Pharmacokinetic and pharmacodynamic relationship of copanlisib in patients with malignant lymphoma and advanced solid tumors: confirmation of on-target PI3K inhibitor activity

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Purpose: Copanlisib is an intravenous pan-class I PI3K inhibitor with efficacy and manageable safety in patients with indolent lymphoma and solid tumors. The pharmacodynamic effects of copanlisib relative to dose and plasma exposure were evaluated.

Patients and Methods: Patients with lymphoma or solid tumors were randomized to 0.4 mg/kg or 0.8 mg/kg copanlisib, 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 tumor-infiltrating lymphocytes in paired tumor biopsies by immunohistochemistry, the relationship between estimated plasma exposure and pharmacodynamic markers, response, and safety.

Results: Sixty-three patients received copanlisib. PRP pAKT levels showed sustained reductions from baseline with 0.4 mg/kg and 0.8 mg/kg copanlisib (median inhibition 73.8% [range −94.9 to 144.0] and 79.6% [range −96.0 to 408.0], respectively). Tumor levels of pAKT ($p<0.05$), pS6 and CD4+ lymphocytes were reduced versus baseline with 0.8 mg/kg copanlisib; there was little effect with 0.4 mg/kg. Dose-related transient plasma glucose elevations were observed following treatment. Estimated copanlisib exposure significantly correlated with changes in plasma pAKT and glucose metabolism markers. There were two complete responses and six partial responses; 7 of 8 responders received 0.8 mg/kg copanlisib. Adverse events (all-grade) included hyperglycemia (52.4%), fatigue (46.0%) and hypertension (41.3%).

Conclusions: Copanlisib demonstrated dose-dependent pharmacodynamic evidence of target engagement and PI3K pathway modulation/inhibition in tumor cells. These results support the use of 0.8 mg/kg copanlisib in patients with solid tumors and hematologic malignancies.

Introduction

The phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway regulates multiple cellular processes, including metabolism, survival, and proliferation (Jellusova and Rickert 2016). PI3K pathway dysregulation causes overactive signaling, seen in many cancers (Thorpe et al. 2015; Vanhaesebroeck et al. 2010), and loss of the tumor-suppressor phosphatase with tensin homology (PTEN) is a key driver of tumorigenesis through the PI3K pathway (Song et al. 2012). 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 (Burris et al. 2018; Flinn et al. 2016; Gopal et al. 2014; Heudel et al. 2017; Juric et al. 2017; Mayer et al. 2017; Miller et al. 2015; Sarker et al. 2015).

Copanlisib is an intravenously administered, highly selective, pan-class I PI3K inhibitor with potent activity against the p110α and p110δ isoforms (Liu et al. 2013). Copanlisib has demonstrated robust single-agent anti-tumor activity and a manageable safety profile, with a response rate of 59.2% reported in a Phase II study in patients with relapsed or refractory indolent lymphoma treated with copanlisib (Dreyling et al. 2017b). Copanlisib is approved in the USA for the treatment of patients with relapsed follicular lymphoma (US Food and Drug Administration 2017).

Copanlisib has previously demonstrated dose-dependent on-target hyperglycemia (Dreyling et al. 2017a; Dreyling et al. 2017b; Patnaik et al. 2016), consistent with the on-target class effect of PI3K pathway inhibitors (Busaidy et al. 2012). 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.

In order to demonstrate target engagement, PI3K pathway modulation, and downstream effects by copanlisib, this study evaluated the pharmacodynamic (PD) effects of single-agent copanlisib relative to dose and plasma exposure in patients with malignant lymphoma and solid tumors.

Methods

Study design

This open-label, single-arm, multi-center Phase I study (NCT02155582) comprised four dose cohorts: two parallel cohorts of non-diabetic patients with either malignant lymphoma or solid tumors, randomized to treatment with 0.4 mg/kg or 0.8 mg/kg copanlisib (body-weight-based dosing, with 0.8 mg/kg the maximum tolerated dose identified in the first-in-human study (Patnaik et al. 2016) and approximately equivalent to a fixed dose of 60 mg). Additionally, two parallel cohorts of diabetic patients were randomized to treatment with fixed doses of 45 mg or 60 mg copanlisib. Forty non-diabetic patients (20 each with lymphoma or solid tumors) and 12 diabetic patients (with lymphoma or solid tumors) were planned to be enrolled.

The primary objective was to evaluate the relationship between exposure and PD 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 diabetic patients, and to evaluate clinical response.

Weight-based dosing was used in non-diabetic patients as the pharmacokinetic (PK) profile of copanlisib is not body-weight-dependent, giving a broad range of individual exposures – this was a prerequisite to achieving the study primary objective. The 45 mg dose in diabetic patients was planned to provide additional dose information in between the since approved 60 mg dose and the median dose of 32 mg received by diabetic patients in the first-in-human study of copanlisib (Patnaik et al. 2016).

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 aged ≥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 ≥1 prior chemo-immunotherapy or immunotherapy-based regimen, or advanced and/or refractory solid tumors with known high prevalence (≥30%) of *PIK3CA* or *PTEN* alteration (including breast and uterine cancers, squamous cell lung cancer, cervical cancer, head and neck cancers, and prostate and ovarian cancers), were eligible for the study. Other key inclusion criteria were: biopsy-accessible tumor; Eastern Cooperative Oncology Group performance status of ≤2; ≥1 bi-dimensionally measurable lesion (Cheson et al. 2007) in case of lymphoma, or ≥1 solid tumor lesion measurable by computed tomography or magnetic resonance imaging (Eisenhauer et al. 2009); and adequate bone marrow, liver, and renal

functions. Diabetic patients had to have diagnosed type 1 or type 2 diabetes mellitus with glycated hemoglobin ≤8.5% and fasting blood glucose ≤160 mg/dL. Patients were not selected based on *PIK3CA* or *PTEN* alteration status at study entry.

Treatment

Copanlisib 0.4 mg/kg or 0.8 mg/kg (non-diabetic patients) or 45 mg or 60 mg (diabetic patients) was administered as a 1-hour intravenous infusion on days 1, 8, and 15 of a 28-day cycle. Patients had to fast for ≥8 hours prior to and 2 hours following the start of the first copanlisib infusion. Fasting blood glucose had to be ≤160 mg/dL prior to the first infusion, and for subsequent infusions, ≤160 mg/dL if fasting or ≤200 mg/dL if non-fasting. Dose reductions from 0.8 mg/kg to 0.6 mg/kg and 0.4 mg/kg, or from 60 mg to 45 mg 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 re-escalation was not permitted.

PD variables

Primary PD variables were maximum change from baseline in expression of phosphorylated AKT (pAKT) in platelet-rich plasma during treatment, and in plasma glucose during the first two treatment cycles. Secondary PD variables included: area under the concentration–time curve from time 0 to 168 hours ($AUC_{(0\text{-}168)}$) of copanlisib after each infusion during two treatment cycles; adverse events (AEs); maximum change from baseline in insulin and C-peptide during the first two treatment cycles; change in [^{18}F]-fluorodeoxyglucose (FDG) uptake after copanlisib dosing (for non-diabetic patients 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 PK 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 PD or safety samples for evaluation (detailed in the Supplementary Methods).

Assessments

Blood for platelet-rich plasma pAKT assays was collected at screening; on cycle 1, day 1 pre-dose and at 1.5 hours after the start of infusion (post-dose); on cycle 1, day 8 at 1.5 hours post-dose; on cycle 1, day 9 within 24 hours post-dose; on cycle 1, day 15 and cycle 2, day 1 at 3 hours post-dose; and within 14 days of last copanlisib dose (end of treatment visit). Plasma samples for glucose, insulin, C-peptide, and PK assessments were collected on cycle 1, day 1 at 10 minutes and 1, 1.5, 2.5, 5, 8, and 24 hours post-dose; pre-dose on cycle 1, day 8; and on cycle 2, day 1 at 1.5 hours after copanlisib infusion. FDG-PET scans were performed at screening and within 24 hours after the start of infusion on cycle 1, day 8. Paired FDG-PET scans were evaluated by central review (ICON plc, Dublin, Ireland). Archival tumor biopsies (where available) and fresh biopsies were collected at screening, and fresh biopsies were collected on cycle 1, day 15 or 16 (within 24 hours after the start of infusion on day 15). Paired tumor samples were used for analysis of potential PD biomarkers by immunohistochemistry (IHC); full details are provided in the Supplementary Methods. Tissues collected at screening also underwent exploratory analysis for DNA mutations by next-generation sequencing (NGS) and expression of PI3K protein isoforms and PTEN by IHC. Plasma for exploratory biomarker evaluation was collected pre-dose, and at 3 hours post-dose on cycle 1, days 1 and 15, and cycle 2, day 1. Plasma analytes including 286 protein markers were measured via multiplexed immunosorbent assay using a Human

DiscoveryMAP® v3.3 platform (Myriad RBM, Inc., Austin, TX, USA). Thirty-two of the analytes were below the limit of detection and were not included in further analysis.

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 (CTCAE) 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 RECIST 1.1 criteria for patients with solid tumors, and the modified Cheson criteria (Cheson et al. 2007) for patients with lymphoma. Tumors were assessed by magnetic resonance imaging, computed tomography, or positron emission tomography-computed tomography at screening, on cycle 2, day 22, and subsequently on day 22 of alternate cycles.

