Abbreviated Title: Copanlisib Window in FL
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Title: A Phase 2 Study of Response-Adapted Therapy with Copanlisib and Rituximab in Untreated Follicular Lymphoma

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Drug Name:	Copanlisib	Rituximab
IND Number:	141280	
Sponsor:	Center for Cancer Research, NCI	
Manufacturer:	Bayer Pharmaceuticals	Genentech
Supplier	Bayer Pharmaceuticals	CC Pharmacy

PRÉCIS

Background:

- Follicular lymphoma (FL) is the most common indolent non-Hodgkin's lymphoma (NHL) with a highly variable clinical course across patients
- Standard frontline therapy for FL includes a monoclonal anti-CD20 antibody with or without chemotherapy that can induce durable remissions but is generally not curable
- The 20% of patients who relapse within 2 years of frontline chemotherapy have an inferior overall survival; molecular profiles and gene-expression signatures can identify patients at high-risk of early treatment failure but are incomplete and require further validation
- The phosphoinositide 3-kinase (PI3K) pathway is critically important in FL; agents that target PI3K show good clinical activity in patients who relapse early after chemotherapy
- Copanlisib is an intravenous therapy targeting both PI3K-α and PI3K-δ isoforms and is FDA-approved for use in adults with relapsed and refractory FL
- Induction therapy with copanlisib and rituximab may produce deep and durable remissions in patients with FL without the use of cytotoxic agents
- Circulating tumor DNA (ctDNA) is a promising modality for monitoring therapy

Objective:

- To determine the complete response (CR) rate after copanlisib and rituximab as induction therapy for patients with untreated follicular lymphoma

Eligibility:

- Patients with histologically confirmed stage II-IV follicular lymphoma, grade 1-2 or 3a that meet criteria for initiation of systemic therapy
- No previous systemic therapy; prior local radiation permitted
- ECOG performance status 0-2
- Adequate bone marrow and organ function

Design:

- Phase 2 study of up to 65 patients with untreated FL who meet standard criteria for treatment
- Patients will first be treated with a "window" of copanlisib monotherapy, followed by induction therapy with copanlisib and rituximab for up to 6 cycles
- Patients who achieve a CR after 6 cycles of induction therapy will stop treatment and be monitored with computed tomography (CT) scans and plasma assays for circulating tumor DNA (ctDNA). Patients who relapse > 6 months from the end of induction can be re-treated with 6 additional cycles of copanlisib and rituximab
- Patients who achieve a partial response after 6 cycles of induction therapy will receive an additional 6 cycles of extended induction therapy with copanlisib and rituximab
- Patients who do not achieve at least a partial response after 6 cycles of induction therapy will stop treatment and be monitored with CT scans and peripheral blood assays for ctDNA
- Patients who progress or relapse after induction therapy and meet criteria for salvage therapy will be treated with standard chemotherapy and a monoclonal anti-CD20 antibody

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES AND ENDPOINTS

1.1.1 Primary Objective

To determine the complete response rate after copanlisib + rituximab induction therapy

1.1.2 Secondary Objectives

- To determine the safety and tolerability of induction therapy with copanlisib and rituximab
- To determine the clinical activity and depth of response after induction therapy with copanlisib and rituximab
- To determine the durability of responses after induction therapy with copanlisib and rituximab
- To assess time to next treatment, progression-free survival, and overall survival

1.1.3 Exploratory Objectives

- To determine if induction therapy with copanlisib and rituximab can achieve deep and durable responses in patients with “high-risk” FL as defined by published gene expression signatures and mutational profiles
- To identify a mutational or gene-expression signature that predicts early clinical response to copanlisib monotherapy
- To identify a mutational or gene-expression signature that predicts clinical response to induction therapy with copanlisib and rituximab
- To identify a mutational or gene-expression signature associated with intrinsic resistance to induction therapy with copanlisib and rituximab
- To explore the mechanisms of acquired resistance to copanlisib + rituximab

- To determine if ctDNA assays such as clonoSEQ® identify molecular relapse prior to clinical progression in FL
- To determine if copanlisib and rituximab induces durable complete responses in patients with untreated follicular lymphoma characterized by the CCP-32 assay

1.2 BACKGROUND AND RATIONALE

1.2.1 Trial design summary

This study will evaluate the safety and efficacy of copanlisib and rituximab when used as frontline induction therapy for patients with untreated FL. Patients who achieve CR after the initial 6 induction cycles of copanlisib and rituximab will be allowed to stop therapy and will undergo active surveillance for relapse with periodic CT scans and assays for ctDNA. Patients who achieve a PR after the initial 6 induction cycles of copanlisib and rituximab will be treated with an additional 6 cycles of extended induction therapy before stopping therapy and initiating active surveillance. Patients who do not achieve at least a PR after the initial 6 induction cycles of copanlisib and rituximab will stop therapy and initiate active surveillance. All patients who relapse or progress after copanlisib and rituximab induction therapy and meet clinical criteria for salvage therapy will be offered standard treatment with a monoclonal anti-CD20 antibody and chemotherapy. An important objective of this study is to determine the molecular correlates of response to copanlisib.

1.2.2 Follicular lymphoma

Follicular lymphoma (FL) is the second most common non-Hodgkin lymphoma (NHL), accounting for about 20-25% of cases(1). The clinical course is highly variable; many patients have indolent disease that is slowly progressive and defined by a perpetually relapsing and remitting course, while other patients experience rapid growth of lymph nodes, histologic transformation, early recurrences, or refractory disease(2, 3). FL is generally not curable with standard frontline systemic chemotherapy, although some patients may achieve durable remissions after chemotherapy with rituximab(4). The overall prognosis for patients with FL has improved in recent decades and the median overall survival in the modern rituximab era is over 18 years(5). Given the heterogeneity in clinical outcomes, current research focuses on understanding the molecular biology of patients with FL at the highest risk of early disease progression after standard frontline chemotherapy. Patients who meet this clinical definition of “high-risk” FL could thereby be prioritized for treatment with novel agents designed to overcome intrinsic chemotherapy resistance.

