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On-Target Pharmacodynamic Activity of the PI3K Inhibitor Copanlisib in Paired Biopsies from Patients with Malignant Lymphoma and Advanced Solid Tumors

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ABSTRACT

The PI3K inhibitor copanlisib has efficacy and manageable safety in patients with indolent lymphoma and solid tumors. Pharmacodynamic effects relative to copanlisib dose and plasma exposure were evaluated. Patients with lymphoma or solid tumors received copanlisib 0.4 or 0.8 mg/kg on days 1, 8, and 15 of a 28-day cycle. Primary variables were maximum changes in phosphorylated AKT (pAKT) levels in platelet-rich plasma (PRP) and plasma glucose. Other evaluations included PI3K signaling markers and T-lymphocytes in paired tumor biopsies, the relationship between estimated plasma exposure and pharmacodynamic markers, response, and safety. Sixty-three patients received copanlisib. PRP pAKT levels showed sustained reductions from baseline following copanlisib [median inhibition: 0.4 mg/kg, 73.8% (range −94.9 to 144.0); 0.8 mg/kg, 79.6% (range −96.0 to 408.0)]. Tumor pAKT was reduced versus baseline with copanlisib 0.8 mg/kg in paired biopsy samples ($P < 0.05$). Dose-related transient plasma glucose elevations were observed. Estimated copanlisib plasma exposure significantly correlated with changes in plasma pAKT and glucose metabolism markers. There were two complete responses and six partial responses; seven of eight responders received copanlisib 0.8 mg/kg. Adverse events (all grade) included hyperglycemia (52.4%), fatigue (46.0%), and hypertension (41.3%). Copanlisib demonstrated dose-dependent pharmacodynamic evidence of target engagement and PI3K pathway modulation/inhibition in tumor and immune cells. Results support the use of copanlisib 0.8 mg/kg (or flat-dose equivalent of 60 mg) in solid tumors and lymphoma, and provide a biomarker hypothesis for studies of copanlisib combined with immune checkpoint inhibitors (NCT03711058).

Introduction

The PI3K/protein kinase B (AKT)/mTOR signaling pathway regulates multiple cellular processes, including metabolism, survival, and proliferation (1). PI3K pathway dysregulation causes overactive signaling, seen in many cancers (2, 3), and loss of the tumor-suppressor PTEN is a key driver of tumorigenesis through the PI3K pathway (4). Targeted inhibition of PI3K signaling has been explored as a therapeutic strategy for patients with cancer, and several PI3K inhibitors are in development or have been approved for the treatment of solid tumors or hematologic malignancies, including both isoform-selective agents and those with broader activity (5–13).

Copanlisib is an intravenously administered, highly selective, pan-class I PI3K inhibitor with potent activity against p110α and p110δ isoforms (14). Copanlisib has demonstrated robust single-agent antitumor activity and a manageable safety profile in phase I and II studies in patients with relapsed or refractory indolent lymphoma treated with copanlisib at the MTD of 0.8 mg/kg, or the flat-dose equivalent of 60 mg, infused weekly, 3 weeks on, 1 week off (15–17).

Intravenously administered copanlisib has previously demonstrated dose-dependent on-target hyperglycemia (15–17), consistent with the on-target class effect of PI3K pathway inhibitors (18). However, identification of additional pharmacodynamic biomarkers directly demonstrating PI3K pathway activity, including in the relevant target tumor tissues, could support better delineation and characterization of the biologically active dose.

To validate the MTD and demonstrate target engagement, PI3K pathway modulation, and downstream effects by copanlisib, this study evaluated the pharmacodynamic effects of single-agent intravenous copanlisib relative to dose and plasma exposure in patients with malignant lymphoma and solid tumors.

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Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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Materials and Methods

Study design

This open-label, single-arm, multicenter phase I study (NCT02155582) comprised four dose cohorts: two parallel cohorts of patients without diabetes with malignant lymphoma or solid tumors, randomized to copanlisib 0.4 or 0.8 mg/kg. In addition, two parallel cohorts of patients with diabetes were randomized to fixed doses of copanlisib 45 or 60 mg. Forty patients without diabetes (20 each with lymphoma or solid tumors) and 12 patients with diabetes (with lymphoma or solid tumors) were planned to be enrolled.

The primary objective was to evaluate the relationship between exposure and pharmacodynamic biomarkers following treatment with copanlisib monotherapy in patients with malignant lymphoma or solid tumor types with high likelihood of PI3K pathway activation. Secondary objectives were to assess the safety and tolerability of copanlisib, including in patients with diabetes, and to evaluate clinical response. The use of randomized dosing at 0.4 or 0.8 mg/kg of copanlisib produces a spread in doses and hence a spread in exposures, allowing the evaluation of the relationship between exposure and pharmacodynamics.

The 45 mg dose in patients with diabetes aimed to provide additional dose information in between the 60 mg dose and the median dose of 32 mg received by patients with diabetes in the first-in-human study of copanlisib (16).

This study was conducted in accordance with Good Clinical Practice guidelines and applicable local laws and regulations, and under the guiding principles detailed in the Declaration of Helsinki. The study protocol and all amendments were reviewed and approved by each site's institutional ethical committee/review board. All participants provided written, informed consent.

Patients

Patients ages $\geq$18 years with a histologically confirmed diagnosis of lymphoma [follicular lymphoma (all grades), lymphoplasmacytic lymphoma/Waldenström macroglobulinemia, transformed indolent lymphoma, diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma, mantle cell lymphoma, or peripheral T-cell lymphoma], relapsed or refractory, with $\geq$1 prior chemo-immunotherapy or immunotherapy-based regimen, or advanced and/or refractory solid tumors with known high prevalence ($\geq$30%) of *PIK3CA* or *PTEN* alteration (including ovarian, breast, uterine, cervical, head and neck, prostate, and squamous cell lung cancer), were eligible. Additional inclusion criteria are detailed in the Supplementary Materials and Methods. Patients were not selected on the basis of *PIK3CA* or *PTEN* alteration status at study entry.

Treatment

Copanlisib 0.4 or 0.8 mg/kg or 45 or 60 mg was administered as a 1-hour intravenous infusion on days 1, 8, and 15 of a 28-day cycle. Copanlisib was provided by Bayer AG (14). Patients fasted for $\geq$8 hours prior to and 2 hours following the start of the first infusion. Fasting blood glucose had to be $\leq$160 mg/dL prior to the first infusion, and for subsequent infusions, $\leq$160 mg/dL if fasting or $\leq$200 mg/dL if nonfasting. Dose reductions from 0.8 to 0.6 and 0.4 mg/kg, or from 60 to 45 and 30 mg, were permitted in case of toxicities and mandatory in case of grade 4 asymptomatic glucose increase >500 mg/dL; subsequent occurrence of this event was to result in permanent discontinuation. Dose reescalation was not permitted.

Pharmacodynamic variables

Primary pharmacodynamic variables were maximum change from baseline in expression of phosphorylated AKT (pAKT) in platelet-rich plasma (PRP) during treatment, and in plasma glucose during the first two treatment cycles. Secondary pharmacodynamic variables included: area under the concentration–time curve from time 0 to 168 hours [$AUC_{(0-168)}$] of copanlisib after each infusion during two treatment cycles; adverse events (AE); maximum change from baseline in insulin and C-peptide during the first two treatment cycles; change in 2[^{18}F]fluoro-2-deoxy-d-glucose (FDG) uptake after copanlisib dosing [for patients without diabetes with detectable FDG tumor uptake at baseline, measured as maximum standardized uptake values (SUV_{max})]; change from baseline in phosphorylation of PI3K pathway proteins and markers of apoptosis and proliferation (pAKT-T308, pAKT-S473, pS6, cleaved caspase-3, and Ki67) in paired tumor biopsies; and clinical response. Exploratory variables included copanlisib pharmacokinetic profile, the time course of glucose metabolism markers (plasma glucose, insulin, and C-peptide) during the first two treatment cycles, and change from baseline in tumor infiltrating lymphocytes ($CD4^+$, $CD8^+$, and $CD3^+$) and in a panel of plasma proteins. Patients could be replaced if they had insufficient pharmacodynamic or safety samples for evaluation (detailed in the Supplementary Materials and Methods).