PK/PD analysis

A population PK model (developed using data from >297 patients treated with copanlisib in Phase I and II trials) (Reif et al. 2016), along with PK observations from this study, were used to derive individual PK parameter estimates and copanlisib exposure variables, including the inter-patient variability of copanlisib PK, using NONMEM version 7.3 (ICON plc, Dublin, Ireland) (see Supplementary Materials 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 PD 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., NC, USA). PKPD regression analyses were performed using R 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). All patients who received ≥1 dose of copanlisib (full analysis set) were included in analyses of safety and efficacy, with exact 95% confidence intervals provided for efficacy. All treated patients with valid pAKT platelet-rich plasma samples (at both baseline and post-dose within the first two cycles, with total AKT count above the lower limit of quantitation [LLOQ, ≥5000] in both samples) were included in the PD analysis of pAKT. All treated patients with valid plasma glucose measurement at baseline and post-dose during the first two cycles, and who did not receive systemic corticosteroids from pre-dose or before the last valid plasma glucose measurement during the first two cycles, were included in PD analysis of plasma glucose. All other PD analyses were performed on the full analysis set using descriptive statistics. All treated patients with valid PK data (≥1 PK measurement post-dose during the first two cycles) were included in PK analysis. Unless otherwise indicated, PK means were calculated if at least two-thirds of measured individual data were above the LLOQ. Response to treatment was correlated to time under treatment and individual exposure estimates, and individual exposure estimates were correlated to PD biomarkers, using Pearson's correlation coefficient.

Results

Patients and treatment

Sixty-four patients were randomized to copanlisib treatment; one patient had a serious AE during screening and was not treated. Sixty-three patients received ≥1 copanlisib infusion (full analysis set), 33 with lymphoma and 30 with solid tumors, including two diabetic patients with solid tumors. Thirty-four non-diabetic 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 diabetic patient each received 45 mg and 60 mg; no additional diabetic patients could be recruited and enrolled for treatment, and the diabetic cohorts 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 lymphoma patients (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 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 had fresh tumor samples at baseline, and 44 had archival samples; up to 47 patients had samples of sufficient quality for baseline analysis of PI3K/AKT/PTEN by IHC and/or mutation testing by NGS. Positive tumor-cell staining for PI3Kα protein was observed in 94.7% of lymphoma sample (18/19) and 88.9% of solid tumor samples (24/27), whereas PI3Kδ protein expression was detected in 94.7% (18/19) and 29.6% (8/27) of samples, respectively (Supplementary Table 1). PI3Kβ and PI3Kγ protein expression was detected in a proportion of all samples, but to a slightly 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 not detected in any lymphoma 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 2).

PD changes in PI3K biomarkers

pAKT in platelet-rich plasma

Fifty-four patients were evaluable for PD analysis of pAKT levels in platelet-rich plasma. Overall, pAKT levels rapidly reduced by >50% from baseline following the first copanlisib infusion on cycle 1, day 1 in patients receiving copanlisib 0.4 mg/kg and 0.8 mg/kg; the reduction in pAKT was maintained during 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 post-infusion (Figure 1). Inhibition was pronounced at 1.5 hours post-dose, and was sustained for approximately 24 hours. Following the end of treatment, pAKT levels returned to near baseline levels at both doses. Inter-patient variability was high throughout treatment at both dose levels, mainly attributed to low pAKT levels (≤3%) at

baseline in seven patients. In the two diabetic patients treated with 45 mg or 60 mg copanlisib, median inhibition from baseline reached 90.0% following the first infusion in the patient receiving 45 mg copanlisib, and 94.6% following the cycle 1, day 8 infusion in the patient receiving 60 mg (data not shown).

IHC analysis of tumor signaling and immune environment

Thirty-one patients had paired tumor biopsy samples evaluable for immunohistochemical 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 0.4 mg/kg copanlisib, 14 treated with 0.8 mg/kg, and one diabetic patient treated with 45 mg. H-scores for pAKT-S473 were significantly reduced at cycle 1, day 15 versus baseline in samples from patients receiving 0.8 mg/kg copanlisib (group mean reduction of 73.4%; 95% CI −94.7, −7.2; $p<0.05$), with little effect seen in patients receiving 0.4 mg/kg (group mean increase of 31.1%; 95% CI −31.3, 64.8 *[Bayer: please provide P-value]*) (Figure 2A).

H-scores for pS6 in paired biopsies were more visibly reduced at day 15 versus baseline in patients receiving 0.8 mg/kg copanlisib (group mean reduction of 72.4%; 95% CI −34.2, −2.4) compared with patients receiving 0.4 mg/kg (group mean increase of 19.5%; 95% CI −8.3, 13.2) *[Bayer: please provide P-value]* (Figure 2B).

Following copanlisib treatment, a dose-dependent reduction in the proportion of CD4+ cells in tumor biopsies was generally observed versus baseline (Figure 2C), with a mean reduction of 71% staining in samples from lymphoma patients receiving 0.8 mg/kg copanlisib *[Bayer: please provide P-values if available]*. 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 (Figure 2D), or on the proportion of CD3+ cells (data not shown).

H-scores for pERK were increased in at day 15 versus baseline in samples from patients receiving 0.4 mg/kg copanlisib (mean H score increase of 74%, p=0.06), although there was a trend towards decreased pERK H-scores in samples from patients receiving 0.8 mg/kg (mean H-score reduction of -37%, p=0.22; data not shown). H-scores for Ki67 were generally reduced at day 15 versus baseline, more commonly from lymphoma samples at the 0.8 mg/kg dose (reductions observed in 5/7 [71.0%] lymphoma samples). Some 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 (data not shown). High inter- and intra-subject variability was observed for IHC results for pERK, Ki67 and cleaved caspase-3.

PD 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 continuously increased, peaking at 5-8 hours post infusion, at both 0.4 mg/kg and 0.8 mg/kg (Figure 3); the increase was more pronounced at the 0.8 mg/kg dose. Levels gradually decreased thereafter, approaching baseline at cycle 1, day 8, pre-dose. In the 0.8 mg/kg group, following the first treatment on cycle 1, day 1, pre-infusion values for subsequent treatments through to cycle 2, day 1 remained close to baseline pre-treatment levels of mean plasma glucose (Figure 3). Similar results were observed in one diabetic patient (treated with 60 mg), with mean plasma glucose increasing from baseline 100.0 mg/dL to a maximum of 305.0 mg/dL at 5 hours post infusion on cycle 1, day 1; levels continually decreased to 135.0 mg/dL before the second infusion on cycle 1, day 8 (data not shown). 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 (data not shown).

Forty-five non-diabetic 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 generally 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 0.4 mg/kg and 0.8 mg/kg copanlisib, respectively (data not shown).

PD 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 both 0.4 mg/kg and 0.8 mg/kg, peaking at approximately 2 hours post-infusion (Supplementary Figure 1). At 0.8 mg/kg, pre-infusion values for subsequent treatments remained close to baseline mean systolic blood pressure, through to cycle 3, day 15, indicating that post-dose blood pressure elevations were not prolonged and were reversible.

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 the 0.8 mg/kg dose in lymphoma patients. These included macrophage inflammatory protein-1 beta, T-cell-specific protein RANTES, and CD27 ($p<0.05$ each) (Supplementary Table 3), 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 increases, returning to near baseline levels at C1D15 and

C1D2, pre-dose) and immune cell markers (e.g. markers associated with regulatory T-cells [Tregs], CD27 and IL-2Rα; showing prolonged decreases and slowly returning to baseline but generally remaining below baseline) following copanlisib treatment (Supplementary Figure 2). High baseline levels of CD27 and IL-2Rα, with maximal decrease from baseline in these levels, were associated with maximal reduction in tumor area following copanlisib treatment in lymphoma patients ($p<0.05$, unadjusted) (Supplementary Table 4), suggesting that copanlisib-mediated PI3K inhibition may modulate T-cell numbers in plasma.

PK

Copanlisib PK profile simulation was based on 506 plasma concentrations from 62 treated patients who were evaluable for PK analysis (PK set): 33 receiving 0.4 mg/kg copanlisib, 27 receiving 0.8 mg/kg, and the two diabetic patients who received 45 mg or 60 mg. One lymphoma patient in the 0.4 mg/kg group was not valid for PK assessment due to missing values. Approximately 90% of all observed copanlisib plasma concentration values fit within the 90% confidence intervals of the simulated population PK profile (Supplementary Figure 3). A three-compartment model best described the observed concentration of copanlisib (Reif et al. 2016). Application of the population PK model to the observed PK data provided estimates of exposure variables following the first copanlisib infusion (Supplementary Table 5). Dose-proportional increases in C_{max} and AUC exposure were observed following infusion from 0.4 mg/kg to 0.8 mg/kg (Supplementary Figure 4); similar exposure was observed for two diabetic patients (data not shown).

PKPD correlations

Estimates of copanlisib plasma exposure, C_{max} or C_{av}, were related to PD variables in all patients in the PK 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), while only

copanlisib C_{max} was significantly correlated with changes in plasma glucose, SUV_{max} by FDG-PET and pAKT-S743 in tumor biopsies ($p<0.05$ each) (Figure 4). Copanlisib exposure was not correlated with pS6 in tumor biopsies.

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 Figure 5), suggesting that ongoing copanlisib exposure led to tumor shrinking. Change in tumor size was correlated with C_{max} though the association did not reach statistical significance.