The Follicular Lymphoma International Prognostic Index (FLIPI) is a prognostic score that incorporates baseline clinical variables for the purpose of identifying subgroups with differences in survival(6). The FLIPI scoring system was determined on the basis of a retrospective multivariate analysis of over 4000 patients with newly diagnosed FL and separates patients into three major risk groups (low, intermediate, high) on the basis of age, stage, serum lactate dehydrogenase (LDH), hemoglobin, and the number of nodal sites. The FLIPI scoring system has been validated to predict clinical outcomes in FL, but was developed before rituximab, and cannot be used as a guide for individual treatment decisions. Another international project was conducted on over 1000 patients with newly diagnosed FL treated with rituximab-based regimens termed the FLIPI-2 that incorporates measures of tumor bulk and beta-2-microglobulin(7). Both the FLIPI and FLIPI-2 are unable to perform at the individual patient level and are not biologic classifiers.(7) Another important limitation of the FLIPI scores is the inability to separate age from underlying

biology. Although, patients under 40 years old are a small subset of patients with FL, these patients remain largely understudied, and represent an unmet clinical need as they may have more favorable features that respond to novel targeted therapies and can avoid the long-term risks associated with chemotherapy(8, 9).

A retrospective analysis of 588 patients with FL in the National LymphoCare database demonstrated that approximately 20% of patients experience progression of disease within 2 years of diagnosis (POD24) despite therapy with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP).(10) The patients with POD24 had significantly worse overall survival including a hazard ratio for early death of 7.17 (95% CI, 4.83 to 10.65) compared with the reference group(10). A follow-up analysis known as the Follicular Lymphoma Analysis of Surrogacy Hypothesis (FLASH) study performed a pooled analysis of 13 randomized clinical trials of patients in both the pre and post rituximab era (total sample size of 5,453) to validate POD24 as an early clinical endpoint in FL datasets(11). A landmark analysis confirmed the association between POD24 and overall survival (OS) in patients who were alive 24 months after trial registration. Patients alive without progression at 24 months were younger and more commonly had favorable PS, limited stage, low FLIPI risk score, normal baseline hemoglobin, and normal baseline B2M. Taken together, these results indicate that early progression after induction chemotherapy is an early clinical indicator of poor survival in FL. However, the biology underlying this poor-risk group of FL patients is unknown, and it cannot be applied prospectively to identify high-risk patients who have not yet undergone systemic treatment.

The first biologic-based classifier for FL was established with gene-expression profiling (GEP) performed on baseline tumor samples in 191 patients(12). This landmark study represented the first evidence that survival outcomes in FL correlate with the molecular features of the nonmalignant immune cells (termed the Immune Response survival predictor score; IR-1 and IR-2) in the baseline biopsy specimen, and was independent of clinical variables(12). These signatures have not been translated to the clinic, in part, because the technology used was based on fresh-frozen tissue samples and a clinically applicable, reproducible assay will need validation on paraffin-embedded tissue specimens. Novel GEP signatures have recently been reported that identify a subset of high-risk patients that make up 25% of those undergoing first-line therapy(13).

The prognostic value of combining individual somatic mutations with clinical variables was retrospectively assessed in two separate cohorts of patients with FL who underwent first-line therapy(14). A prognostic model was created by combining clinical variables with the result of next-generation DNA sequencing panels that tested the mutation status of 74 relevant genes(14). Patients were treated uniformly, and biopsies were within 1 year of starting therapy. The resulting risk model, termed the m7-FLIPI, combines the ECOG performance status, the FLIPI score, and the mutational status of 7 genes (*EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP*, and *CARD11*). The m7-FLIPI demonstrated superior predictive power over clinical factors alone and identified a subgroup of FL patients (28% of total cohort) who had a 5-year failure-free survival of only 38.29% (95% CI 25.31-57.95). These findings were validated in a separate cohort and identified a subgroup of FL patients (22% of total cohort) with a 5-year failure-free survival of 25% (95% CI 12.5-49.99).(14). The m7-FLIPI, was recently analyzed for its ability to predict POD24 after initial therapy(15) The study confirmed the poor overall survival of FL patients who progress within 2 years after initial therapy, but 39% and 57% of cases with POD24 within the two cohorts tested, respectively, were categorized as “low-risk” by the m7-FLIPI(15). Conversely, 14% and 21% of patients within the two cohorts, respectively, were considered “high-risk” by m7-

FLIPI, did not progress early after initial therapy.

Taken together, these findings highlight the need for further refinement of biologic-based risk assessments of prognosis from the time of diagnosis. Precise characterization of the molecular biology of "high-risk" FL could facilitate the proper selection of patients for novel treatment regimens designed to overcome intrinsic chemotherapy resistance (16).

1.2.3 Non-cytotoxic combination therapy in FL

The standard approach to frontline treatment of FL is combination chemotherapy with the addition of a monoclonal antibody targeting CD20, such as rituximab(17). Although some patients are treated with rituximab monotherapy, this approach is most commonly applied to patients with low tumor burden and/or with advanced age or co-morbid conditions that preclude the safe use of conventional cytotoxic chemotherapy(17, 18). Randomized phase III studies have demonstrated that rituximab added to combination chemotherapy platforms improves both PFS and OS compared to chemotherapy alone(19-21). Newer chemotherapy platforms and novel anti-CD20 antibodies have been tested and demonstrate high overall response rates including the ability to achieve remission in many patients(22-24). However, no evidence exists that combination chemotherapy + anti-CD20 monoclonal antibodies can reliably cure patients with FL(4, 23, 25). Further, cytotoxic chemotherapy is associated with significant short-term toxicities and long-term complications including secondary malignancies and increased risk of infections(4, 24). Since achieving a prolonged remission duration while maintaining quality of life is important in FL therapy, novel combinations are needed(26). The incorporation of novel targeted agents that target oncogenic pathways such as B-cell receptor signaling raise the intriguing possibility of "chemotherapy-free" combinations that can be tailored to individual subsets of FL patients and possibly result in deep and durable remissions.

Phase II studies have investigated frontline treatment regimens that incorporate non-cytotoxic agents such as lenalidomide combined with rituximab (R^2) and demonstrated very high overall response rates.(27, 28). Lenalidomide-rituximab was tested in a single institution study of 46 patients with previously untreated FL and demonstrated an overall response rate of 95% including 87% rate of complete response(27). A second multi-center study by the Alliance for Clinical Trials in Oncology tested the same regimen in 66 patients with previously untreated FL and showed an ORR of 95%, a CR rate of 72%, and a 2-year PFS of 86%(28). Based on these promising phase II results, lenalidomide plus rituximab was compared to rituximab with combination chemotherapy in an ongoing randomized phase III study (RELEVANCE study), that has recently reported no difference in CR rates at 30 months (CR30) or progression-free survival (*press release*). It is unknown if lenalidomide plus rituximab benefits certain subsets of patients or provides benefits regarding toxicities or quality of life.