Assessments

Details of sample collection, assessment schedules and methods for PRP for pAKT assays, plasma samples for glucose, insulin, C-peptide, pharmacokinetic assessments, and exploratory biomarker evaluation, FDG-PET scans, and tumor biopsies for IHC and exploratory mutation testing are in the Supplementary Materials and Methods. Submission of fresh tumor biopsies at screening was mandatory per protocol, with signed, written, informed consent. Safety was assessed throughout the study and included toxicity/AEs, vital signs (including systolic blood pressure), clinical laboratory variables (including flow cytometric analysis of lymphocyte subsets in paired tumor biopsies), and concomitant medications. AEs were reported and graded according to the Common Terminology Criteria for Adverse Events version 4.03. Safety follow-up was performed within 30 days of but no later than 48 days after the last dose of copanlisib. Tumor or lymphoma lesions were assessed according to the RECIST version 1.1 criteria (19) for patients with solid tumors, and the modified Cheson criteria (20) for patients with lymphoma. Tumors were assessed by MRI, CT, or PET-CT at screening, on cycle 2, day 22, and subsequently on day 22 of alternate cycles.

Pharmacokinetic/pharmacodynamic analysis

A population pharmacokinetic model (developed using data from >297 patients treated with copanlisib in phase I and II trials; ref. 21) and pharmacokinetic observations from this study were used to derive individual pharmacokinetic parameter estimates and copanlisib exposure variables, including the interpatient variability of copanlisib pharmacokinetics, using NONMEM version 7.3 (ICON plc; see Supplementary Materials and Methods, for further details). Estimated plasma exposures of copanlisib [maximum concentration during a dosing interval (C_{max}) and average concentration during a dosing interval (C_{av})] during the first two treatment cycles were related to pharmacodynamic variables via linear regression with estimation of intercept.

Statistical analysis

All statistical analyses were prespecified and were performed using SAS release 9.2 (SAS Institute Inc.). Pharmacokinetic/

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pharmacodynamic regression analyses were performed using R 3.3.1 (R Foundation for Statistical Computing). No formal sample size could be estimated as the evaluation of the relationship between exposure and pharmacodynamics was not on the basis of hypothesis testing. All patients who received ≥1 dose of copanlisib (full analysis set) were included in analyses of safety and efficacy, with exact 95% confidence intervals (CI) provided for efficacy. All treated patients with valid pharmacodynamic sample measurements and pharmacokinetic data were included in pharmacodynamic and pharmacokinetic analyses (detailed in the Supplementary Materials and Methods). Response to treatment was correlated to time under treatment and individual exposure estimates; individual exposure estimates were correlated to pharmacodynamic biomarkers using Pearson correlation coefficient.

Results

Patients and treatment

Eighty-four patients were enrolled in this study, of whom 64 were randomized to copanlisib treatment; 1 patient had a serious AE during screening and was not treated (Supplementary Table S1). Sixty-three patients received ≥1 copanlisib infusion (full analysis set), 33 with lymphoma and 30 with solid tumors, including 2 patients with diabetes with solid tumors. Thirty-four patients received 0.4 mg/kg (20 with lymphoma and 14 with solid tumors) and 27 received 0.8 mg/kg (13 with lymphoma and 14 with solid tumors). One patient with diabetes each received 45 and 60 mg; no additional patients with diabetes could be recruited and enrolled for treatment, and the cohorts with diabetes were prematurely closed. Demographics and baseline characteristics are shown in **Table 1**. The most common cancer types were DLBCL (18/63; 28.6%) and breast cancer (16/63; 25.4%).

The median number of infusions was 6 (range 2–65), the median duration of treatment was 7 weeks (range 2–87; median of 13.3 weeks at the 0.8 mg/kg dose and 7 weeks at 0.4 mg/kg), and the median number of cycles was 1.75 (range 0.5–21.8). The mean actual dose per administration was 28.9 mg in patients receiving 0.4 mg/kg and 49.8 mg in patients receiving 0.8 mg/kg; this was higher in patients with lymphoma (51.0 mg). Twenty-six patients (41.3%) had ≥1 dose modification. Eight patients (12.7%) had dose reductions and 26 (41.3%) had dose interruptions or delays. AEs were attributed as the cause of all dose reductions (100%) and the majority of interruptions or delays (93.2%). The most common drug-related treatment-emergent AE (TEAE) leading to dose interruption and/or reduction was hypertension (grade 2 or 3), reported for 8 patients (12.7%).

Baseline tumor PI3K/AKT/PTEN characterization

Sixty-two treated patients consented to provide fresh tumor samples at baseline, and 44 had archival samples; up to 47 patients had sufficient quality fresh or archival tumor samples for baseline analysis of PI3K/AKT/PTEN by IHC and/or mutation testing by next-generation sequencing. Positive tumor-cell staining for PI3Kα protein was observed in 94.7% of lymphoma samples (18/19) and 88.9% of solid tumor samples (24/27), whereas PI3Kδ protein expression was detected in 94.7% (18/19) of lymphoma samples and 29.6% (8/27) of solid tumor samples (Supplementary Table S2). PI3Kβ and PI3Kγ protein expression was detected in a proportion of all samples, but to a lesser extent than the –α and –δ isoforms. *PIK3CA*- or *AKT1*-activating mutations were detected in 21.1% of solid tumor samples (4/19), as were *PTEN* low copy number or loss-of-function mutations (4/19; 21.1%); these mutations were mutually exclusive and were only detected in solid tumor samples. PTEN protein loss was more frequent in solid tumor samples [18.5% (5/27)] than in lymphoma samples [5.3% (1/19); Supplementary Table S2]. Among the five solid tumor samples with PTEN protein loss, two had *PTEN* gene loss-of-function mutations (K144fs*31 and R130*), two had *PTEN* copy number loss, and one had wild-type *PTEN*. The lymphoma sample with PTEN protein loss had wild-type *PTEN*.

Table 1. Patient demographics and baseline disease characteristics.

	Total ($N = 63$)
Females, *n* (%)	42 (66.7)
Median age, years (range)	61.0 (38–80)
Race, *n* (%)[a]	
White	40 (63.5)
Asian	2 (3.2)
ECOG performance status, *n* (%)	
0	22 (34.9)
1	33 (52.4)
2	8 (12.7)
Lymphoma cohort, *n*	33
Histology, *n* (%)	
DLBCL	18 (54.5)
Malignant DLBCL—not otherwise specified	15 (45.5)
DLCBL transformed from follicular lymphoma	3 (9.1)
T-cell lymphoma	7 (21.2)
Angioimmunoblastic T-cell lymphoma	4 (12.1)
Anaplastic large-cell lymphoma, T-/null-cell type	2 (6.1)
Mature T-cell lymphoma—not otherwise specified	1 (3.0)
Follicular lymphoma, grade 3[b]	3 (9.1)
Mantle cell lymphoma[c]	3 (9.1)
Burkitt lymphoma—not otherwise specified	2 (6.1)
Ann Arbor classification, *n* (%)[d,e]	
Stage I	1 (3.0)
Stage II	1 (3.0)
Stage III	8 (24.2)
Stage IV	20 (60.6)
Solid tumor cohort, *n*	30
Histology, *n* (%)[d]	
Breast cancer	16 (25.4)
Ovarian cancer	4 (6.3)
Endometrial cancer	3 (4.8)
Other[f]	7 (11.1)
Breast cancer stage at initial diagnosis, *n* (%)[g,h]	
I	3 (18.8)
II	3 (18.8)
III	5 (31.3)
IV	2 (12.5)

[a]Race not reported for 20 patients and missing for 1 patient.
[b]Includes 1 grade 3a and 2 grade 3b.
[c]Includes all variants: blastic, pleomorphic, and small cell.
[d]Percentages expressed as a proportion of the number of patients with lymphoma.
[e]Ann Arbor stage unknown for 3 patients.
[f]Includes cervical cancer ($n = 1$), adenoid cystic carcinoma of the tongue ($n = 1$), head and neck cancer ($n = 1$), non–small cell lung cancer ($n = 1$), maxillary osteosarcoma ($n = 1$), prostate cancer ($n = 1$), and cancer of unknown primary site ($n = 1$).
[g]Percentages expressed as a proportion of the number of patients with breast cancer.
[h]Stage missing for 3 patients.