Efficacy

All thirty-three patients with lymphoma were evaluable for efficacy. Two patients (6.1%) achieved a complete response as best response, one with peripheral T-cell lymphoma (angioimmunoblastic lymphadenopathy-type) and one with DLBCL, both receiving copanlisib 0.8 mg/kg (equivalent to 60 mg absolute dose). Five patients (15.2%) achieved a partial response: four at the 0.8 mg/kg dose (two patients with DLBCL and one each with follicular lymphoma [grade 3a] or mantle cell lymphoma), and one patient with DLBCL at the 0.4 mg/kg dose; the patient with follicular lymphoma had a partial response for over 10 treatment cycles. One DLBCL patient with a partial response showed reduced immunohistochemical staining for pAKT and pS6 in tumor tissue (Supplementary Figure 6) *[Bayer: please confirm paired biopsy results for other responders, if available]*. 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 nine (27.3%) were not assessed or not evaluable (not all target lesions were assessed in two patients and seven patients had discontinued treatment prior to the first assessment).

All 30 patients with solid tumors were evaluable for efficacy; none achieved a complete response, and one (3.3%) achieved a partial response as best response (patient with endometrial adenocarcinoma receiving 0.8 mg/kg copanlisib, who achieved partial response over four cycles; this patient had a *TP53* G245S mutation and a frameshift mutation in *ARID1A* [S363fs*36], as well as 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 one patient with endometrial adenocarcinoma receiving 0.4 mg/kg copanlisib who had stable disease over 20 cycles), 14 (46.7%) had disease progression, and two (6.7%) were not assessed due to treatment discontinuation prior to the first assessment.

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.

Safety

Safety was evaluated in all 63 treated patients; 59 (93.7%) had ≥1 treatment-emergent AE (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 eight (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%). There were nine deaths due to grade 5 events; one was considered drug-related: pneumonitis in a patient receiving 0.8 mg/kg copanlisib. One additional death occurred that was not associated with a TEAE and was due to death – 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, the most common being grade 3 alkaline phosphatase increase in two patients (3.2%).

Discussion

This Phase I study examined the relationship between copanlisib exposure and PD 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 (Patnaik et al. 2016) and is an on-target class effect of PI3K inhibitors (Bendell et al. 2012; Brown et al. 2015; Sarker et al. 2015). This relationship was evaluated in lymphoma patients and patients with solid tumors, following two cycles of copanlisib monotherapy over a wide range of approximately 20-65 mg total dose. Dosing in non-diabetic patients used body-weight-based dosing (0.4 and 0.8 mg/kg [the latter equivalent to an absolute dose of 60 mg]), instead of a flat dose. Copanlisib demonstrated clear PI3K pathway PD effects, with rapid and prolonged (~24 h) inhibition of pAKT in surrogate tissue (platelet-rich plasma) through the first two treatment cycles. Inhibition was increased with 0.8 mg/kg copanlisib compared with 0.4 mg/kg, confirming dose-dependent target modulation, irrespective of inter-patient variation, with recovery of pAKT levels to near baseline following the end of treatment, indicating reversible PI3K inhibition after drug clearance. Copanlisib also inhibited pAKT and pS6 in lymphoma and solid tumor biopsies, with greater inhibition at 0.8 mg/kg, irrespective of inter-patient 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 (Ang et al. 2012; Ang et al. 2017), supporting on-target PI3K inhibition by copanlisib in this population.

Consistent with the first-in-human study (Patnaik et al. 2016), 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 (Foukas et al. 2006; Knight et al. 2006), and in tumor shrinkage, causing reduced tumor glucose metabolism.

Copanlisib plasma exposure variables demonstrated dose linearity and dose proportionality from 0.4 mg/kg to 0.8 mg/kg, consistent with previous reports (Patnaik et al. 2016). 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, further 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 0.8 mg/kg copanlisib demonstrated clear reduction in the proportion of CD4+ T-lymphocytes in biopsies from lymphoma patients, with little effect seen with 0.4 mg/kg. CD4+ T-lymphocytes are comprised of regulatory (Treg) and helper (Th) 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 (Han et al. 2014). This preliminary result supports a role for PI3K signaling in Treg-mediated suppression of anti-tumor immune cells (Abu-Eid et al. 2014), 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. Though exploratory, copanlisib treatment also reduced CD4+ T-lymphocyte marker levels in plasma (IL-2Rα 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 lymphoma patients, these results support future validation studies and provide rationale for clinical studies of copanlisib in combination with immune checkpoint inhibitors (Liu et al. 2017).

Reduced levels of proliferation markers and increased levels of apoptosis markers (pERK, Ki67 and cleaved caspase-3, respectively), were observed in some biopsies following copanlisib treatment, generally more frequently in lymphoma patients receiving 0.8 mg/kg, further supporting the greater anti-tumor 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 0.8 mg/kg copanlisib compared with 0.4 mg/kg.

Systolic blood pressure elevations following copanlisib infusion were consistent with the first-in-human study (Patnaik et al. 2016). The mechanism for post-infusion hypertension remains unclear, but may be explained by direct dysregulation of PI3K signaling in vascular endothelial cells (Ha et al. 2011), or insulin-dependent vasoconstriction (Montagnani et al. 2001; Symons et al. 2009). Hypertension has been reported as a TEAE in other clinical studies of PI3K/AKT/mTOR pathway inhibitors (Seront et al. 2016).

Patients with solid tumors and malignant lymphoma, including those with diabetes, were included, with signs of copanlisib efficacy previously reported in these groups (Dreyling et al. 2017a; Dreyling et al. 2017b; Patnaik et al. 2016). Overall, the objective response rate in

lymphoma patients was lower than in previous reports (Dreyling et al. 2017a; Dreyling et al. 2017b; Patnaik et al. 2016). However, copanlisib efficacy was a secondary objective of this study and only one patient with indolent lymphoma was included, unlike in previous reports; the majority of lymphoma patients here had aggressive subtypes (Lenz et al. 2017). Additionally, not all patients received 0.8 mg/kg copanlisib - 22/33 lymphoma patients 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 the higher number of objective responses observed in patients receiving 0.8 mg/kg versus 0.4 mg/kg copanlisib (two complete responses and four partial responses, versus one partial response, respectively). Overall, these data support the improved efficacy of 0.8 mg/kg copanlisib 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 (Dreyling et al. 2017a; Dreyling et al. 2017b; Patnaik et al. 2016); 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 two diabetic patients was feasible; however, the low number of diabetic patients treated precluded evaluation of the safety of copanlisib doses higher than 32mg (median dose in diabetic patients in the Phase I study (Patnaik et al. 2016)), although this is being evaluated in ongoing studies (Dreyling et al. 2018).

In summary, copanlisib has demonstrated PD evidence of dose- and exposure-dependent target engagement, PI3K pathway modulation, and clinical proof of mechanism, with higher efficacy and manageable safety at a dose of 0.8 mg/kg. These findings support the current recommended dosing schedule (US Food and Drug Administration 2017) and the clinical use of copanlisib in patients with solid tumors and hematologic malignancies. These findings also provide rationale for clinical studies of copanlisib in combination with immune checkpoint inhibitors (NCT03711058).

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Tables

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 one patient; [b]Includes one grade 3a and two 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 three 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 three patients

DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group

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)

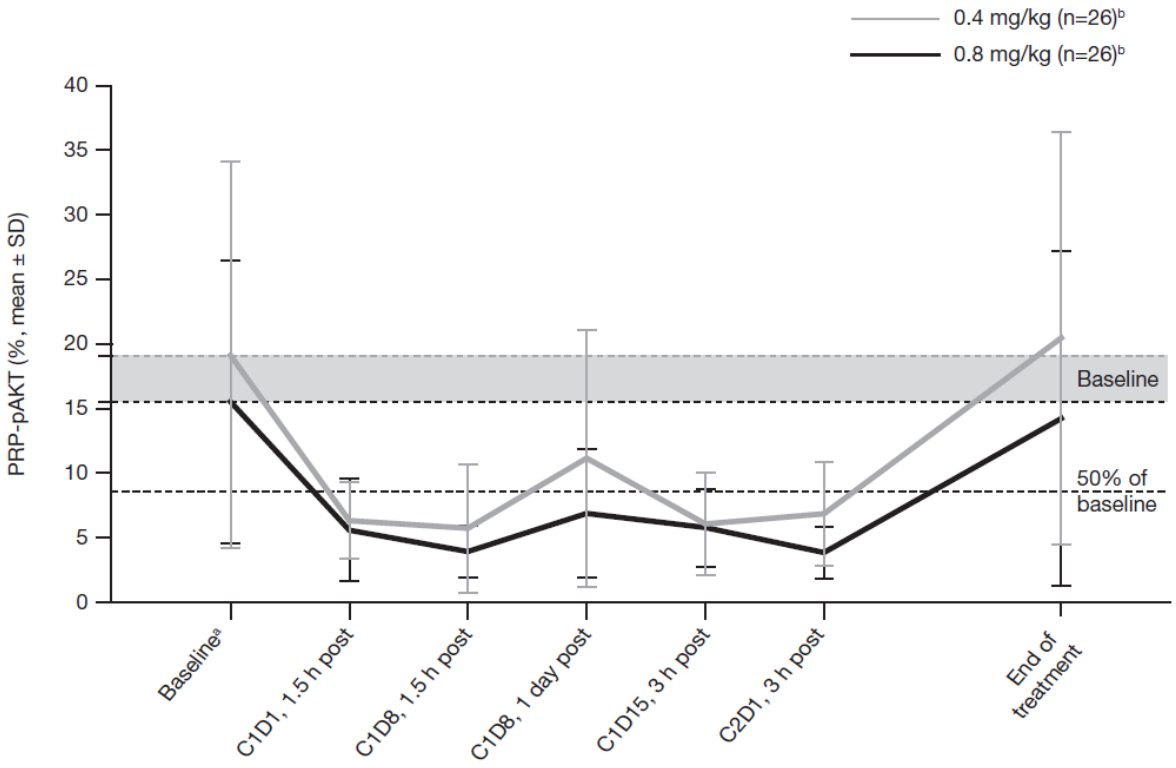
Anemia	8 (23.5)	1 (2.9)	10 (37.0)	5 (18.5)	0	0	0	0	18 (28.6)	6 (9.5)
Dyspnea	8 (23.5)	1 (2.9)	7 (25.9)	0	1 (100)	0	0	0	16 (25.4)	1 (1.6)
Pain	8 (23.5)	0	7 (25.9)	0	1 (100)	0	0	0	16 (25.4)	0
Constipation	8 (23.5)	0	6 (22.2)	0	1 (100)	0	0	0	15 (23.8)	0
Vomiting	6 (17.6)	2 (5.9)	8 (29.6)	0	1 (100)	0	0	0	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 subjects with the specified event starting or worsening between start of treatment and 48 days after end of treatment; [b]Diabetic patient; [c]An additional six deaths were recorded after the 48-day safety follow-up window after permanent treatment discontinuation