Recommendations for clinical trial development in FL highlight the importance of identifying effective therapies for high-risk FL patients as well as the need for comprehensive molecular characterization of the tumors that respond to novel therapies(26). In this way, therapy selection may ultimately be determined by individual risk or disease biology (i.e. precision medicine)(16). Recognizing that precision medicine focuses on subsets of patients that are most likely to respond to targeted therapies, a series of smaller phase II trials with strong translational endpoints are required to better understand the correlation between tumor biology and tumor response(26). An important objective of this trial is to define the complete response rate as well as the complete molecular remission rate, using sensitive markers of minimal residual disease (MRD), after therapy with copanlisib plus rituximab. Further, the durability of those remissions will be

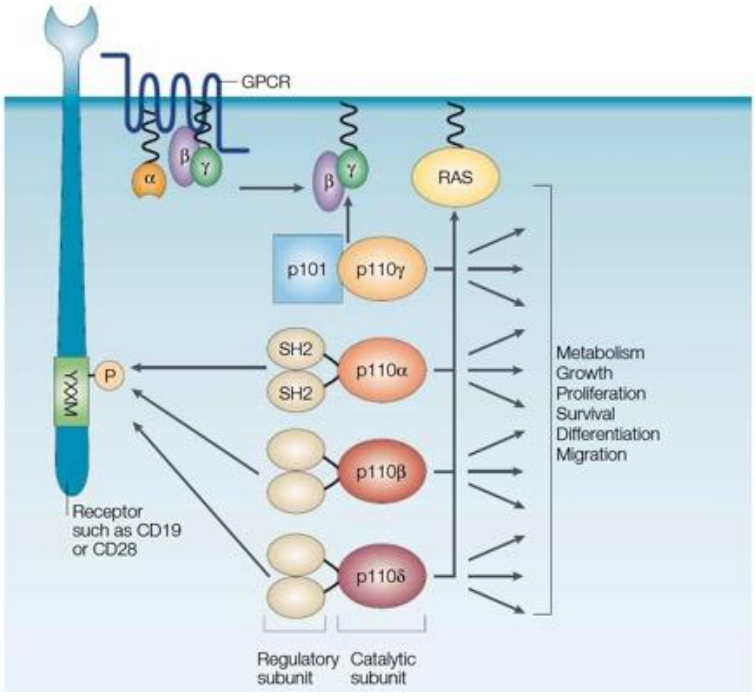
investigated as complete responses that last at least 30 months (CR30); this endpoint was selected as a secondary endpoint of interest since it was recently shown to be a valid surrogate of both PFS and OS(29). Finally, a fundamental need in frontline therapy for FL includes regimens that are effective without a requirement for indefinite therapy or prolonged maintenance schedules(26). For this reason, a defined period of treatment duration was selected for this trial with an option for re-treatment in patients who relapse after complete response.

1.2.4 Phosphoinositide 3-kinase (PI3K) pathway as a therapeutic target

The phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB) pathway, also known as the AKT signaling pathway, holds a central role for normal cellular responses to growth factors and cellular homeostasis(30, 31). As a consequence, aberrant activation of the PI3K pathway enhances cell survival and proliferation, thereby leading to malignant transformation across a variety of cancers.

Human cells express three classes of PI3K enzymes and mammals express four class I catalytic isoforms (p110α, β, γ, and δ). Class IA PI3Ks are heterodimeric enzymes consisting of regulatory p85 and catalytic p110 subunits and are primarily responsible for phosphorylating the second messenger, phosphatidylinositol 4,5-bisphosphate (PIP2) (**Figure 1**). Redundancy exists since each of the catalytic subunits can bind to each of the regulatory subunits and different heterodimers are recruited to the same receptors(32). The p110α and p110β proteins are ubiquitous whereas the expression of p110γ and p110δ are predominantly within immune cells. The PI3K family members participate in a broad range of cellular regulatory processes including growth, proliferation, metabolism, and motility(33). Notably, the immune system relies on PI3K signaling and catalytic isoforms must be properly balanced for normal immune cell development.

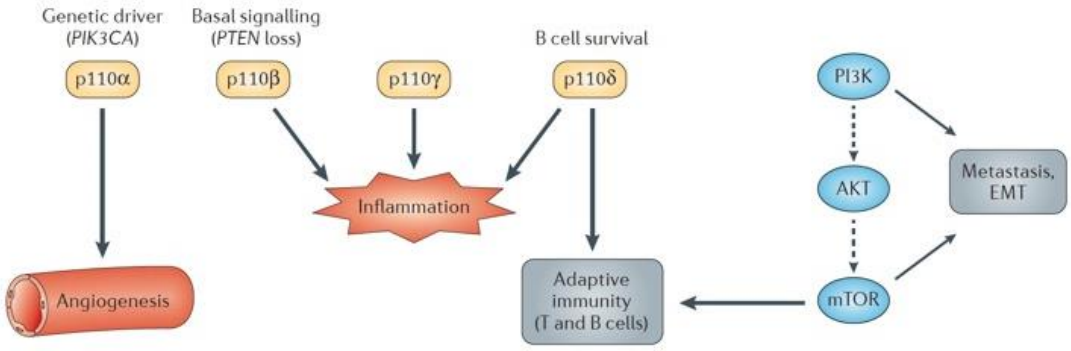
Figure 1: Class IA PI3Ks enzymes



P110α is the only PI3K catalytic subunit isoform that is significantly associated with cancer-associated somatic mutations in its gene, *PIK3CA*(34), but other mechanisms of PI3K activation exist including the loss of the tumor suppressor, phosphatase and tensin homolog gene, *PTEN*(35).

Further, class I PI3Ks are involved in the recruitment of a number of specific proteins to membrane-signaling complexes, and PI3K effectors including serine/threonine kinases of the AKT family, tyrosine kinases of the TEC family (ITK, BTK, etc.), as well as mTORC1 and mTORC2(31). In this way, multiple downstream signaling pathways can be triggered by PI3K activation. The pathway also contributes to cancer-promoting aspects of the tumor microenvironment including angiogenesis and immune cell crosstalk (**Figure 2**).