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Pharmacodynamic changes in PI3K biomarkers

pAKT in PRP

Fifty-four patients were evaluable for pharmacodynamic analysis of pAKT levels in PRP. pAKT levels rapidly reduced by >50% from baseline following the first copanlisib infusion on cycle 1, day 1 in patients receiving copanlisib 0.4 and 0.8 mg/kg; reduced pAKT was observed over two cycles of treatment, with median inhibition reaching 73.8% (range −94.9 to 144.0) and 79.6% (range −96.0 to 408.0), respectively, in cycle 1, day 8, 1.5 hours postinfusion (**Fig. 1**). Inhibition was pronounced at 1.5 hours postdose, and was sustained for approximately 24 hours. Following the end of treatment, pAKT levels returned to near baseline levels at both doses. Interpatient variability was high throughout treatment at both dose levels, mainly attributed to low pAKT levels (≤3%) at baseline in 7 patients.

IHC analysis of tumor signaling and immune environment

Thirty-one patients had paired tumor biopsy samples evaluable for IHC evaluation of pAKT-S473, pS6, pERK, Ki67, and cleaved caspase-3 in tumor cells, and T-lymphocyte markers CD4, CD8, and CD3: 16 treated with copanlisib 0.4 mg/kg, 14 treated with 0.8 mg/kg, and 1 patient with diabetes treated with 45 mg. *H*-scores for pAKT-S473 were significantly reduced at cycle 1, day 15 versus baseline in samples from patients receiving copanlisib 0.8 mg/kg (group mean reduction of 73.4%; 95% CI, −94.7 to −7.2; $P = 0.02$, unadjusted), with little effect in patients receiving 0.4 mg/kg (group mean increase of 31.1%; 95% CI, −31.3 to 64.8; $P = 0.30$; **Fig. 2A**).

H-scores for pS6 in paired biopsies were more visibly reduced at day 15 versus baseline in patients receiving copanlisib 0.8 mg/kg (group mean reduction of 72.4%; 95% CI, −34.2 to −2.4; $P = 0.08$, unadjusted) compared with patients receiving 0.4 mg/kg (group mean increase of 19.5%; 95% CI, −8.3 to 13.2; $P = 0.67$; **Fig. 2B**).

Following copanlisib treatment, a dose-dependent reduction in the proportion of $CD4^+$ cells in tumor biopsies was observed versus baseline (**Fig. 2C**), with a mean reduction of 71% staining in samples from patients with lymphoma receiving copanlisib 0.8 mg/kg ($P = 0.22$, unadjusted). No reduction or little effect was observed on $CD4^+$ cells at the 0.4 mg/kg dose, on the proportion of $CD8^+$ cells at either dose or in either tumor population (**Fig. 2D**).

H-scores for pERK were increased at day 15 versus baseline in samples from patients receiving copanlisib 0.4 mg/kg (mean *H*-score increase of 74%, $P = 0.06$), although there was a trend toward decreased pERK *H*-scores in samples from patients receiving 0.8 mg/kg (mean *H*-score reduction of −37%, $P = 0.22$). *H*-scores for Ki67 were generally reduced at day 15 versus baseline, commonly from lymphoma samples at the 0.8 mg/kg dose [reductions observed in 5/7 (71.0%) lymphoma samples]. Increases in *H*-scores for cleaved caspase-3 were observed, although no dose dependency was observed in any samples, possibly due to low signal detection. High inter- and intrasubject variability was observed for IHC results for pERK, Ki67, and cleaved caspase-3.

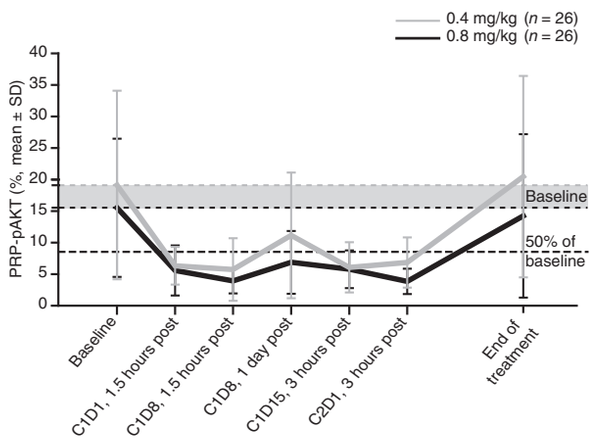

Figure 1.

Mean change from baseline following copanlisib infusion in pAKT in PRP in patients treated with copanlisib 0.4 and 0.8 mg/kg. Baseline was defined as cycle 1, day 1 preinfusion; 52 patients were included in the figure (26 each treated with copanlisib 0.4 and 0.8 mg/kg); 9 patients were not included due to nonevaluable pAKT values (below lower limit of quantification; $n = 8$) or missing values for cycle 1, days 1 and 8 ($n = 1$). C, cycle; D, day; post, postinfusion.

Pharmacodynamic changes in plasma glucose and glucose metabolism

Fifty-four patients were evaluated for analysis of plasma glucose as part of clinical laboratory measurements. Following the first copanlisib infusion, mean plasma glucose levels increased, peaking at 5 to 8 hours postinfusion, at both 0.4 and 0.8 mg/kg (**Fig. 3**); this was more pronounced at the 0.8 mg/kg dose. Levels gradually decreased thereafter. In the 0.8 mg/kg group, following the first treatment on cycle 1, day 1, preinfusion values for subsequent treatments through to cycle 2, day 1 remained close to baseline pretreatment levels of mean plasma glucose (**Fig. 3**). Time courses of mean serum levels of the glucose markers insulin and C-peptide showed similar dose-dependent, transient increases following copanlisib infusion at all dose levels, returning close to baseline at the end of dosing.

Forty-five patients had evaluable paired FDG-PET scans taken at screening and cycle 1, day 8 or 9. Following copanlisib infusion, transient and dose-dependent decreases in SUV_{max} were observed, suggesting reduced tumor glucose metabolism in response to treatment; the maximum decreases from baseline of >25% were observed in 75.0% (18/24) and 85.7% (18/21) of patients treated with copanlisib 0.4 and 0.8 mg/kg, respectively.

Pharmacodynamic changes in safety variables

Sixty-one patients were evaluated for systolic blood pressure changes as part of safety monitoring. A dose-dependent, transient, and reversible increase in mean systolic blood pressure was observed following the first copanlisib infusion at 0.4 and 0.8 mg/kg, peaking at approximately 2 hours postinfusion (Supplementary Fig. S1A and S1B). At 0.8 mg/kg, preinfusion values for subsequent treatments remained close to baseline mean systolic blood pressure, through to cycle 3, day 15, indicating that postdose blood pressure elevations were reversible and not prolonged.