CTCAE, Common Terminology Criteria for Adverse Events; TEAE, treatment-emergent adverse event

Figures

Figure 1. Mean change from baseline following copanlisib infusion in pAKT in platelet-rich plasma in non-diabetic patients treated with copanlisib 0.4 mg/kg and 0.8 mg/kg

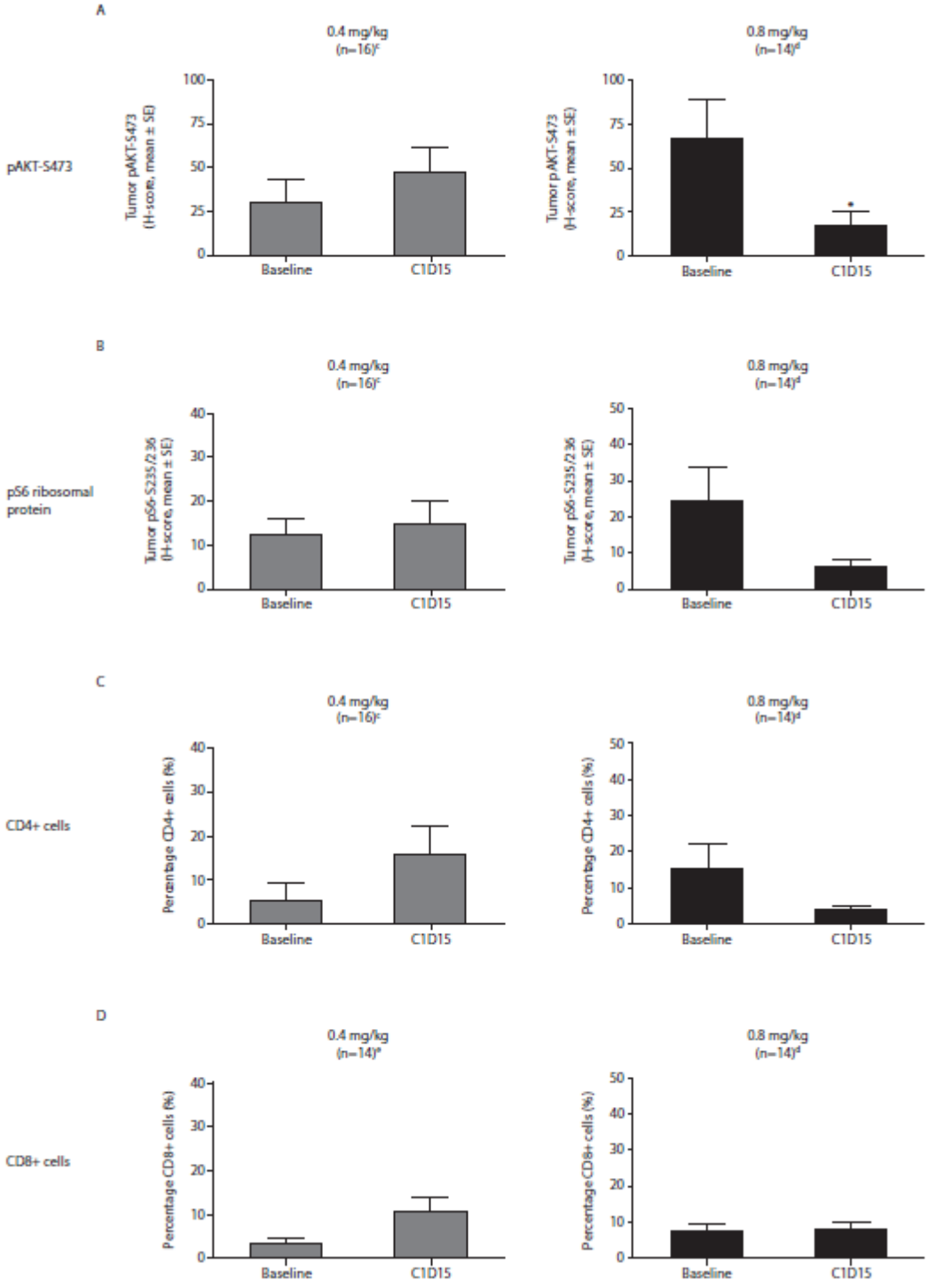


[a]Baseline was defined as cycle 1, day 1 pre-infusion; [b]52 non-diabetic patients were included in the figure (26 each treated with 0.4 mg and 0.8 mg/kg copanlisib); nine patients were not included due to non-evaluable pAKT values (below LLOQ; n=8) or missing values for cycle 1, days 1 and 8 (n=1)

*p≤0.05, Wilcoxon signed-rank test with continuity correction

C, cycle; D, day; pAKT, phosphorylated protein kinase B; post, post-infusion; PRP, platelet-rich plasma; SD, standard deviation

Figure 2. Mean change in immunohistochemistry 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 mg/kg and 0.8 mg/kg by IHC

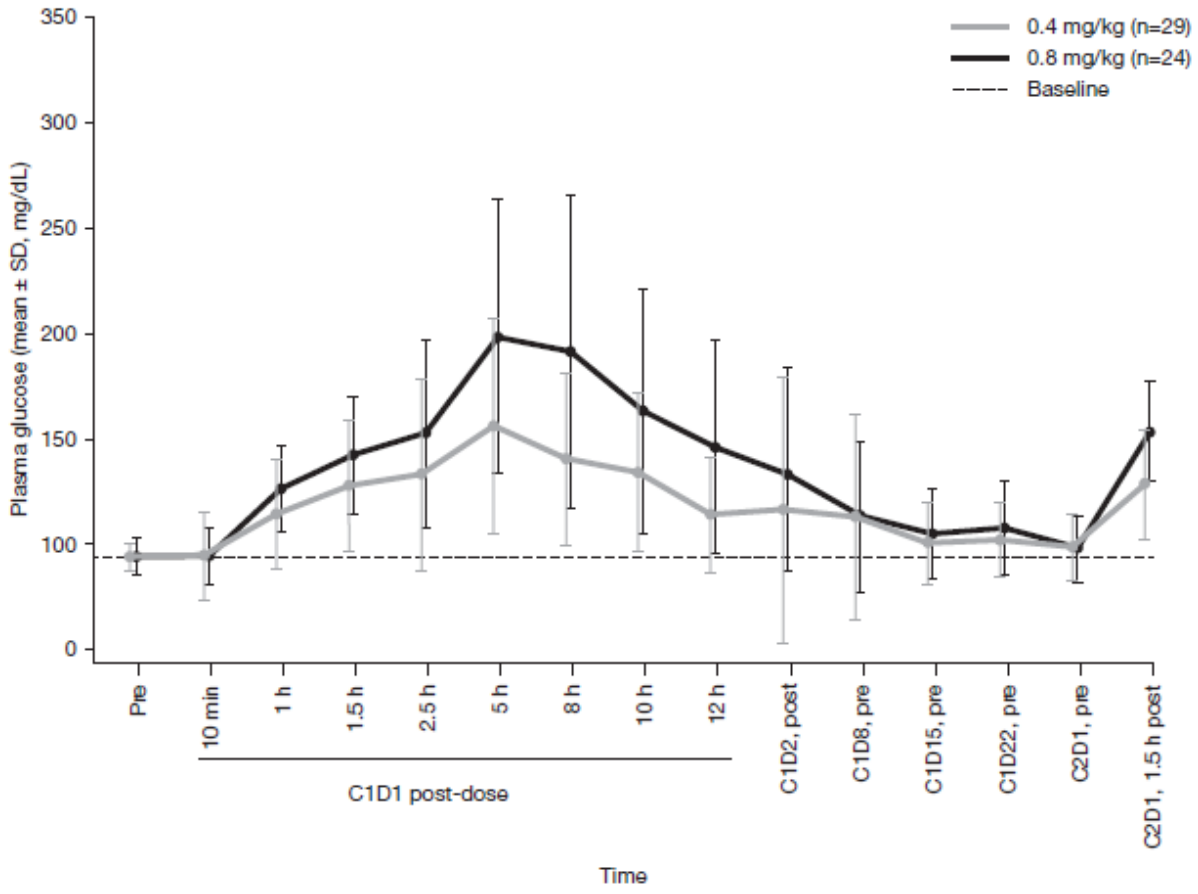


[a]One diabetic patient treated with 45 mg copanlisib had no change in pAKT-S473 H-score and is not included in the figure; [b]The single diabetic patient treated with 45 mg copanlisib had a mild reduction in pS6 H-score from baseline to cycle 1, day 15 (115 versus 105) and is not included in the figure; [c]Includes eight patients each with solid tumors and lymphoma; [d]Includes seven patients each with solid tumors and lymphoma; [e]Includes six patients with solid tumors and eight patients with lymphoma