Figure 2: PI3K activation pathway



Due to its central role in multiple malignancies, a number of agents have been developed that target the various PI3K isoforms. However, since the PI3K pathway is important for the regulation of a variety of physiological processes in virtually all tissues types, the clinical development of targeted PI3K inhibitors has been slowed by the emergence of dose-limiting toxicities and on-target adverse events. Idelalisib (Zydelig®) is a highly selective, orally bioavailable inhibitor of the PI3K-δ isoform, and is FDA-approved for use as monotherapy in relapsed FL in patients who have received at least two prior systemic therapies(36). Idelalisib was approved on the basis of its ability to achieve clinical responses in patients who were "double-refractory" to both rituximab and alkylating agents. The overall response rate to idelalisib in this refractory population was 57% including a 6% rate of complete responses. Potentially more intriguing, however, is the ability of idelalisib to remain effective in patients who relapse within two years of chemotherapy (POD24)(37). In 37 patients with relapsed FL who had experienced early progression after chemotherapy, idelalisib demonstrated an ORR of 59.4% and the median duration of response in these patients was 11.8 months (95% CI, 3.8, not reached). The PI3K pathway serves to attenuate the inflammatory response, however, and the resulting pro-inflammatory effect of targeted inhibition with idelalisib resulted in severe toxicities of autoimmune colitis, mucositis, and pneumonitis in some patients. These toxicities resulted in a black box warning in the FDA label warning of fatal and serious toxicities associated with idelalisib including hepatic, severe diarrhea, colitis, pneumonitis, and intestinal perforation. Taken together, these results establish the PI3K pathway as an attractive target for patients with high-risk FL, but with important caveats regarding potential toxicities.

1.2.5 Copanlisib (BAY-80-6946, Aliqopa®)

Copanlisib is an intraveneous pan-class I PI3K inhibitor with predominant activity against the PI3K-α and PI3K-δ isoforms. Copanlisib uses an intermittent dosing schedule designed to achieve optimal target inhibition within the tumor while sparing normal tissue. Indeed, the strategy of intermittent dosing was more effective in mice bearing breast cancer xenografts than continuous

dosing(38). Another potential benefit of simultaneous targeting of the PI3K-α isoform is that upregulation of alternative isoforms over time may be a mechanism of resistance to selective PI3K-δ inhibitors(39, 40). Studies in mantle cell lymphoma cell lines and from patient samples have shown that p110α expression increased significantly upon relapse after treatment with idelalisib, suggesting p100α plays a mechanistic role in relapse. In addition, combined inhibition of both p110-α and p110-δ isoforms was a more effective strategy for inhibiting constitutive PI3K activation in cell lines(39).

1.2.5.1 Pre-clinical studies of copanlisib

A set of primary PD studies was performed to characterize and assess the efficacy and specificity of copanlisib as a single agent and in combination with investigational or established therapeutic agents in various models in vitro and in vivo. Secondary PD was addressed in the frame of the primary PD and safety pharmacology. The safety pharmacology studies addressed the impact of copanlisib on vital organ functions (CNS, cardiovascular system [including ECG], respiratory system) as well as on supplemental organ systems and functions. Pharmacodynamic interaction studies with various antidiabetics have been performed. Results of the program are summarized below.

Table 4–1: Primary PD: listing of *in vitro* studies with copanlisib (BAY 80-6946) and noteworthy findings

Title/Test system	Test article/ concentration	Noteworthy findings	Reference[a]
Biochemical activities in Class I PI3K enzymatic assays	BAY 80-6946	Inhibition of Class I PI3K isoforms with sub- or single-digit nanomolar IC_{50}	MRC-01363
Inhibition of PI3K downstream signaling molecule phosphorylation	BAY 80-6946	Potent cellular activity against PI3K signaling (IC_{50} of 1-5 nM in phospho-AKT (T308 and S473) and phospho-4E-BP1 (T70) cellular mechanistic assays)	A46057
Panel of 220 kinases	BAY 80-6946	No significant activities, exception mTOR (IC_{50} 45 nM)	A53636
Selectivity against PI3K vs. mTOR kinase in rat ELT3 cells	BAY 80-6946	100- to 1000-fold cellular selectivity of PI3K vs. mTOR signaling and therefore it is not a dual PI3K/mTOR inhibitor	A53636
Anti-proliferative effect in tumor cell lines	BAY 80-6946	BAY 80-6946 exhibited selective anti-proliferative activity in a human tumor cell line panel, with 40-fold greater activity in breast cancer lines with PIK3CA mutations and/or HER2 overexpression than HER2-negative, wild-type PIK3CA cells	A46057
In vitro cytotoxic activity of BAY 80-6946 against human hematological tumor cell lines	BAY 80-6946, idelalisib	BAY 80-6946 exhibited potent cytotoxic activity against a broad range of hematological tumor types, including ALL, AML, NHL, and myeloma. BAY 80-6946 had a lower IC_{50} than idelalisib (CAL-101) in all cell lines tested.	PH-37232
Differential anti-proliferative profile in NHL-DLBCL tumor cell lines	BAY 80-6946, idelalisib, ibrutinib	BAY 80-6946 is more potent and has a broader anti-tumor spectrum	PH-39090
In vitro combination effect in NHL-DLBCL tumor cell lines	BAY 80-6946, BAY 86-9766, ibrutinib	Synergistic antitumor effect was observed in the tumor cell lines responding to BTK inhibition. Antagonistic effect was observed in BTK inhibitor-resistant tumor cell lines. Very strong synergistic combination with the MEK inhibitor BAY 86-9766 was demonstrated in MyD88-and CARD11-mutant OCI-Ly3 DLBCL cell lines.	PH-39090
Rapid induction of apoptosis by PI3K inhibitors is dependent upon their transient inhibition of RAS-ERK signaling	BAY 80-6946 MK2206 PD0325901	PI3K inhibition, but not AKT inhibition, causes rapid inhibition of wild-type RAS and of RAF-MEK-ERK signaling. Inhibition of RAS-ERK signaling is transient, rebounding a few hours after drug addition, and is required for rapid induction of apoptosis.	PH-39091