Plasma biomarker analysis

Fifty-eight patients were evaluable for exploratory analysis of plasma proteins. Decreased levels of several cytokines, chemokines, and immune cell markers were observed at cycle 1, day 15 versus baseline following copanlisib treatment, particularly at 0.8 mg/kg in patients with lymphoma. These included macrophage inflammatory protein-1β, T-cell–specific protein RANTES, and CD27 ($P < 0.05$ each; Supplementary Table S3), suggesting a potential immunomodulatory effect of copanlisib treatment.

Different dynamics in changes in plasma levels were observed between metabolic markers (e.g., C-peptide, showing transient

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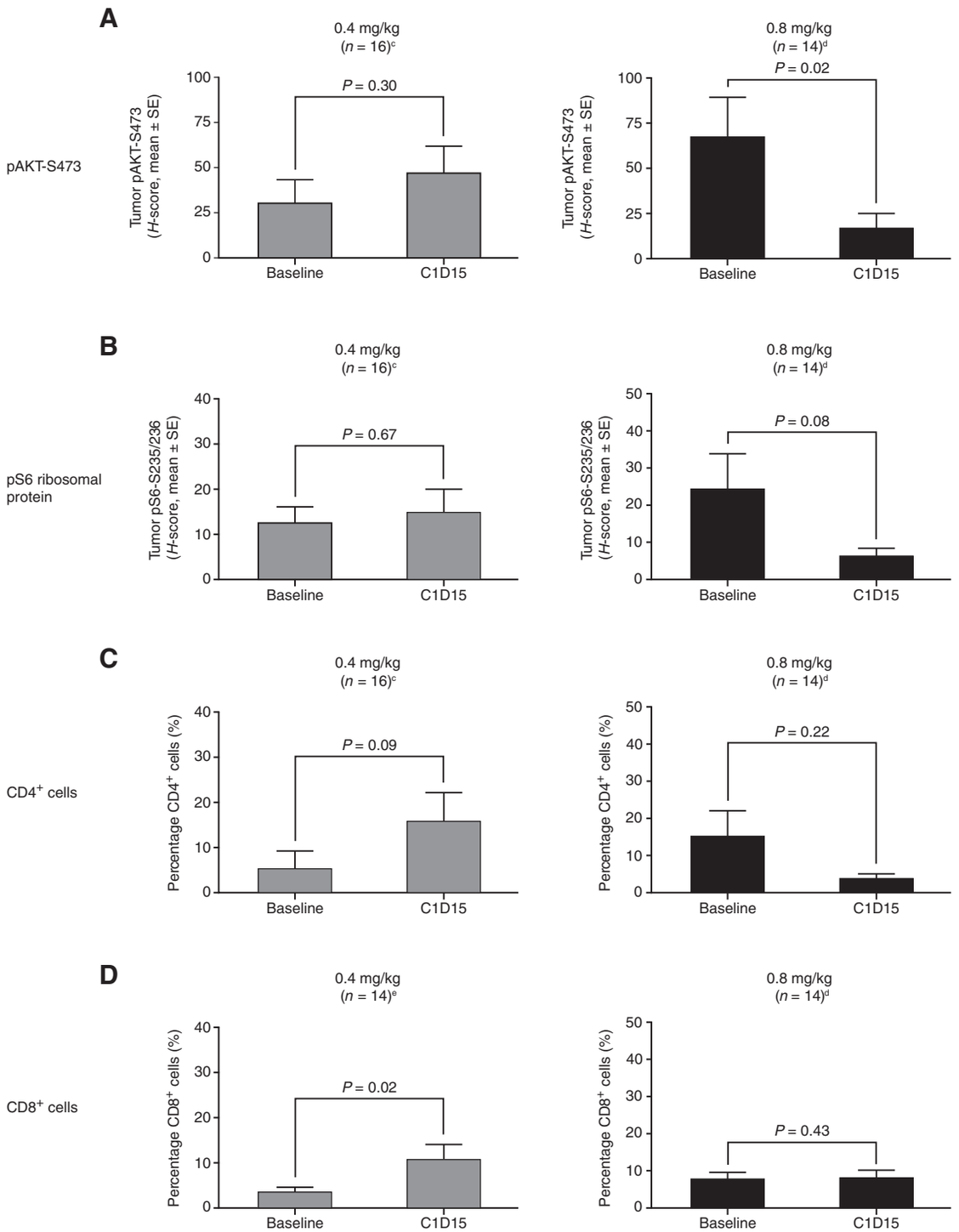

Figure 2.

Mean change in IHC *H*-scores for pAKT-S473[a] (**A**) and pS6[b] ribosomal protein (**B**) and percentages of $CD4^+$ (**C**) and $CD8^+$ lymphocyte subsets (**D**) in paired tumor biopsies from patients receiving copanlisib 0.4 and 0.8 mg/kg by IHC. *P* values (unadjusted) for the difference of *H*-scores or percentage of biomarker positive cells between the cycle 1, day 15, and baseline groups for each paired tumor were calculated using the Wilcoxon signed-rank test with continuity correction. [a]One patient with diabetes treated with copanlisib 45 mg had no change in pAKT-S473 *H*-score and is not included in the figure. [b]The single patient with diabetes treated with copanlisib 45 mg had a mild reduction in pS6 *H*-score from baseline to cycle 1, day 15 (115 vs. 105), and is not included in the figure. [c]Includes 8 patients each with solid tumors and lymphoma. [d]Includes 7 patients each with solid tumors and lymphoma. [e]Includes 6 patients with solid tumors and 8 patients with lymphoma. C, cycle; D, day.

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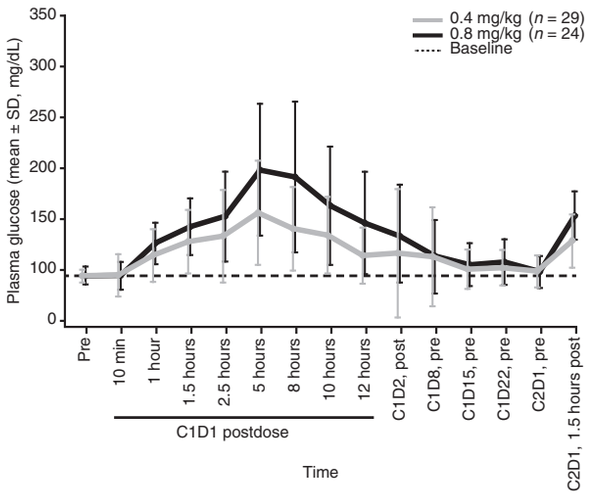

Figure 3.

Mean (±SD) plasma glucose over the first two cycles of treatment in patients receiving copanlisib 0.4 and 0.8 mg/kg. Fifty-four patients were included in this analysis (29 treated with copanlisib 0.4 mg/kg, 24 treated with 0.8 mg/kg, and 1 patient with diabetes treated with 60 mg); 53 patients are included in this figure (1 patient with diabetes treated with copanlisib 60 mg was not included); 9 patients were excluded from the analysis due to the use of systemic corticosteroids from predose or prior to the last plasma glucose measurement during the first two cycles of treatment. C, cycle; D, day; pre, preinfusion; post, postinfusion.

increases, returning to near baseline levels at cycle 1, day 15 and cycle 1, day 2, predose) and immune cell markers (e.g., markers associated with Tregs, CD27, and IL2Rα, showing prolonged decreases and slowly returning to baseline but generally remaining below baseline) following copanlisib treatment (Supplementary Fig. S2A–S2C). High baseline levels of CD27 and IL2Rα, with maximal decrease from baseline in these levels, were associated with maximal reduction in tumor area following copanlisib treatment in patients with lymphoma ($P < 0.05$, unadjusted; Supplementary Table S4), suggesting that copanlisib-mediated PI3K inhibition may modulate T-cell numbers in plasma.