*, $p<0.05$; SE, standard error

[Bayer: please provide p values for all comparisons, if available]

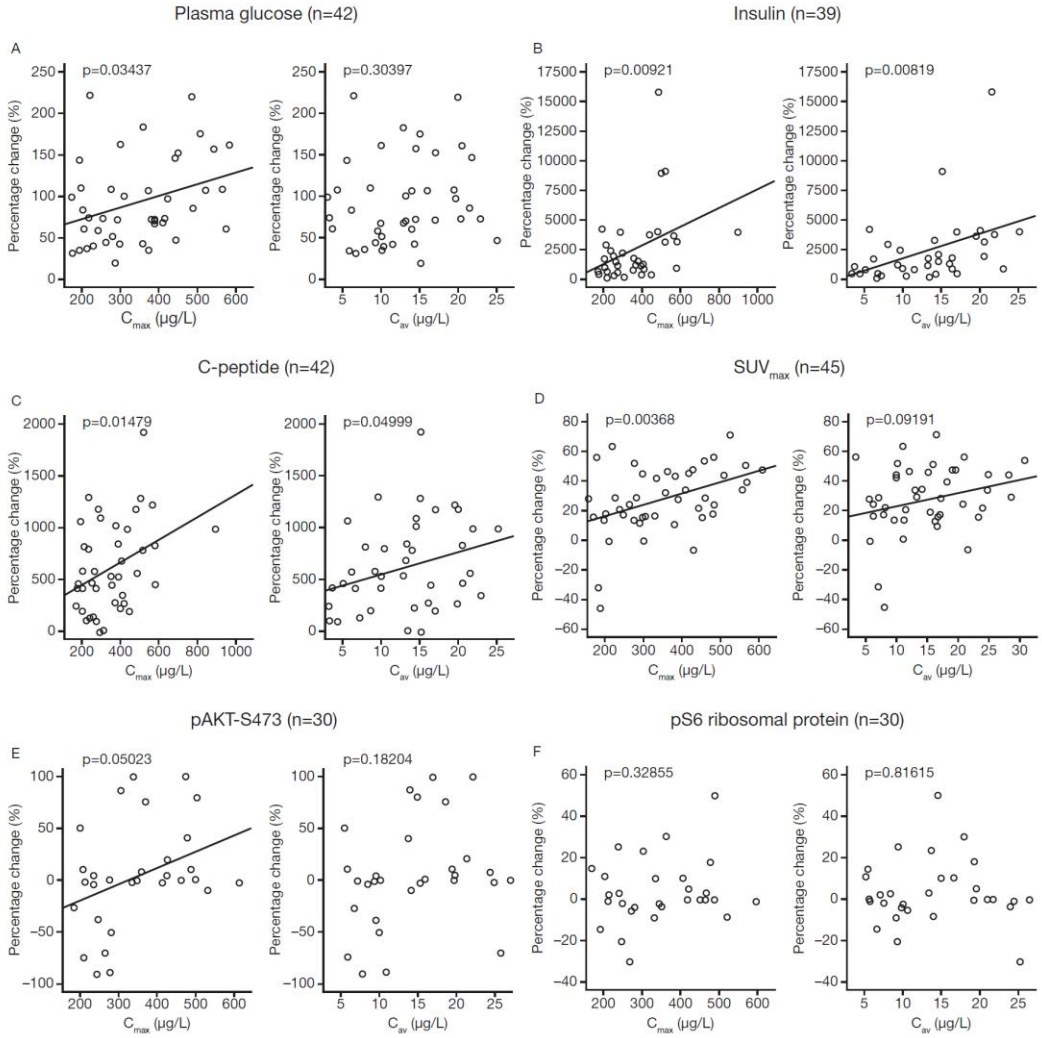
Figure 3. Mean (±standard deviation) plasma glucose pre- and over the first 24 hours post-infusion at cycle 1 day 1, and pre- and post-infusion at later cycles in non-diabetic patients receiving copanlisib 0.4 mg/kg and 0.8 mg/kg[a]



[a]Fifty-four patients were included in this analysis (29 treated with 0.4 mg/kg copanlisib, 24 treated with 0.8 mg/kg, and one diabetic treated with 60 mg copanlisib); only 53 non-diabetic patients are included in this figure - one diabetic patient treated with 60 mg copanlisib was not included; nine patients were excluded from the analysis due to the use of systemic corticosteroids from pre-dose or prior to the last plasma glucose measurement during the first 2 cycles of treatment.

C, cycle; D, day; pre, pre-infusion; SD, standard deviation

Figure 4. Correlation between maximal % 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 PD change



p values shown for Pearson's correlation coefficients. Regression lines with intercept are only shown when $p<0.1$

C_{av}, average observed concentration following single dosing; C_{max}, maximum observed concentration following single dosing; pAKT, phosphorylated protein kinase B; SUV_{max}, maximum standardized uptake

Supplementary materials

Methods

Criteria for replacement of patients

Patients could be replaced if: of the 10 patients with lymphoma or solid tumors per dose group, fewer than six had evaluable paired [^{18}F]-fluorodeoxyglucose positron emission tomography or evaluable fresh tumor biopsies, or the minimum required evaluable phosphorylated protein kinase B (pAKT) in surrogate tissue; or if a patient had missing pAKT values at screening and cycle 1, day 1, or at 1.5 hours after the start of copanlisib infusion on cycle 1, days 1 or 8. Diabetic patients could be replaced if fewer than six with lymphoma or solid tumors at 45 mg or 60 mg were evaluable for safety after the first two treatment cycles.

Pharmacodynamic (PD) assessments

Platelet-rich plasma (PRP) for pAKT assays was prepared through centrifugation of 5 mL blood samples. PRP was then divided into two tubes (500 µL per tube). One tube was treated with thrombin receptor activating peptide (TRAP) (final concentration 40 µM), whereas the other tube was untreated, labelled as mock. Both tubes were incubated at 37ºC for 10 minutes followed by centrifugation. PRP aliquots were analyzed in duplicate using a phospho(S473)/total AKT assay kit (K15100D-2; Meso Scale Diagnostics, LLC, Maryland, USA). Mean percentage pAKT results were calculated from the average of the TRAP and mock duplicates as follows: per replicate sample, total AKT and pAKT raw values were used to calculate percentage pAKT based on %pAKT = ((2 × phospho-signal)/(phospho-signal + total signal)) × 100.

Fresh or archival tumor biopsies were prepared as formalin-fixed, paraffin-embedded tissue for analysis by single-stain immunohistochemistry (IHC), performed by Mosaic Laboratories (Lake Forest, CA, USA). Biomarkers evaluated by IHC included phosphoproteins downstream of PI3K (pAKT-T308, pAKT-S473, pS6, pMAPK, pERK-T202/Y204), T-cell markers (CD3, CD4, CD8), and apoptosis / proliferation markers (cleaved caspase-3 and Ki67). Single-stain IHC testing was performed by Mosaic Laboratories (Lake Forest, CA, USA) using the following antibodies: anti-pAKT-S473 rabbit clone 14-5 (#M3628, Dako, CA, USA), anti-pAKT-T308 rabbit clone C31E5E (#2965, Cell Signaling, Danvers, MA, USA), anti-pS6-S235/236 rabbit clone D57.2.2E (#4858, Cell Signaling), anti-pERK-T202/Y204 rabbit clone 20G11 (#4376, Cell Signaling), anti-Ki-67 mouse clone MiB-1 (#M7240, Dako), anti-CD3 mouse clone LN10 (#CD3-565-L-CE-S, Leica), anti-CD4 mouse clone 4B12 (#M7310, Dako), anti-CD8 mouse clone C8/144B (#M7103, Dako), and anti-cleaved caspase-3 rabbit clone 5A1E (#9664, Cell Signaling). IHC evaluation of PI3K protein isoforms was performed using the following antibodies: anti-PTEN rabbit clone 138G, anti-p110α mouse clone D55D5, and anti-p110δ rabbit clone A-8 *[Bayer to provide supplier details for the PI3K protein isoform antibodies]*.

IHC-stained tissues were evaluated for staining intensity using H-scores; H-scores were based on the average intensity of positively stained cells each sample, weighted as high [3+], medium [2+], or low [1+] intensity. Group mean reductions in H-score from baseline to cycle 1, day 15 were calculated using the formula 100 × ((baseline−cycle 1, day 15)/baseline). Negative values represent a decrease in tumor biomarker expression following copanlisib, whereas positive values represent an increase.

