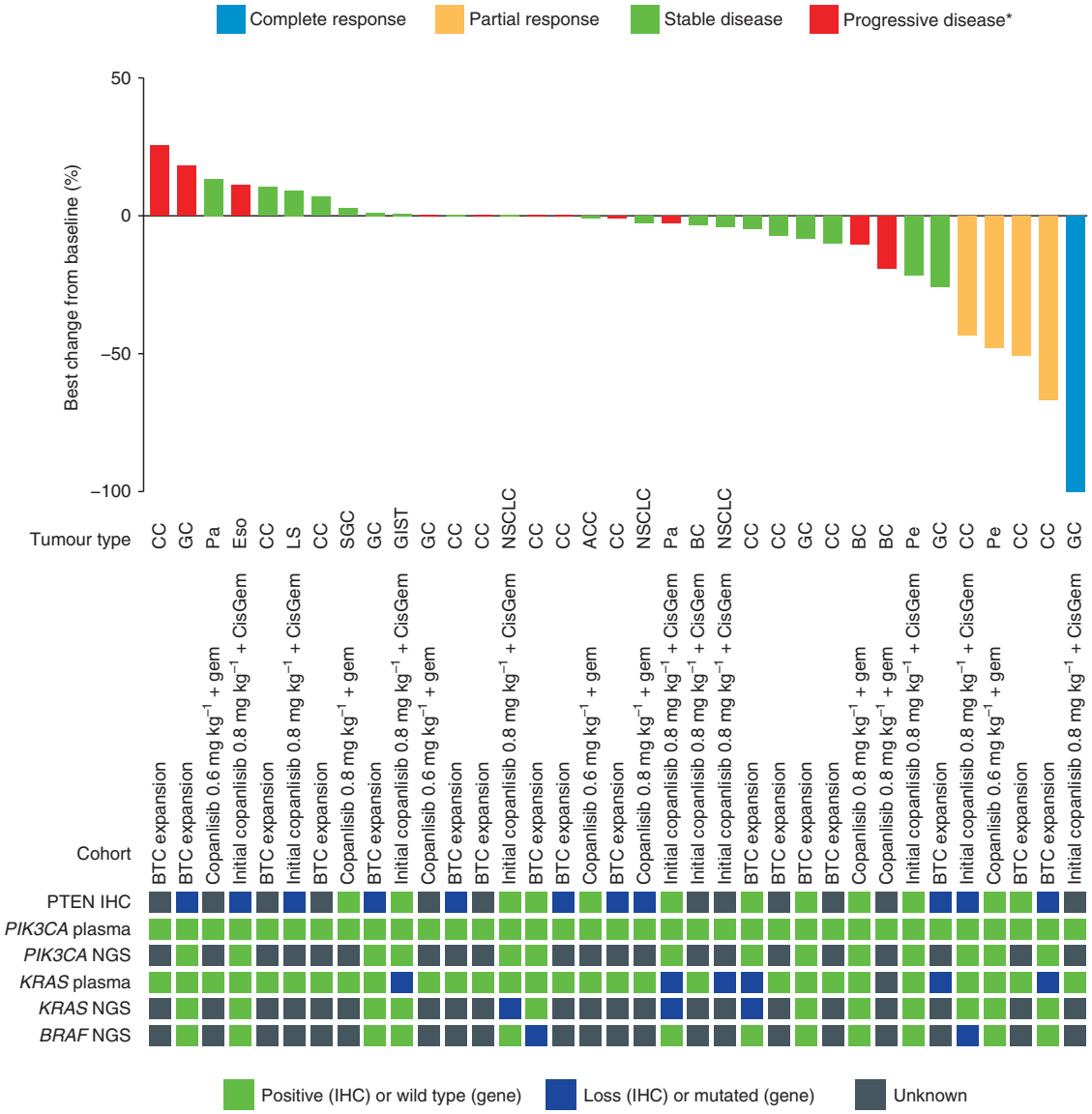

Figure 3. Waterfall plot of best change in target lesion size from baseline *vs BRAF, KRAS* and *PIK3CA* mutation status and PTEN protein status for all patients with data for both biomarkers and change in target lesion size. ACC = adenoid cystic cancer; BC = breast cancer; BTC = biliary tract cancer; CC = cholangiocarcinoma; CisGem = cisplatin (25 mg m^{-2}) plus gemcitabine (1000 mg m^{-2}); Eso = oesophageal cancer; GIST = gastrointestinal stromal tumour; GC = gallbladder cancer; gem = gemcitabine (1000 mg m^{-2}); IHC = immunohistochemistry; LS = liposarcoma; NGS = next-generation sequencing; NSCLC = non-small-cell lung cancer; Pa = pancreatic cancer; Pe = peritoneal cancer; SGC = salivary gland cancer (parotid gland). *The majority of patients with disease progression progressed because of non-target lesion growth.

DISCUSSION

This phase I, open-label study determined the MTD and RP2D of copanlisib plus gemcitabine and the RP2D of copanlisib plus CisGem. The MTD and RP2D of copanlisib plus gemcitabine 1000 mg m^{-2} was 0.8 mg kg^{-1} on days 1, 8 and 15 of a 28-day treatment cycle. The one DLT reported was posterior reversible encephalopathy syndrome, attributed to copanlisib and gemcitabine, which has previously been associated with gemcitabine (Rajasekhar and George, 2007; Marrone *et al*, 2011). The RP2D for the triplet combination was copanlisib 0.8 mg kg^{-1} + gemcitabine 1000 mg m^{-2} + cisplatin 25 mg m^{-2} on days 1 and 8 of a 21-day treatment cycle.