Pharmacokinetics

Copanlisib pharmacokinetic profile simulation was based on 506 plasma concentrations from 62 treated patients evaluable for pharmacokinetic analysis (pharmacokinetic set): 33 receiving copanlisib 0.4 mg/kg, 27 receiving 0.8 mg/kg, and the 2 patients with diabetes who received 45 or 60 mg. One patient with lymphoma (0.4 mg/kg group) was not valid for pharmacokinetic assessment due to missing values. Approximately 90% of all observed copanlisib plasma concentration values fit within the 90% CIs of the simulated population pharmacokinetic profile (Supplementary Fig. S3). A three-compartment model best described the observed concentration of copanlisib (21). Application of the population pharmacokinetic model to the observed pharmacokinetic data provided estimates of exposure variables following the first copanlisib infusion (Supplementary Table S5). Dose-proportional increases in C_{max} and $AUC_{(0-168)}$ exposure were observed following infusion from 0.4 to 0.8 mg/kg, with similar exposure observed for 2 patients with diabetes. Specifically, the geometric means of C_{max} and $AUC_{(0-168)}$ for the dose groups 0.4 and 0.8 mg/kg were 250 and 479 μg/L and 1,554 and 3,127 μg·h/L, respectively (Supplementary Table S5; ref. 22). The respective C_{av} were $AUC_{(0-168)/168\ hours}$ or 9.25 and 18.6 μg/L.

Pharmacokinetic/pharmacodynamic correlations

Estimates of copanlisib plasma exposure, C_{max} or C_{av}, were related to pharmacodynamic variables in patients in the pharmacokinetic set with evaluable samples. C_{max} and C_{av} were significantly correlated with changes from baseline during treatment in insulin and C-peptide ($P <$ 0.05 each), whereas only copanlisib C_{max} was significantly correlated with changes in plasma glucose, SUV_{max} by FDG-PET, and pAKT-S743 in tumor biopsies ($P \leq 0.05$ each; **Fig. 4A–E**). Copanlisib exposure was not correlated with changes in pS6 levels in tumor biopsies (**Fig. 4F**).

The relationship between tumor size and copanlisib exposure was examined for 42 patients with evaluable data. Decreased tumor size from baseline following two treatment cycles was significantly correlated ($P < 0.05$) with time under treatment and AUC from time 0 to first tumor assessment (AUC_TS; Supplementary Fig. S4A and S4B), suggesting that copanlisib exposure led to tumor shrinking. Change in tumor size was correlated with C_{max}, although the association was not statistically significant.

Efficacy

All 33 patients with lymphoma were evaluable for efficacy. Two patients (6.1%) achieved a complete response as best response, 1 with peripheral T-cell lymphoma (angioimmunoblastic lymphadenopathy type) and 1 with DLBCL, both receiving copanlisib 0.8 mg/kg (equivalent to 60 mg absolute dose). Five patients (15.2%) achieved a partial response: 4 at the 0.8 mg/kg dose [2 patients with DLBCL and 1 each with follicular lymphoma (grade 3a) or mantle cell lymphoma], and 1 patient with DLBCL at the 0.4 mg/kg dose; the patient with follicular lymphoma had a partial response for over 10 treatment cycles. The objective response rate in lymphoma patients was 21.2% (7/33). Five patients (15.2%) had stable disease, 12 (36.4%) had disease progression, and 9 (27.3%) were not assessed or not evaluable (not all target lesions were assessed in 2 patients and 7 patients had discontinued treatment prior to the first assessment). The duration of treatment in patients with non-Hodgkin lymphoma receiving copanlisib 0.8 mg/kg demonstrated prolonged and durable stable disease in some patients (**Fig. 5A** and **B**).

All 30 patients with solid tumors were evaluable for efficacy; none achieved a complete response, and 1 (3.3%) achieved a partial response as best response (patient with endometrial adenocarcinoma receiving copanlisib 0.8 mg/kg, who achieved partial response over four cycles; this patient had a *TP53* G245S mutation and a frameshift mutation in *ARID1A* [S363fs*36], and a *PIK3CA* M1004V mutation). The objective response rate in solid tumor patients was 3.3% (1/30). Thirteen patients (43.3%) had stable disease (including 1 patient with endometrial adenocarcinoma receiving copanlisib 0.4mg/kg who had stable disease over 20 cycles), 14 (46.7%) had disease progression, and 2 (6.7%) were not assessed due to treatment discontinuation prior to the first assessment (**Fig. 5C** and **D**).

In non-Hodgkin lymphoma and solid tumor patients treated at 0.8 mg/kg, 24 patients had baseline tumor biomarker data, of which 21 patients had an evaluable response status. All patients with non-Hodgkin lymphoma ($n = 11$) had wild-type status of any genes in the PI3K/AKT/PTEN pathways, presence of PTEN protein, and presence of both PI3Kα/δ isoforms; therefore, no association of these biomarkers with response could be established in these patients.

DLBCL cell of origin classification into germinal center B-cell like (GCB) and activated B-cell like (ABC) subtypes has biological, prognostic, and potential therapeutic implications for copanlisib. Six patients with DLBCL were treated with copanlisib 0.8 mg/kg, of which 4 had tumor cell of origin data, 2 each were classified as ABC DLBCL

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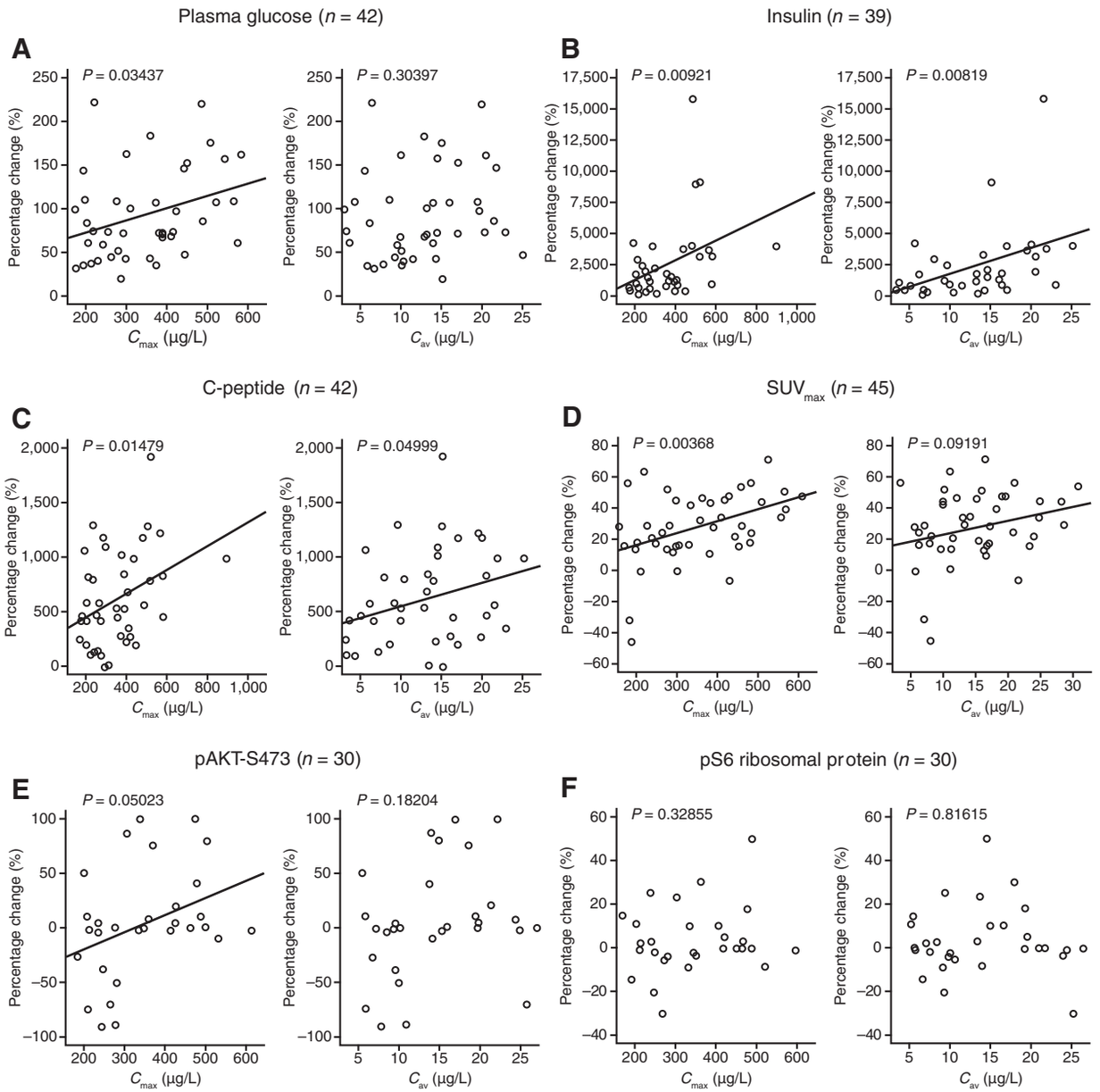