Pharmacokinetic (PK) methods and PKPD analysis

Copanlisib plasma concentrations were determined following protein precipitation followed by separation employing high-pressure liquid chromatography and tandem mass spectrometric detection. The nominal calibration range for copanlisib was from 0.503 μg/L and 0.508 μg/L (lower limit of quantitation) to 107 μg/L and 109 μg/L (upper llimit of quantitation).

PK parameter estimates were as follows: maximum observed concentration after single-dose administration (C_{max}); average observed concentration after single-dose administration (C_{av}); C_{av} from time 0 to first tumor assessment (C_{av}_TS); area under the concentration–time curve (AUC) from time 0 to infinity; AUC from 0 to 168 h ($AUC_{(0-168)}$); and AUC from time 0 to first tumor assessment (AUC_TS).

Supplementary Tables

Supplementary Table 1. Summary of baseline PI3K isoform protein expression in tumor tissues collected at screening

PI3K isoform	Positive samples, n (%)[a]	Median H-score	Mean (SD) H-score
Lymphoma cohort (n=19)			
α	18 (94.7)	40	66.6 (64.6)
δ	18 (94.7)	70	90.3 (77.3)
β	9 (47.4)	0	22.5 (38.2)
γ	12 (63.2)	10	45.0 (65.6)
Solid tumor cohort (n=27)			
α	24 (88.9)	60	71.5 (47.6)
δ	8 (29.6)	0	6.4 (14.4)
β[b]	16 (64.0)	10	30.7 (43.0)
γ[b]	21 (84.0)	30	58.6 (70.4)

[a]Positive staining was defined by the presence of any detectable level of staining; [b]n=25

PI3K, phosphatidylinositol-3 kinase; SD, standard deviation

Supplementary Table 2. Summary of baseline PTEN protein loss, *PTEN* loss of function mutations and *PIK3CA*- and *AKT*-activating mutations

n/total number of samples (%)	Lymphoma	Solid tumors
PTEN protein loss by IHC	1/19 (5.3)	5/27 (18.5)
PTEN mutations by NGS	0/19 (0)	4/19 (21.1)
PIK3CA or *AKT1/3* mutations by NGS	0/19 (0)	4/19 (21.1)
Total	1/20 (5.0)	10/27 (37.0)

IHC, immunohistochemistry; NGS, next-generation sequencing

Supplementary Table 3. Summary of plasma biomarkers with decreased levels at cycle 1, day 15 versus baseline[a] following copanlisib infusion

	Lymphoma, 0.4 mg/kg (n=17)		Solid tumors, 0.4 mg/kg (n=14)		Lymphoma, 0.8 mg/kg (n=13)		Solid tumors, 0.4 mg/kg (n=14)	
Plasma analyte	**Fold change**	**p value**	**Fold change**	**p value**	**Fold change**	**p value**	**Fold change**	**p value**
MIP-1-β	0.57	0.00	0.47	0.00	0.32	0.00	0.44	0.00
T-cell specific protein RANTES	0.85	0.60	0.58	0.06	0.43	0.01	0.61	0.05
CD27	0.84	0.23	0.87	0.04	0.52	0.01	0.76	0.00
IL-10	0.78	0.08	1.01	0.64	0.56	0.00	1.03	0.45
MCP-1	0.85	0.32	0.79	0.04	0.67	0.00	0.77	0.04
IL-2 receptor-α	0.83	0.10	0.83	0.02	0.69	0.03	0.76	0.00
HCC-4	1.01	0.86	0.93	0.30	0.76	0.00	0.89	0.07
CCL15	0.92	0.47	0.89	0.06	0.77	0.01	0.84	0.01
MIP-3-β	0.88	0.46	0.69	0.00	0.78	0.07	0.83	0.04
CD163	0.92	0.18	0.99	0.90	0.79	0.07	0.84	0.00
CD5L	0.92	0.39	0.98	0.79	0.80	0.00	0.88	0.01
CD40L	1.17	0.40	0.84	0.18	0.81	0.27	0.68	0.15

CCL15, C-C motif chemokine 15; CD5L, CD5 antigen-like; CD40L, CD40 ligand; HCC-4, chemokine CC-4; IL, interleukin; MCP-1, monocyte chemotactic protein; MIP, macrophage inflammatory protein

[a]Change versus baseline is shown as fold change

Supplementary Table 4. Plasma cytokine/chemokine markers significantly associated with the maximum change in tumor area, and whose maximum change from baseline were associated with the maximum change in tumor area following copanlisib treatment at cycle 1 day 15 in patients with lymphoma[a]

Plasma analyte	Log2(fold difference)	Fold difference	SD	p value	p value (adjusted)
Analytes at baseline significantly associated with maximum change in tumor area[b]					
CD27	-2.28	0.21	0.5	<0.001	0.17
IL-2Rα	-1.53	0.35	0.65	0.03	0.61
Macrophage inflammatory protein-3 α (MIP-3α)	0.93	1.91	0.43	0.04	0.61
Erythropoietin	1.44	2.71	0.6	0.03	0.61
Monocyte chemoattractant protein 2 (MCP-2)	1.65	3.14	0.73	0.04	0.61
Myeloid progenitor inhibitory factor 1 (MPIF-1)	2.11	4.32	0.84	0.02	0.61
Fibulin-1C	2.38	5.22	0.94	0.02	0.61
TNF ligand superfamily member 13 (APRIL)	3.06	8.35	1.04	0.008	0.61
TNF ligand superfamily member 12 (Tweak)	3.13	8.73	1.09	0.01	0.61
Prostasin	4.42	21.40	1.33	0.004	0.61
Analytes with maximal change from baseline significantly associated with maximum change in tumor area[c]					
Prostasin	-1.95	0.26	0.48	<0.001	0.48
Periostin	-1.47	0.36	0.54	0.01	0.59
TNF ligand superfamily member 12 (Tweak)	-1.43	0.37	0.38	0.001	0.48

TNF ligand superfamily member 13 (APRIL)	-1.02	0.49	0.40	0.02	0.59
Myeloid progenitor inhibitory factor 1 (MPIF-1)	-0.89	0.54	0.37	0.03	0.59
Glucose-6-phosphate isomerase (G6PI)	0.49	1.40	0.21	0.03	0.59
Myeloperoxidase	0.49	1.41	0.23	0.05	0.59
IL-2Rα	0.60	1.52	0.33	0.08	0.59
Lactoferrin	0.75	1.68	0.26	0.01	0.59
CD27	1.02	2.03	0.30	0.003	0.59
Apolipoprotein H	1.11	2.16	0.46	0.03	0.59
Fatty-acid binding protein (FABP), liver	1.30	2.46	0.50	0.02	0.59
Mast/stem cell growth factor receptor	1.44	2.72	0.66	0.04	0.59
Sclerostin	1.62	3.08	0.71	0.03	0.59

[a]Includes 23 patients in total; 13 patients receiving 0.4 mg/kg copanlisib and 10 patients receiving 0.8 mg/kg copanlisib; [b]Decreasing direction of change in fold difference indicates higher baseline levels of protein are associated with greater decreases in tumor area (e.g. CD27 and IL-2Rα), whereas increasing direction indicates low baseline levels of protein are associated with greater decreases in tumor area (e.g. Tweak and prostasin); [c]Decreasing direction of change in fold difference indicates greater increases from baseline in protein levels are associated with greater decreases in tumor area (e.g. prostasin and periostin), and increasing direction of change indicates greater decreases from baseline in protein levels are associated with greater decreases in tumor area (e.g. sclerostin).

SD, standard deviation; TNF, tumor necrosis factor

Supplementary Table 5. Estimation of copanlisib exposure variables following the first infusion or up to the time of the first tumor size assessment (TS) based on the population PK model

Dose	Exposure metric[a]	n	Minimum	Maximum	Median	Geometric mean	Geometric CV
0.4 mg/kg	C_{max} (µg/L)	33	171.8	389.0	251.7	250.1	0.224
	C_{av} (µg/L)	33	3.29	20.48	9.72	8.96	0.493
	$AUC_{(0-168)}$ (µg·h/L)	33	575.0	3509.8	1570.1	1553.7	0.489
	AUC (µg·h/L)	33	575.3	4793.3	1730.7	1677.6	0.536
	C_{av}_TS (µg·h/L)	20	2.32	20.16	7.63	7.56	0.455
	AUC_TS (µg·h/L)	20	2785.0	23687.0	9425.7	8894.2	0.460
0.8 mg/kg	C_{max} (µg/L)	27	355.6	897.4	487.9	478.9	0.199
	C_{av} (µg/L)	27	10.17	36.43	19.19	18.27	0.302
	$AUC_{(0-168)}$ (µg·h/L)	27	1721.6	4780.7	3331.2	3127.0	0.291
	AUC (µg·h/L)	27	1749.3	5189.2	3489.5	3312.5	0.311
	C_{av}_TS (µg·h/L)	21	8.90	24.90	15.31	15.72	0.264
	AUC_TS (µg·h/L)	21	10461.5	29309.1	18135.9	18622.0	0.274
Diabetic patients[b]	C_{max} (µg/L)	2	441.4	480.8	461.1	460.7	0.060