[a]Bayer HealthCare, report number
PD: Pharmacodynamics; AML: acute myeloid leukemia; PI3K: phosphatidylinositol 3-kinase; mTOR: mammalian target of rapamycin; HER2: human epidermal growth factor receptor 2; IC_{50}: half-maximal inhibitory concentration; MEK: mitogen-activated protein kinase kinase; PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; NHL: non-Hodgkin's lymphoma; DLBCL: diffuse large B-cell lymphoma; BTK: Bruton's tyrosine kinase; 4E-BP1: eukaryotic translation initiation factor 4E-binding protein 1; MyD88: myeloid differentiation primary response gene (88); CARD11: caspase recruitment domain-containing protein 11; OCI-Ly3: Ontario Cancer Institute-lymphoma 3; AKT: protein kinase B; phospho-AKT: phosphorylated AKT; ALL: acute lymphoblastic leukemia; ERK: extracellular signal-regulated kinase

1.2.5.2 Metabolism of copanlisib

In vitro investigations revealed oxidation and/or dealkylation reactions at the alkylmorpholine side chain as the most important biotransformation reactions in liver microsomes and hepatocytes of various animal species and man. No major species differences were observed. Based on the in vitro data, oxidative metabolism of copanlisib was predominantly catalyzed by CYP3A4 (>90%) and to a minor extent by CYP1A1 (<10%). In vitro investigations revealed

that enzymatic activities of major CYP and UGT isoforms as well as DPD were not affected in the presence of copanlisib (IC50 >50 μM) or metabolite M-1 (IC50 > 20 μM). Copanlisib and its metabolite M-1 showed no induction of CYP1A2 and CYP3A4 in the in vitro test system up to concentrations of 1111 μg/L. Therefore, clinically relevant PK DDI through inhibition/induction of major CYP and UGT isoforms or DPD (IC50 > 20 μM) caused by copanlisib and metabolite M-1 are unlikely.

1.2.5.3 Absorption of copanlisib

The pharmacokinetics of copanlisib were very similar in rats, dogs and Cynomolgus monkeys. The low to moderate clearance and a high volume of distribution resulted in moderate to long elimination half-lives of parent compound between 4 and 17 hours. The pharmacokinetic in mice was different and showed a very high CL. Thus, elimination occurred with a very short t1/2 of about 1 hour. The pharmacokinetics of copanlisib were almost linear in rats and dogs after administration of the lower dose levels. With increasing doses, a slight tendency to an over-proportional increase of the AUC was observed in rats and dogs. This trend was not reflected by the Cmax Pharmacokinetics was not dependent on sex and no relevant drug accumulation was observed in the rat and dog 1 Cycle, repeat-dose toxicity studies.

The extent of plasma protein binding of copanlisib was low in various species with free fractions between 10% and 42% (15.8% in human plasma). With the exception of Cynomolgus monkeys, free fractions of copanlisib were 2 to 3 times higher in the various animal species in comparison with man. There was no saturation of plasma protein binding of copanlisib within the investigated pharmacological and toxicological relevant concentration range (100-10000 μg/L). The plasma protein binding of the metabolite M-1 was similar to the unchanged copanlisib. The free fractions ranged between 20% and 56% (20.0% in human plasma).

1.2.5.4 Excretion of copanlisib

In rats, dogs, and man, radioactivity was excreted mainly via the biliary/fecal route after i.v. administration of [14C]copanlisib. About 10% of the administered radioactivity was excreted by the renal route in rats and dogs. In man, 22% of the administered radioactivity were excreted via urine. In rats, considerable direct radioactivity excretion into the gastrointestinal contents was observed.

1.2.5.5 Pharmacokinetics of copanlisib

The pharmacokinetics of copanlisib (BAY 80-6946) were studied in vivo in CD-1 mice, Wistar rats, Beagle dogs, and Cynomolgus monkeys after i.v. administration copanlisib or [14C]copanlisib. The free base, BAY 80-6946, was formulated in a mannitol solution after addition of hydrochloric acid, so that the hydrochloride/dihydrochloride salt was formed in the administration solution. The dihydrochloride salt of BAY 80-6946 (BAY 84-1236) was used in some of the in vitro studies. All doses and equivalent concentrations refer to BAY 80-6946 (i.e., the pharmacologically active component). Data on PK after repeated administration were derived from the exposure determination performed as part of the toxicity studies in Wistar rats and Beagle dogs. Distribution studies in rats were performed by means of autoradiography. Additionally, in vitro studies were performed to investigate plasma protein binding, blood cell/plasma partitioning, drug metabolism in several species including humans and drug transporter characteristics as well as pharmacokinetic drug-drug interaction potential towards metabolizing enzymes and transporters.

Results of the program are briefly summarized as:

- Almost linear pharmacokinetics in rats and dogs after i.v. administration, tendency to over-proportional increase in the AUC after higher doses.
- Low extent of plasma protein binding of copanlisib in various species with free fractions between approximately 10% and 42% (15.8% in human plasma). Protein binding of metabolite M-1 was observed with free fractions between 20% and 56% (20.0% in human plasma).
- Significant distribution of radioactivity (copanlisib and radioactive metabolites) from blood into the organs and tissues of rats, with higher concentrations in organs and tissues in comparison with blood. Moderate penetration of the blood-brain barrier; high affinity to melanin-bearing tissues.
- Penetration of the placental barrier in rats to a low to moderate extent, lower fetal organ and tissue radioactivity concentrations than in maternal organs and tissues, with the exception of brain (higher radioactivity concentrations in fetuses than in dams).
- Oxidation and/or dealkylation at the alkylmorpholine side chain are major biotransformation pathways in liver microsomes and hepatocytes of various animal species and man; with no major species differences. CYP3A4 is major metabolizing enzyme in man (>90%), while CYP1A1 (<10%) contributes to a minor extent based on the in vitro data.
- Copanlisib was the predominating component in plasma of mouse, rat, dog, and man (>84% of total radioactivity AUC). Metabolite M-1 was the only relevant circulating metabolite in plasma of rat and man (about 10% and 5% of total radioactivity AUC in rat and man, respectively). Metabolites M-1 and M-3 were occasionally found in mouse plasma.
- In man and rat, balanced excretion via unchanged copanlisib and oxidative biotransformation products. In dogs, mainly oxidative biotransformation and less excretion of unchanged copanlisib. Metabolites almost solely formed by dealkylation and/or oxidation at the alkylmorpholine side chain. Biotransformation of copanlisib is mediated by CYP3A4 (>90% of metabolism) and to a minor extent by CYP1A1 (<10% of metabolism). Radioactivity excreted mainly via the biliary/fecal route in rat (85%), dog (75%) and man (64%) after i.v. administration of [14C] copanlisib. About 10% of the administered radioactivity excreted by the renal route in rats and dogs. In man, 22% of the administered radioactivity excreted via urine.
- Unchanged copanlisib represented approximately 30% of the administered dose in feces and 15% in urine, respectively.
- [14C]Copanlisib-related radioactivity was secreted into the milk of lactating rats only to a low extent (1.7% of dose).
- Based on in vitro studies with different cell lines, copanlisib is a substrate of P-gp and of BCRP. Copanlisib is not a substrate of OCT, OAT or OATP. Copanlisib is not a substrate of MATE1 or MATE2K
- Low risk for clinically relevant PK DDI through inhibition (IC50 > 50 μM) or induction (assessable up to 1111 mg/L in vitro) of major CYP isoforms, inhibition of major UGT

isoforms (IC50 > 50 μM) and inhibition of DPD (IC50 > 20 μM) caused by copanlisib (clinically relevant Cmax: about 1 μM in patients).