Figure 4.

Correlation between maximal percentage change in plasma glucose (**A**), insulin (**B**), C-peptide (**C**), SUV_{max} (**D**), pAKT-S473 (**E**), and pS6 ribosomal protein (**F**) from baseline during the first two treatment cycles and copanlisib plasma exposure (C_{max} or C_{av}) during the dosing interval of the maximal pharmacodynamic change. *P* values shown for Pearson correlation coefficients. Regression lines with intercept are only shown when $P < 0.1$.

and GCB DLBCL; both ABC DLBCL patients were nonevaluable for response, whereas 1 patient with GCB DLBCL had a partial response and the other had progressive disease. The patient with DLBCL with a partial response showed reduced tumor IHC staining for pAKT and pS6.

In solid tumor patients treated with copanlisib 0.8 mg/kg ($n = 13$), 1 patient with endometrial cancer with a partial response had a *TP53* G245S mutation, a frameshift mutation of *ARID1A* (S363fs*36), an unknown functional impact mutation in *PIK3CA* (M1004V), and high protein expression levels of PI3Kα and PI3Kγ in the tumor. Four of the 7 patients with stable disease had evidence of PI3K pathway activation: two with *PIK3CA* activation mutations of E542K and E545K, respectively, one with both *PTEN* copy number loss and PTEN protein loss, and one with *PTEN* copy number loss. All 5 patients with progressive disease had no detectable mutations in these genes or PTEN protein loss. Because of the low number of patients evaluated, none of these markers could be correlated with response.

Overall, an association between individual response to treatment and tumor PI3K pathway biomarker mutational status at baseline could not be established due to the low prevalence of *PIK3CA* or *PTEN* mutations.

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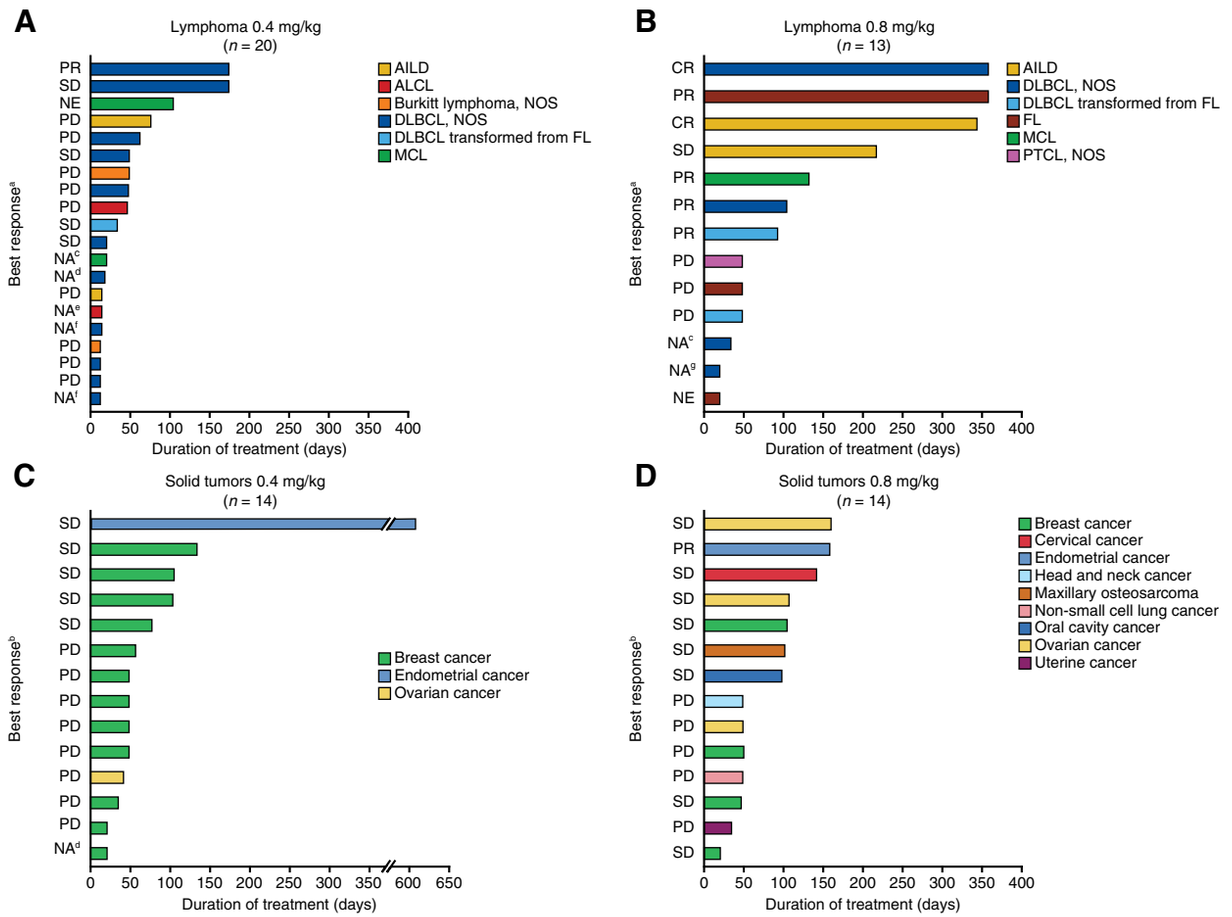

Figure 5.

Duration of treatment and best overall response of patients with non-Hodgkin lymphoma treated with copanlisib 0.4 mg/kg (**A**) or 0.8 mg/kg (**B**) and patients with solid tumors treated with copanlisib 0.4 mg/kg (**C**) or 0.8 mg/kg (**D**). [a]Best response according to modified Cheson criteria (20). [b]Best response according to the RECIST version 1.1 criteria (19). [c]Discontinued due to AE associated with clinical disease progression. [d]Discontinued due to consent withdrawal. [e]Discontinued due to AE not associated with clinical disease progression. [f]Discontinued due to PD—radiologic progression seen on the FDG-PET for pharmacodynamics at C1D8. [g]Discontinued due to PD—clinical progression. AILD, angioimmunoblastic lymphadenopathy-type; ALCL, anaplastic large cell lymphoma; CR, complete response; FL, follicular lymphoma; MCL, mantle cell lymphoma; NA, not assessed; NE, not evaluable; NOS, not otherwise specified; PD, progressive disease; PR, partial response; PTCL, peripheral T-cell lymphoma; SD, stable disease.