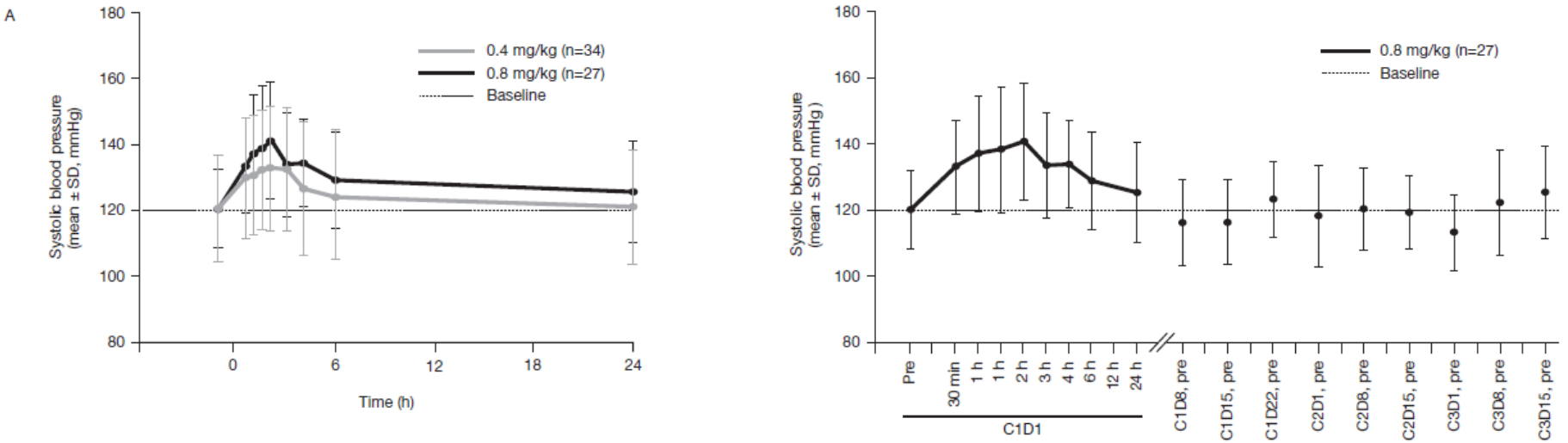
	C_{av} (µg/L)	2	18.78	22.61	20.69	20.61	0.132
	$AUC_{(0-168)}$ (µg·h/L)	2	3018.6	4225.4	3622.0	3571.4	0.241
	AUC (µg·h/L)	2	3379.6	4641.1	4010.3	3960.4	0.227
	C_{av}_TS (µg·h/L)	1[b]	13.88	13.88	13.88	13.88	NA
	AUC_TS (µg·h/L)	1	16006.3	16006.3	16006.3	16006.3	NA
All patients	C_{max} (µg/L)	62	171.8	897.4	355.8	338.5	0.400
	C_{av} (µg/L)	62	3.29	36.43	13.73	12.55	0.572
	$AUC_{(0-168)}$ (µg·h/L)	62	575.0	4780.7	2495.6	2164.3	0.563
	AUC (µg·h/L)	62	575.3	5189.2	2569.7	2319.5	0.586
	C_{av}_TS (µg·h/L)	42	2.32	24.90	12.21	11.06	0.539
	AUC_TS (µg·h/L)	42	2785.0	29309.1	14178.3	13051.0	0.547

[a]C_{max} and C_{av} were estimated using actual dosing, $AUC_{(0-168)}$ was estimated from a single nominal dose, and AUC was calculated from dose/plasma clearance; [b]One patient received a dose of 45 mg and one received a dose of 60 mg

AUC, area under the concentration–time curve; C_{av}, average observed concentration following single dosing; CV, coefficient of variation; C_{max}, maximum observed concentration following single dosing; NA, not available; PK, pharmacokinetic; TS, from time 0 to first tumor assessment

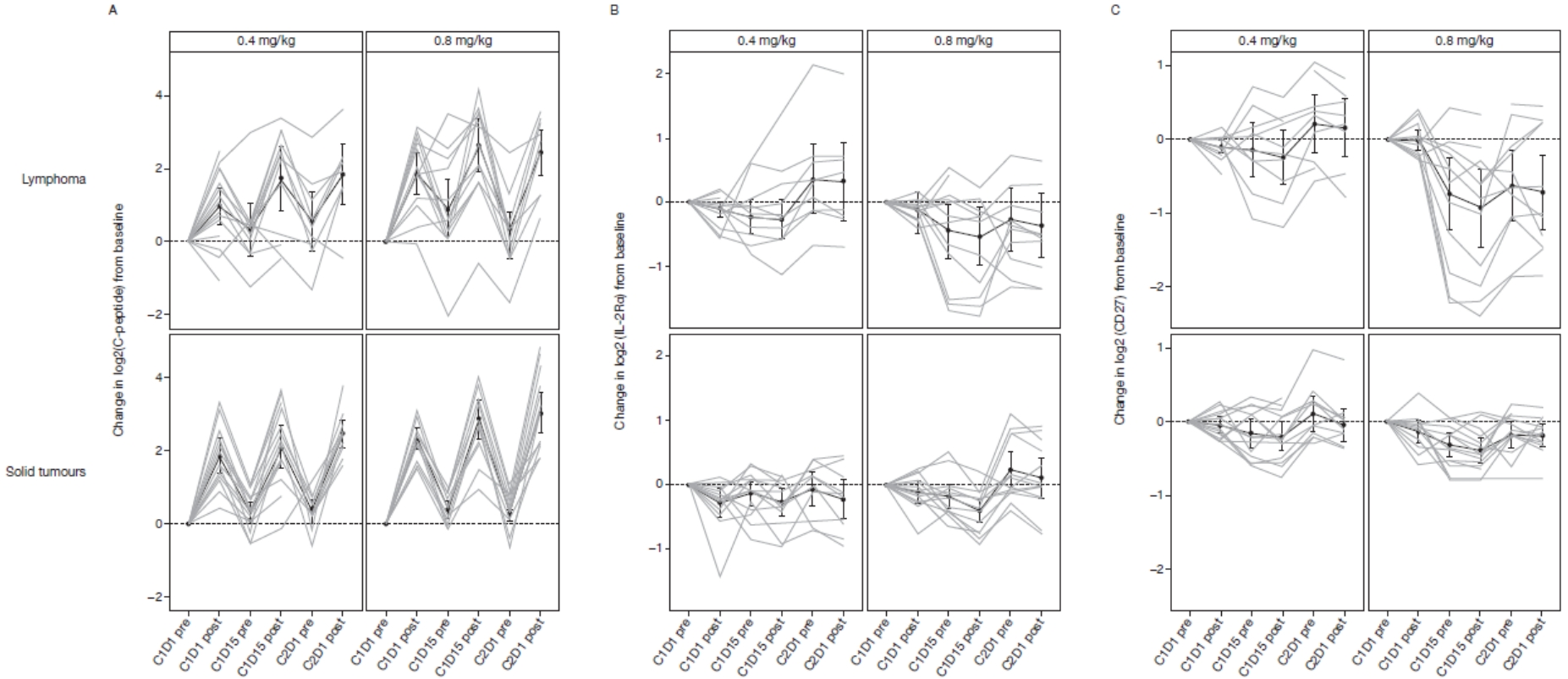
Supplementary Figures

Supplementary Figure 1. Mean systolic blood pressure over the first 24 hours post-infusion and at pre-infusion at later cycles in non-diabetic patients receiving copanlisib 0.4 mg/kg and 0.8 mg/kg



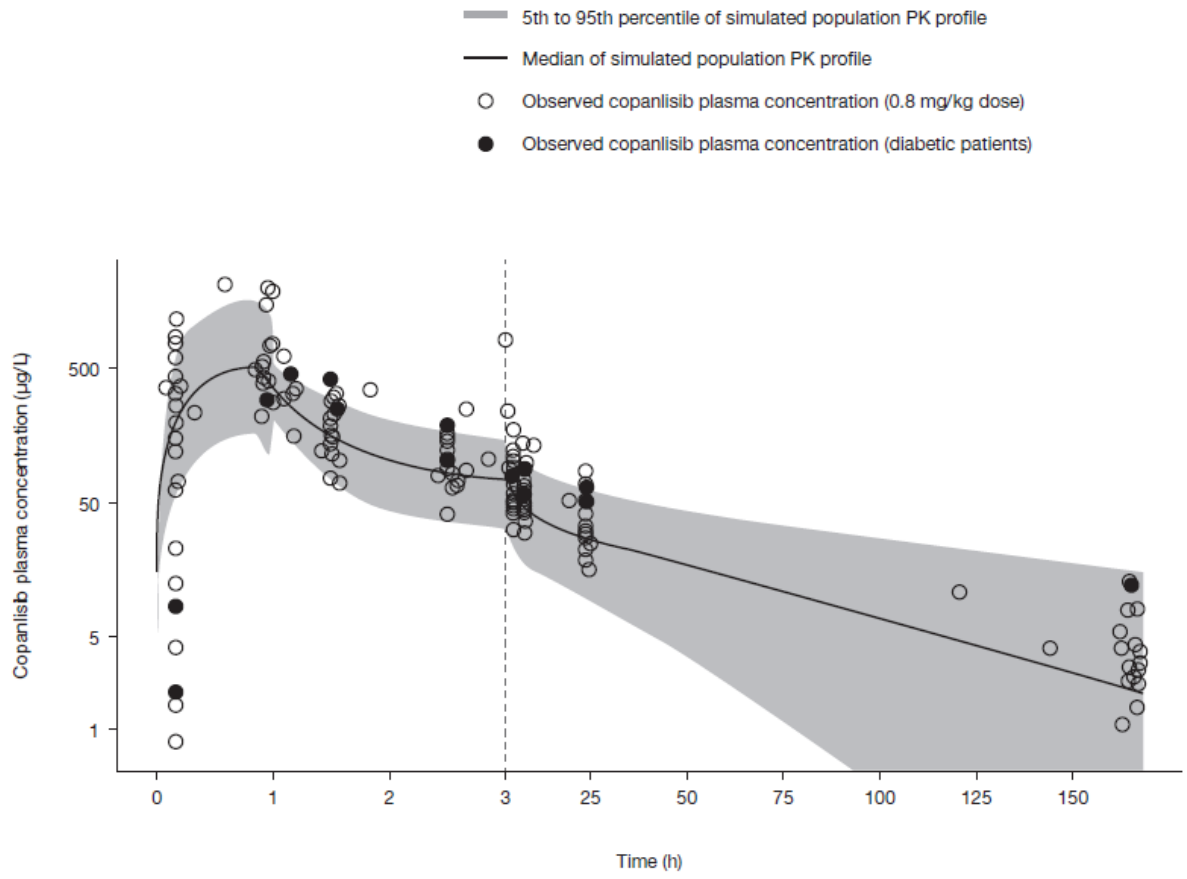
C, cycle; D, day; pre, pre-infusion; SD, standard deviation