Copanlisib plus CisGem demonstrated an acceptable safety profile. Most toxicities were manageable through dose modifications and remedial drug treatment. No new or unexpected safety signals were observed. The most common TEAEs were nausea, hyperglycaemia and decreased platelet count. Most patients experienced at least one grade ⩾3 TEAE, most commonly decreased neutrophil count, hypertension and decreased platelet count. The observed safety profile of copanlisib plus CisGem was generally consistent with previously observed toxicities of copanlisib monotherapy (hyperglycaemia, nausea and hypertension), although the incidence of haematological TEAEs was higher than previously reported with either gemcitabine or copanlisib alone (Valle *et al*, 2010; Patnaik *et al*, 2016). This is likely to be related to the combination of the well-known safety profiles of gemcitabine (haematological toxicities and flu-like symptoms) and cisplatin (haematological toxicities and nausea) (Valle *et al*, 2010; Lilly USA, LLC, 2014).

The acceptable safety profile of the copanlisib plus CisGem combination is in contrast to that of the approved PI3K-δ inhibitor idelalisib, which demonstrated an increased rate of AEs, including deaths, in combination with other anti-cancer therapies in clinical trials, resulting in the termination of six trials in patients with chronic lymphocytic leukaemia, small lymphocytic lymphoma and

indolent non-Hodgkin's lymphomas (US Food and Drug Administration, 2016).

Infusion-related increases in plasma glucose and plasma insulin were observed in the majority of patients (~80%), in line with the expected mechanism of action and consistent with observations from previous clinical studies (Patnaik *et al,* 2016; Dreyling *et al,* 2017a). Few patients received systemic corticosteroids as concomitant medication; therefore, the observed increase in glucose is likely attributed to copanlisib. Although a direct analysis of the correlation between copanlisib exposure or tumour response and plasma glucose or insulin levels was not performed and some confounding factors such as corticosteroid and insulin administrations were present, plasma glucose and plasma insulin remain useful pharmacodynamic markers of on-target copanlisib exposure.

Copanlisib exhibited favourable PK properties in combination with CisGem, with rapid absorption, a nearly dose-proportional increase in exposure in the dose-escalation cohorts and minimal differences between the two drug combinations tested. There was no indication of any clinically relevant PK interactions between copanlisib and gemcitabine or cisplatin, consistent with the known metabolic pathway for copanlisib.

Recent analyses indicate that an intermittent dose schedule of 60 mg weekly on days 1, 8 and 15 of a 28-day cycle was likely to achieve a similar risk–benefit ratio as 0.8 mg kg^{-1} weight-based dosing (Reif *et al,* 2016), and fixed dosing is being explored in ongoing studies.

The objective response rate was 10% overall, 12% with the copanlisib plus CisGem combination and 6.3% with the copanlisib plus gemcitabine combination; however, many of these patients had already been treated with CisGem as first-line therapy. The response rate was 36% in patients with BTC with no prior anticancer therapy (4 out of 11 patients). BTC is a heterogeneous group of malignancies, so direct comparison with other studies is subject to selection bias. A large phase III study of patients with advanced BTC receiving CisGem demonstrated a response rate of 26% (Valle *et al,* 2010). Further investigation of copanlisib plus CisGem is warranted.

The exploratory biomarker analysis observed PTEN loss in 41% of evaluable patients (69% in patients with BTC). This observation is in line with previous reports in gallbladder cancer, where PTEN loss was found in 72% of patients (Ali *et al,* 2015), suggesting that this tumour type is widely reliant on PI3K pathway signalling through this mechanism. The prevalence of *KRAS* mutations detected in patients with BTC (18%) is also similar to what has been previously reported (~20%), whereas the *PIK3CA* mutation rate (0%) is lower and the *BRAF* mutation rate (22%) is higher than what has been reported in previous studies (~10% and 5%, respectively) (Jain and Javle, 2016; Javle *et al,* 2016; Zhao *et al,* 2016). Although none of the biomarkers evaluated associated clearly with outcome in this cohort, these analyses may be limited by the mixed tumour types and small numbers of patients included in the molecular subgroups.

In summary, copanlisib demonstrated an acceptable safety profile and potentially promising clinical response when administered at a dose of 0.8 mg kg^{-1} (approximately equivalent to 60 mg fixed dose) in combination with CisGem. Pharmacokinetic profiles for copanlisib and its metabolite M-1 were favourable, and co-administration of CisGem had no influence on copanlisib and metabolite M-1 exposure. A phase II study investigating the clinical benefits of copanlisib plus CisGem in patients with advanced cholangiocarcinoma (NCT02631590) is currently underway.

ACKNOWLEDGEMENTS

Medical writing assistance was provided by Laura Badtke, PhD (Complete HealthVizion, Chicago, IL, USA), based on detailed discussion and feedback from all the authors. We thank Ron Dubowy, Camille Granvil and Jie Cheng.

CONFLICT OF INTEREST

RDK reports grants and personal fees from Bayer and Bristol-Myers Squibb, personal fees from Lilly and grants from Eisai. JEG-O reports institutional clinical trial support from Bayer, Novartis, Peregrine Pharmaceuticals, NanoCarrier, Genentech, Seattle Genetics, MedImmune and Pfizer. CP, CX and AK are employees of Bayer HealthCare. IG and AA-H are employees of Bayer AG. SRA declares no competing financial interest.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)