- P-gp (IC50: 7 μM and 7.6 μM for digoxin and dipyridamol, respectively) and BCRP (IC50: 11.5 μM for topotecan) mediated transport was inhibited by copanlisib in vitro. Metabolite M-1 showed only a marginal inhibitory effect on P-gp and BCRP-mediated transport (IC50: > 40 μM). Copanlisib was a potent inhibitor of MATE2K (IC50: 0.09 μM for metformin, 0.58 μM for MPP+) and an inhibitor of MATE1 (IC50: 10.8 μM for metformin). The uptake transporters OATP1B1, OATP1B3, OAT1, OAT3, OCT1, and OCT2 were not inhibited by copanlisib. No inhibition of BSEP and MRP2 by copanlisib was observed. Clinically relevant PK DDI with P-gp, BCRP, MATE1, BSEP and MRP2 substrates as well as substrates of hepatic and renal uptake transporters are unlikely. Copanlisib plasma concentrations in patients exceed the IC50 for MATE2K-inhibition (up to 6 hours after copanlisib infusion at the 60 mg dose). The clinical relevance of this observation is unknown.
- According to the copanlisib package insert, itraconazole (CYP3A and BCRP inhibitor) is associated with a 53% increase in copanlisib AUC and rifampin (induces CYP3A and P-gp) causes a 63% decrease in AUC. So deactivating variants in CYP3As would plausibly be associated with PK as well. There are several SNPs in CYP3A4 and CYP3A5 with robust levels of clinical evidence for association with PK of some medications. Copanlisib is also a known substrate of P-gp and BCRP. The PharmacoScan platform (Thermo) is able to test 4,627 variants in 1191 different pharmacogenes, including all of those involved in copanlisib metabolism and transport, and will be utilized to understand copanlisib pharmacogenetics.

1.2.5.6 Pharmacodynamics of copanlisib

The pharmacodynamic effects on glucose and plasma insulin levels as well as [18F]FDG-CT/PET have been investigated in the first-in-man Study 12871 using copanlisib monotherapy at different doses. All 47 non-diabetic patients treated at ≥0.4 mg/kg copanlisib experienced a PD effect as well as all 6 diabetic patients treated at 0.4 mg/kg copanlisib. Peak plasma glucose values were seen 5 to 8 hours after the start of the copanlisib infusion. Hyperglycaemia was reversible, i.e., not observed after copanlisib treatment had been permanently discontinued. Dose-related increases in glucose and insulin as well as copanlisib exposure-related increases in plasma glucose were observed. Treatment with copanlisib resulted in a reduction in the pharmacodynamic marker pAKT in surrogate tissue platelet rich plasma (PRP) with a median decrease up to 81.5% at the 0.4 mg/kg dose level and up to 77.8% at the 0.8 mg/kg dose level compared with baseline, indicative of a potent inhibition of the PI3K pathway in surrogate tissue at both tested dosages of copanlisib (Study 16790). Treatment with copanlisib was associated with modulation of blood glucose with corresponding changes in C-peptide and insulin, showing a trend towards a dose dependency (0.8 mg/kg >0.4 mg/kg). Copanlisib has been shown to prolong the QT/QTc interval, which may lead to an increased risk for ventricular arrhythmias. For this reason, it is recommended to use copanlisib with caution in patients who have, or may develop prolongation of QT, such as patients with a congenital long QT syndrome, patients taking certain anti-arrhythmic medicines or other medicinal products that lead to QT prolongation, and those with electrolyte disturbances such as hypokalemia, hypocalcemia, or hypomagnesemia.

Clinical studies of copanlisib

A phase I study of copanlisib was done in patients with advanced solid tumors and various subsets of NHL demonstrated to determine the safety, tolerability, pharmacokinetics, and maximum tolerated dose (MTD) of copanlisib(41). This study included a cohort of patients with type 2 diabetes mellitus. Patients received three weekly intravenous infusions of copanlisib per 28-day cycle over the dose range 0.1–1.2 mg/kg and plasma copanlisib levels were analyzed for pharmacokinetics. Fifty-seven patients were treated and the MTD of copanlisib was established at 0.8 mg/kg. Copanlisib typically reached maximum plasma concentration (Cmax) between 0.5 and 1 h. The terminal half-life was 38.2 h (coefficient of variation 43%), and no accumulation was observed after once-weekly administration. Patients received a median of two treatment cycles (range, 1–49) of copanlisib, and the median duration of treatment was 6 weeks (range, 0.1–2051). Peak exposure of copanlisib correlated with transient hyperglycemia post-infusion. Blood glucose values typically peaked 5–8 h after the start of copanlisib infusion and declined to pre-diabetic levels 24–48 h after infusion and to normal levels before the start of the next infusion. The most common drug-related adverse events (AEs) were metabolic (hyperglycemia), gastrointestinal (nausea and diarrhea), and cardiovascular (hypertension). The overall incidence of drug-related all-grade and grade 3 hypertension was 21% and 14%, respectively. The incidence and severity of gastrointestinal toxicities due to copanlisib were low; nausea was the most common drug-related gastrointestinal toxicity (37%; all events grade ≤2) and diarrhea was less common (all-grade: 16%; grade ≥3: 2%). Elevated aminotransferase was primarily an incidental laboratory toxicity finding and was mostly grade 1; grade ≥3 in five patients. Drug-related pneumonitis was reported in two cases. Most AEs were managed with dose modifications, and there was only one drug-related AE leading to permanent discontinuation [left ventricular systolic dysfunction], which resolved after discontinuation. In this phase 1 study, all patients (n=6) with FL responded (one CR and five PRs)(41). One patient with FL was treated for 47 months, and another went off study after having been treated for a total of 43 months.