Supplementary Figure 2. Changes in plasma levels of metabolic marker C-peptide (A) and immune cell markers IL-2Rα (B) and CD27 (C) following treatment with 0.4 mg/kg and 0.8 mg/kg copanlisib



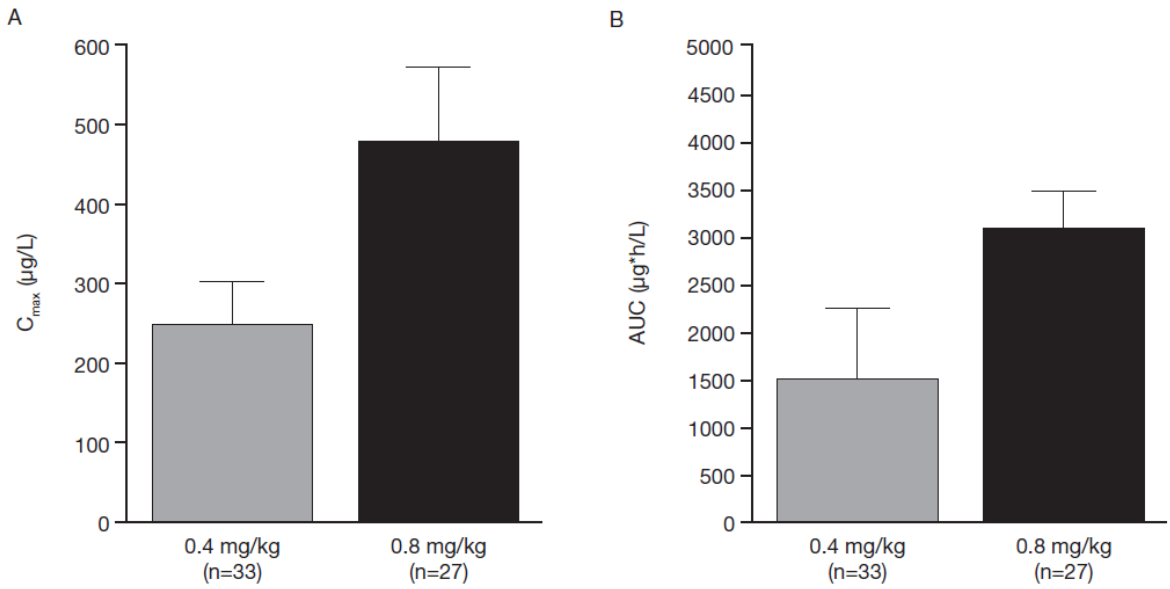
Post, 3 hours post dose

Supplementary Figure 3. Copanlisib plasma concentration—time profile following infusion during the first week of treatment



PK, pharmacokinetic

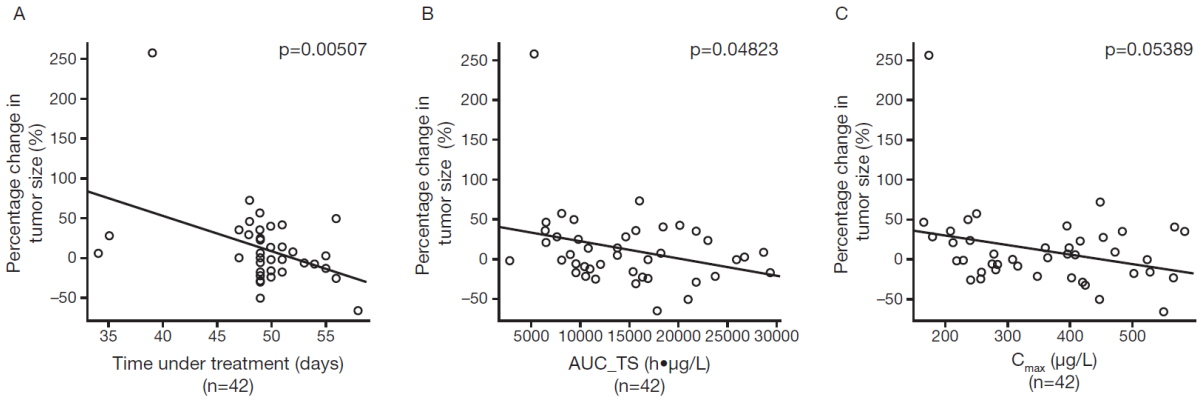
Supplementary Figure 4. Copanlisib plasma exposure C_{max} (A) and AUC (B) after the first infusion on cycle 1, day 1



60 patients were included in the evaluation of C_{max} and AUC; two patients were not included because of diabetes and one patient was not evaluable

AUC, area under the concentration–time curve; C_{max}, maximum observed concentration following single dosing

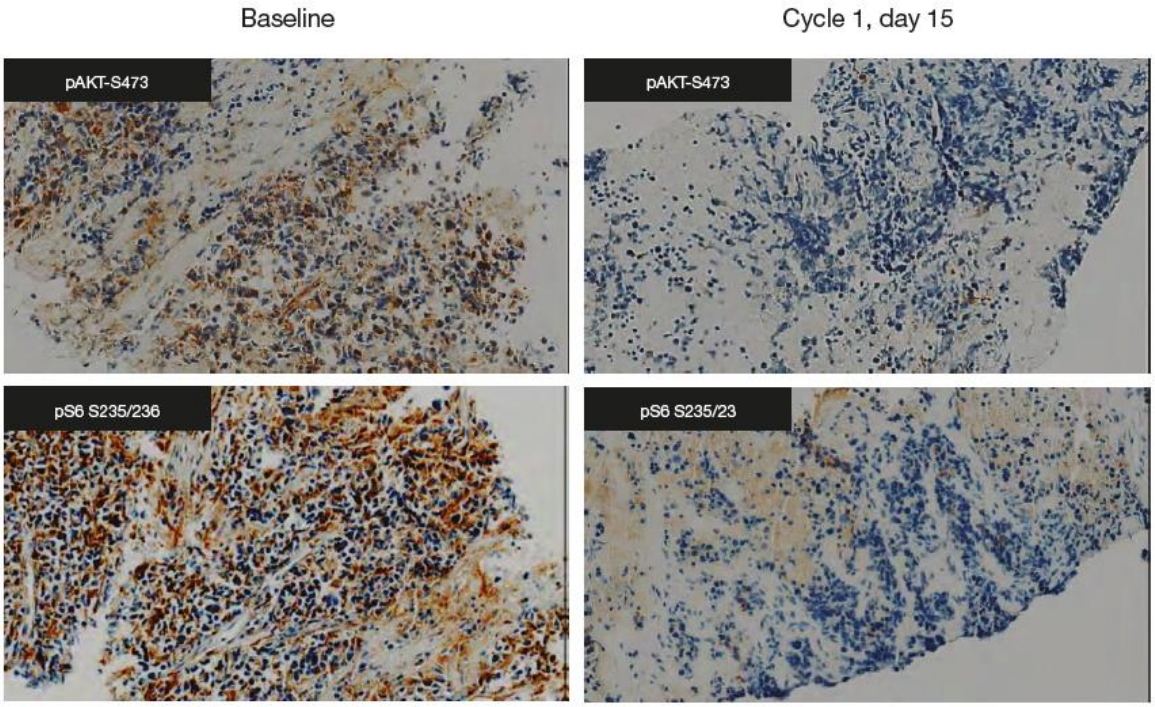
Supplementary Figure 5. Correlation between percentage change in tumor size from baseline and time under treatment (A), AUC_TS (B), and C_{max} (C)



p values shown for Pearson's correlation coefficients

AUC_TS, area under the concentration–time curve from time 0 to first tumor assessment; C_{max}, maximum observed concentration following single dosing

Supplementary Figure 6. Immunohistochemical staining of pAKT-S473 and pS6-S235/236 in paired tumor tissues at baseline and day 15 from one DLBCL patient who achieved a partial response



DLBCL, diffuse large B-cell lymphoma; pAKT, phosphorylated protein kinase B