Safety

Safety was evaluated in all 63 treated patients; 59 (93.7%) had ≥1 TEAE of any grade (**Table 2**) and 53 (84.1%) had ≥1 drug-related TEAE of any grade. Thirty-three patients (52.4%) had TEAEs of worst grade 3 and 8 (12.7%) had TEAEs of worst grade 4. Serious TEAEs were reported in 36 patients (57.1%), with grade 3 as worst grade in 18 (28.6%) and grade 4 as worst grade in 3 (4.8%). Nine deaths due to grade 5 events occurred and 1 was considered drug related: pneumonitis in a patient receiving copanlisib 0.8 mg/kg. One additional death not associated with a TEAE occurred and was not otherwise specified. The most common TEAEs (all grade), irrespective of causality, included hyperglycemia (52.4%), fatigue (46.0%), and hypertension (41.3%; **Table 2**). The most common grade 3 or 4 TEAEs (≥10% combined) were hypertension (30.2%/0), hyperglycemia (22.2%/1.6%), hypophosphatemia (11.1%/0), and lymphocyte count decreased (7.9%/4.8%).

Fifteen patients (23.8%) experienced TEAEs leading to permanent discontinuation, most commonly grade 3 alkaline phosphatase increase in 2 patients (3.2%).

Discussion

This phase I study examined the pharmacodynamic effect of copanlisib on the PI3K signaling pathway, the relationship between copanlisib plasma exposure and pharmacodynamic biomarkers, including downstream targets of PI3K (pAKT in surrogate tissue and pAKT and pS6 in tumor biopsies), and hyperglycemia, which has previously been observed following copanlisib treatment (16) and is an on-target class effect of PI3K inhibitors (7, 23, 24). This was evaluated in patients with lymphoma and patients with solid tumors, following two cycles of copanlisib monotherapy over a wide range of approximately 20 to 65 mg total dose. Dosing was body-weight based [0.4 and 0.8 mg/kg (the latter equivalent to an absolute dose of 60 mg)] instead

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Table 2. Summary of safety and incidence of all-grade and grade 3 or 4 TEAEs occurring in ≥20% of patients overall.

n (%)[a]	Copanlisib 0.4 mg/kg (*n* = 34)		Copanlisib 0.8 mg/kg (*n* = 27)		Copanlisib 45 mg (*n* = 1)[b]		Copanlisib 60 mg (*n* = 1)[b]		Total (*N* = 63)	
Any TEAE	31 (91.2)		26 (96.3)		1 (100)		1 (100)		59 (93.7)	
Worst CTCAE grade										
1	0		0		0		0		0	
2	7 (20.6)		2 (7.4)		0		0		9 (14.3)	
3	15 (44.1)		17 (63.0)		1 (100)		0		33 (52.4)	
4	3 (8.8)		4 (14.8)		0		1 (100)		8 (12.7)	
5	6 (17.6)		3 (11.1)		0		0		9 (14.3)[c]	
TEAEs occurring in ≥15% of patients	All grade	Grade 3 or 4	All grade	Grade 3 or 4	All grade	Grade 3 or 4	All grade	Grade 3 or 4	All grade	Grade 3 or 4
Hyperglycemia	14 (41.2)	2 (5.9)	17 (63.0)	11 (40.7)	1 (100)	1 (100)	1 (100)	1 (100)	33 (52.4)	15 (23.8)
Fatigue	13 (38.2)	1 (2.9)	14 (51.9)	1 (3.7)	1 (100)	0	1 (100)	0	29 (46.0)	2 (3.2)
Hypertension	11 (32.4)	7 (20.6)	13 (48.1)	10 (37.0)	1 (100)	1 (100)	1 (100)	1 (100)	26 (41.3)	19 (30.2)
Nausea	11 (32.4)	1 (2.9)	12 (44.4)	0	1 (100)	0	0	0	24 (38.1)	1 (1.6)
Diarrhea	5 (14.7)	0	16 (59.3)	1 (3.7)	0	0	0	0	21 (33.3)	1 (1.6)
Anemia	8 (23.5)	1 (2.9)	10 (37.0)	5 (18.5)	0	0	0	0	18 (28.6)	6 (9.5)
Dyspnea	8 (23.5)	1 (2.9)	7 (25.9)	0	1 (100)	0	0	0	16 (25.4)	1 (1.6)
Pain	8 (23.5)	0	7 (25.9)	0	1 (100)	0	0	0	16 (25.4)	0
Constipation	8 (23.5)	0	6 (22.2)	0	1 (100)	0	0	0	15 (23.8)	0
Vomiting	6 (17.6)	2 (5.9)	8 (29.6)	0	1 (100)	0	0	0	15 (23.8)	2 (3.2)
Anorexia	7 (20.6)	0	6 (22.2)	0	1 (100)	0	0	0	14 (22.2)	0
Decreased appetite	7 (20.6)	0	6 (22.2)	0	1 (100)	0	0	0	14 (22.2)	0
Oral mucositis	3 (8.8)	0	11 (40.7)	0	0	0	0	0	14 (22.2)	0
Abdominal pain	4 (11.8)	0	7 (25.9)	1 (3.7)	1 (100)	0	0	0	12 (19.0)	1 (1.6)
Cough	6 (17.6)	0	6 (22.2)	1 (3.7)	0	0	0	0	12 (19.0)	1 (1.6)
Metabolism and nutrition disorders—other, specify	4 (11.8)	1 (2.9)	7 (25.9)	0	0	0	0	0	11 (17.5)	1 (1.6)
Fever	4 (11.8)	0	6 (22.2)	1 (3.7)	0	0	0	0	10 (15.9)	1 (1.6)
Headache	2 (5.9)	0	8 (29.6)	0	0	0	0	0	10 (15.9)	0
Hypophosphatemia	2 (5.9)	0	7 (25.9)	6 (22.2)	0	0	1 (100)	1 (100)	10 (15.9)	7 (11.1)

[a]Number (%) of patients with the specified event starting or worsening between start of treatment and 48 days after end of treatment.
[b]Patient with diabetes.
[c]An additional 6 deaths were recorded after the 48-day safety follow-up window after permanent treatment discontinuation.

of a flat dose. Copanlisib demonstrated clear PI3K pathway pharmacodynamic effects, with rapid and prolonged (~24 hours) inhibition of pAKT in surrogate tissue (PRP) through the first two treatment cycles. Inhibition was increased with copanlisib 0.8 mg/kg compared with 0.4 mg/kg, confirming dose-dependent target modulation, irrespective of interpatient variation, with recovery of pAKT levels to near baseline following the end of treatment, indicating reversible PI3K inhibition after drug clearance. Copanlisib inhibited pAKT and pS6 in lymphoma and solid tumor biopsies, with greater inhibition at 0.8 mg/kg, irrespective of interpatient variation, further supporting dose-dependent target modulation by copanlisib. Detectable changes in these markers that can be directly attributed to PI3K pathway inhibition have been reported following treatment with other mTOR/PI3K inhibitors (25, 26), supporting on-target PI3K inhibition by copanlisib in this population.

Consistent with the first-in-human study (16), copanlisib infusion led to dose-dependent, transient increases in plasma glucose. Additional markers of plasma glucose (insulin and C-peptide) and tumor glucose metabolism (FDG-PET) showed similar, transient, dose-dependent effects. These data support a role for copanlisib-mediated PI3K inhibition in blood glucose elevations through inhibition of insulin signaling (27, 28), and in tumor shrinkage, causing reduced tumor glucose metabolism.

Copanlisib plasma exposure variables demonstrated dose linearity and dose proportionality from 0.4 to 0.8 mg/kg, consistent with previous reports (16). Copanlisib C_{max} and C_{av} were associated with dose-dependent changes in glucose metabolism biomarkers (plasma glucose, insulin, C-peptide, and SUV_{max}) and the PI3K pathway biomarker pAKT-S473, supporting direct modulation of PI3K activity by copanlisib. Change in tumor size was significantly correlated with AUC_TS, supported by the observed reductions in tumor glucose metabolism (SUV_{max}) following treatment.