Copanlisib was further evaluated in a multicenter phase II study for patients with indolent lymphoma that was relapsed or refractory after two previous lines of therapy(42). In this study, 142 patients (n=104 with FL) were enrolled and treated with copanlisib 60mg IV on days 1, 8, 15 of a 28-day cycle until disease progression or unacceptable toxicity. The primary end point of the study was objective response rate (ORR). The ORR was 59% and 12% of patients achieved a complete response. The median time to response was 53 days and the median duration was 22.6 months. The toxicity profile of copanlisib was manageable with the most frequent treatment-related adverse events of grade 3 or higher were transient hyperglycemia (41%), transient hypertension (24%), neutropenia (24%), and lung infection (15%)(42). The median duration of treatment was 22 weeks and patients received 96% of the planned dosing. Dose delays occurred in 74% of patients and most of these were due to adverse events. In 25% of patients, adverse events led to discontinuation of copanlisib. An adverse event of special interest was noninfectious pneumonitis which occurred in 8% of patients. Three opportunistic infections occurred on this study: two *P. jirovecii* infections and one asperillosis infection. Six patients (4%) died during treatment or within 35 days of treatment discontinuation. Of these 6 deaths, three were considered treatment-related: lung infection, respiratory failure, and a cerebral thromoembolic event. Since p110α mediates most tissue responses to insulin and maintains glucose homeostasis, hyperglycemia was an expected on-target effect of copanlisib but successfully managed in most cases with the use of intravenous fluids and only occasional

use of insulin was required. Gene set enrichment analysis (GSEA) was performed in all patients with available gene expression data, and patients with high PI3K/BCR gene expression scores were the likeliest to respond to copanlisib. These data led to the FDA to approve the use of copanlisib for patients with relapsed or refractory FL after treatment with at least two lines of prior therapy.

1.2.5.7 Copanlisib in Combination with Rituximab

Study 17067 (CHRONOS 3) is a randomized, double-blind, placebo-controlled, two-arm, phase III study to evaluate the efficacy and safety of copanlisib in combination with rituximab in patients with relapsed indolent NHL (iNHL). Approximately 567 (435 FL and 132 other iNHL) patients will be randomly assigned in a 2:1 ratio to one of the double blinded treatment arms: copanlisib plus rituximab or placebo plus rituximab. To date, more than 270 patients have been enrolled on this study since February 2015 with close monitoring of safety by the study's data monitoring committee. No interruption or suspension of enrollment has been noted on the study. When more detailed toxicity data is available, and if there is a safety signal of concern, a revision to this protocol to include stopping rules may be considered.

1.2.6 Circulating tumor DNA (ctDNA)

Small fragments of DNA from normal and diseased tissue is constantly shed into the bloodstream as cell-free DNA (cfDNA) through processes of apoptosis, necrosis, and secretion.(43, 44) A very small fraction of the cell-free DNA in patients with FL will originate from malignant cells, and can be captured as circulating tumor DNA (ctDNA)(45-47). Modern next-generation sequencing (NGS)-based assays have enabled the detection and quantification of ctDNA in the majority of patients with FL(48). We will use a modern ctDNA platform that combines universal PCR primers for the variable-dense-joining (VDJ) region of the immunoglobulin receptor with NGS technologies (i.e. clonoSEQ®)(49). This ctDNA assay is highly tumor-specific and can be used as a method for disease detection at the molecular level in a variety of B-cell lymphomas(48, 50, 51).

Assessment of ctDNA along critical clinical landmark timelines, including use as an active surveillance monitoring tool for patients who are in remission, offers the promise to overcome fundamental limitations of our current definitions of disease response. Namely, radiographic imaging scans are unable to measure minimal residual disease (MRD). Many patients who achieve a complete response to therapy based on imaging scans will have the persistence of MRD, highlighting the need for more sensitive methods for disease detection(52, 53). Since the goal of our study is to determine patients who are uniquely sensitive to copanlisib, assays for ctDNA will be used to determine early signs of biologic activity during the copanlisib window, during induction therapy, and at the end of induction therapy to determine the rate of complete molecular remission (defined as patients who achieve complete response and are MRD-negative). After the completion of induction therapy, patients will stop treatment and will be active monitored with both imaging scans as well as assays for ctDNA. In this way, ctDNA can be used as a non-invasive method to monitor tumor response kinetics during therapy, define the depth of response to induction therapy, and be used as a surveillance tool for early disease detection after therapy cessation. Although clinical decisions regarding response to induction therapy and timing of salvage therapy will not be made based on the results of ctDNA in this study, we hypothesize that these assays may outperform conventional methods of determining prognosis prior to starting therapy, response to induction therapy, and early detection of disease relapse. The data generated

from this study is expected to inform future clinical trials that test the hypothesis of making clinical decisions based, in part, on the results of ctDNA assays.

1.2.7 CCP-32 Gene Expression Profiling Assay in FL

Follicular lymphoma (FL) is a clinically and biologically heterogeneous B-cell lymphoma and survival is improving with modern therapy. Yet patients who progress within 2 years of frontline therapy (POD24) have a 5-year overall survival of only 50%. The CCP-32 is a gene-expression profiling assay performed on FFPE tissue to identify FL patients who are high-risk for early progression (POD24) when treated with standard chemoimmunotherapy. We hypothesize that copanlisib and rituximab will induce durable complete responses in patients with untreated follicular lymphoma including those characterized as high-risk by the CCP-32 assay.

1.2.8 Scientific correlates during the copanlisib window

A critical component of this trial will be the comprehensive assessment of molecular and/or immunologic correlates that predict response to copanlisib in both tissue and peripheral blood(16). As with most targeted therapies, the accurate identification of patients most likely to respond to treatment will be paramount for the success of precision medicine(54). Similarly, the identification of resistance mechanisms will be critical since both intrinsic and acquired resistance to PI3K inhibitors is commonly associated with mutations or copy number alterations of regulatory genes within the pathway or parallel oncogenic pathways(39). To reach these goals, our study design will utilize a "window of opportunity" in which patients will first receive copanlisib as a single agent in order to characterize predictive biomarkers including the potential molecular correlates of response. The Center for Cancer Research is uniquely positioned to overlay the strong translational endpoints within this trial. The Staudt lab will perform comprehensive molecular characterization of the tumors at baseline on FFPE blocks, and this evaluation will include whole-exome sequencing of tumor DNA, RNA-sequencing, and methylation arrays for copy number abnormalities. Further, the study will allow for an optional tissue biopsy after the window of copanlisib monotherapy to better assess early predictors of response, drug penetrance into tissue, and potential insights into the mechanisms of intrinsic resistance to copanlisib. We aim to study the molecular correlates of response both in pre-treatment tumor tissue as well as through "liquid biopsies" obtained via ctDNA(49).