Treatment with copanlisib 0.8 mg/kg demonstrated a trend of reduction in the proportion of $CD4^+$ T-lymphocytes in biopsies from patients with lymphoma, with little effect seen with 0.4 mg/kg. $CD4^+$ T-lymphocytes are comprised of Treg and helper subsets, and total $CD4^+$ cell numbers have been positively associated with tumor grade in other tumor types, suggesting that reduced $CD4^+$ lymphocyte numbers in tumor tissue may be associated with improved prognosis (29). This preliminary result suggests a role for PI3K signaling in Treg-mediated suppression of antitumor immune cells (30), and supports a role for copanlisib-mediated inhibition of PI3K signaling in alleviating this mechanism of immunosuppression; however, the small sample sizes prevent conclusive interpretations. Although exploratory, copanlisib treatment reduced proinflammatory marker levels in plasma (IL2Rα and CD27), suggesting a broad immunomodulatory effect of copanlisib. However, a more detailed subset analysis of $CD4^+$ T-lymphocytes was not performed (the $FOXP3^+$ antigen was not evaluated), limiting conclusive interpretations on which Treg populations may be modulated by copanlisib activity. Together with

the high PI3K isoform expression observed in patients with lymphoma, these results support future validation studies and provide rationale for clinical studies of copanlisib in combination with immune checkpoint inhibitors (31).

Reduced levels of proliferation markers and increased levels of apoptosis markers (pERK, Ki67, and cleaved caspase-3, respectively) were observed in biopsies following copanlisib treatment, more frequently in patients with lymphoma receiving 0.8 mg/kg, further supporting the greater antitumor activity of the 0.8 mg/kg dose compared with 0.4 mg/kg. Although preliminary, these results may be associated with the higher response rate observed in lymphoma patients treated with copanlisib 0.8 mg/kg compared with 0.4 mg/kg.

Systolic blood pressure elevations following copanlisib infusion were consistent with the first-in-human study (16). The mechanism for postinfusion hypertension remains unclear, but may be explained by direct dysregulation of PI3K signaling in vascular endothelial cells (32), or insulin-dependent vasoconstriction (33, 34). Hypertension has been reported as a TEAE in other clinical studies of PI3K/AKT/mTOR pathway inhibitors (35).

Patients with solid tumors and malignant lymphoma, including those with diabetes, were included, with signs of copanlisib efficacy previously reported in these groups (15–17). The objective response rate in lymphoma patients was lower than in previous reports (15–17). However, copanlisib efficacy was a secondary objective of this study and included only 1 patient with indolent lymphoma, unlike in previous reports; the majority of patients with lymphoma here had aggressive subtypes (36). In addition, not all patients received copanlisib 0.8 mg/kg; 22 of 33 patients with lymphoma and 14/30 solid tumor patients received 0.4 mg/kg, hence exposure could have been insufficient for an objective response in some patients. Increased inhibition of PI3K pathway targets at the 0.8 mg/kg dose was consistent with greater objective responses observed in patients receiving copanlisib 0.8 mg/kg versus 0.4 mg/kg (two complete responses and five partial responses, vs. one partial response, respectively). These data support the improved efficacy of copanlisib 0.8 mg/kg over 0.4 mg/kg. However, the lack of association between inhibition of PI3K pathway biomarkers with individual response to treatment suggests that copanlisib-mediated inhibition of PI3K is insufficient as a marker of response.

Copanlisib exhibited a manageable safety profile at all doses tested, consistent with previous studies (15–17); hyperglycemia, fatigue, hypertension, and nausea were among the most common TEAEs and there were no unexpected safety findings. Although hyperglycemia and hypertension were frequent TEAEs (all grade/grade 3: 52.4%/22.2% and 41.3%/30.2%, respectively), they did not cause any discontinuations from treatment. The treatment of 2 patients with diabetes was feasible; however, the low number of patients with diabetes treated precluded evaluation of the safety of copanlisib doses higher than 32 mg (median dose in patients with diabetes in the phase I study; ref. 16), although this is being evaluated in ongoing studies (37).

In summary, copanlisib has demonstrated pharmacodynamic evidence of dose- and exposure-dependent target engagement, PI3K pathway modulation, and clinical proof of mechanism, with manageable safety, and higher efficacy at a dose of 0.8 mg/kg (equivalent to a 60 mg fixed dose). These findings support the current recommended dosing schedule and the clinical use of copanlisib in patients with solid tumors and hematologic malignancies (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209936s000lbl.pdf). These findings also provide a biomarker hypothesis for clinical studies of copanlisib in combination with immune checkpoint inhibitors (NCT03711058).

Disclosure of Potential Conflicts of Interest

F. Morschhauser is an employee/paid consultant at Celgene, is a consultant at Roche, and is a member of advisory board of Epizyme. J.-P. Machiels has an unpaid consultant/advisory board relationship at MSD, Janssen, Cue Biopharma, ALX Oncology, Roche, AstraZeneca, Bayer, Innate, Merck Serono, BI, BMS, and Novartis. G. Salles has received speakers bureau honoraria from Amgen, Abbvie, Novartis, Roche, Servier, Takeda, Autolus, Celgene, Gilead, Epizyme, Janssen, Karyopharm, Merck, and Morphosys. D. Cunningham reports receiving other commercial research support from Amgen (REAL 3: Trial for the Royal Marsden NHS Foundation Trust), Sanofi (Trial for the Royal Marsden NHS Foundation Trust), Janssen (IMYC Trial: Trial for the Royal Marsden NHS Foundation Trust), Merck (ICONIC/POLEM Trial: Trial for the Royal Marsden NHS Foundation Trust), Merrimack (PLATFORM Trial: Trial for the Royal Marsden NHS Foundation Trust), AstraZeneca (FRGR Trial: Trial for the Royal Marsden NHS Foundation Trust), MedImmune (PLATFORM Trial: Trial for the Royal Marsden NHS Foundation Trust), Celgene (PROSPECT R Trial: Trial for the Royal Marsden NHS Foundation Trust), Bayer (PROSPECT R Trial: Trial for the Royal Marsden NHS Foundation Trust), 4SC (EMERGE Trial: Trial for the Royal Marsden NHS Foundation Trust), Clovis (PLATFORM Trial: Trial for the Royal Marsden NHS Foundation Trust), and Eli Lilly (PLATFORM Trial: Trial for the Royal Marsden NHS Foundation Trust). F. Peyrade has received speakers bureau honoraria from Janssen and Merck. H.-T. Arkenau is an Executive Medical Director at HCA/Sarah Cannon and has an unpaid consultant/advisory board relationship at Roche, Guardant, Bayer, and Bicycle. I. Genvresse is a Clinical Development Leader at Bayer AG. L. Liu is a Director at Bayer US LLC. K. Köchert is an employee/paid consultant at Bayer AG and has ownership interest (including patents) in Bayer AG. K. Shen is a Lead Statistician at Bayer U.S. LLC. C. Kneip is an Assay Technology Expert at Bayer AG. C.E. Peña is a Director, Global Biomarkers Representative, at Bayer AG, and has ownership interest (including patents) in Bayer AG. J. Grevel is a Senior Scientist at BAST Inc. Ltd. G. Cisternas is a Clinical Pharmacology Medical Lead at Bayer AG. C. Granvil is a Director Clinical Pharmacology Lead at Bayer U.S. LLC. A. Awada is has an advisory or speaker role at and has received research grants to his institute from at Eli Lilly, AstraZeneca, Bayer AG, Leo Pharma, Amgen, EISAI, BMS, Pfizer, Novartis, MSD, Genomic Health, and Ipsen; reports receiving a commercial research grant from MSD and BMS; and has received speakers bureau honoraria from Eli Lilly. No potential conflicts of interest were disclosed by the other authors.

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