The tumor microenvironment (TME) within FL may also influence response or resistance to targeted therapy since FL is a proliferation of malignant germinal center B-cells admixed with a varying proportion of nonmalignant immune cells such as T-cells, follicular dendritic cells, macrophages and stromal cells(55). The malignant cells in FL nodes retain a substantial inter-dependence with the nonmalignant cells and other stromal elements that constitute the tumor microenvironment in a pattern recently described as "re-education"(56). The spatial arrangement of the TME in FL varies across patients, however, and depends on genetic aberrations within the tumor cells as well as dependence on external stimuli for survival, proliferation, and immune escape(56). Early in the disease course, the lymph node architecture resembles that of normal reactive germinal centers, but the organization and dependence of the tumor cells on the TME is hypothesized to wane over the course of disease. We plan to explore the three-dimensional relationship of immune cells and stromal cells of interest in FL using a unique three-dimensional histo-cytometry method in collaboration with the Center for Advanced Tissue Imaging here at NIH. The novel imaging technology will enable highly multiplexed, quantitative image analysis of tissues samples and can be performed serially.

1.2.9 Rationale for response-adapted therapy design

In order to develop a precision approach to therapy in FL, it is important to identify patients who are most sensitive to copanlisib and rituximab therapy. The primary endpoint of our study is the CR rate after induction therapy since these are the patients who are likely to benefit most from this novel combination. Other trials testing the use of novel agents in upfront treatment of FL have used treatment durations of 1 year, 2 years, or indefinite therapy(27, 57). These extended durations of treatment lead to exorbitant costs of treatment as well as longer periods of exposure to agents that may impair immune function. Extended schedules of treatment may not be justified in patients with FL who have an indolent disease course and regimens that offer an option to discontinue therapy are justified.

In our study, we plan to stop therapy in patients who achieve CR after the first 6 cycles of therapy. These patients will have received 9 doses of rituximab which is a standard treatment option for low tumor burden FL and will serve as a rational comparison to this combination(18). Further, discontinuing therapy in patients who achieve deep remissions allows for the testing of novel surveillance strategies including monitoring with non-invasive tests such as ctDNA(49). If such a monitoring schedule proves itself to be feasible and effective, it could be further tested and developed as a method for rationally deciding treatment durations instead of empiric decision-making that currently guides maintenance therapy in FL(58).

It is also possible that treatment duration longer than 6 months is required to achieve a CR to induction therapy. For this reason, patients who achieve at least a PR to therapy, but do not meet criteria for CR will be treated with an additional 6 cycle of copanlisib and rituximab at a reduced dose schedule of copanlisib of twice per cycle. The modified dosing schedule of copanlisib is hypothesized to decrease the number of adverse events associated with treatment, should improve patient adherence to long treatment durations, and will provide an opportunity to test if some patients require more than 6 cycles of copanlisib and rituximab to achieve a remission.

Patients that do not respond to copanlisib and rituximab after 6 cycles of induction therapy will not be given further cycles of induction, since the likelihood of achieving a first response beyond 6 cycles would be unlikely. In some situations, however, these patients may have derived clinical benefit and do not necessarily need to initiate immediate salvage therapy. For this reason, these patients will stop therapy with copanlisib and rituximab, but will undergo active surveillance with ctDNA to further understand the correlations between quantitative results on ctDNA and the clinical criteria governing the need for initiation of salvage chemotherapy.

1.2.10 Rationale for salvage chemotherapy

The current standard of care for frontline treatment of patients with advanced-stage and high tumor burden FL remains combination chemotherapy with the addition of an anti-CD20 monoclonal antibody(26, 59). Select patients are treated with rituximab monotherapy, but this approach is typically reserved for patients with low tumor burden or with co-morbidities that prevent the safe administration of chemotherapy(26). The most commonly used frontline regimens consist of R-CHOP, rituximab, cyclophosphamide, vincristine, and prednisone (R-CVP), and rituximab, bendamustine (BR). A recent randomized study demonstrated that the use of an alternative anti-CD20 monoclonal antibody, obinutuzumab, prolonged the PFS of FL patients when used as frontline therapy, but since no overall survival benefit has yet to be determined we consider rituximab-based therapy as the current standard of care(24).

In this protocol, we plan to treat all patients who relapse or progress after induction therapy with copanlisib and rituximab with either BR or R-CHOP as standard salvage chemotherapy. The rationale for providing treatment with standard chemotherapy on this protocol is to ensure that patients willing to participate in our study of experimental treatment can be assured of an opportunity to get standard chemotherapy. Although standard salvage chemotherapy is not considered curative, it can induce long durations of remission in some patients. Further, it is of scientific interest to determine if patients who are not sensitive to copanlisib-based therapy can achieve quality depths and duration of response to standard therapy.

1.2.11 Study Summary and Hypotheses

We hypothesize that induction therapy with copanlisib and rituximab will result in complete responses and durable remissions for most patients with untreated follicular lymphoma. We also anticipate that induction therapy with copanlisib and rituximab will be effective in patients with high-risk follicular lymphoma as defined by published gene expression and molecular signatures. In addition, we anticipate that circulating tumor DNA via the clonoSEQ® assay will appear in plasma months prior to clinical or radiographic relapse, and that progressive rises in ctDNA via clonoSEQ® will predict the timing of second-line therapy. Finally, we hypothesize that patients who achieve a complete response to induction with copanlisib and rituximab lasting at least 6 months will remain sensitive to re-treatment with copanlisib and rituximab.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have a confirmed histologic diagnosis of FL, grade 1-2 or 3a, according to the criteria established by the most recent version of the World Health Organization (WHO) classification system. Pathologic diagnosis must be confirmed by Laboratory of Pathology, NCI

2.1.1.2 Stage II-IV disease. **NOTE:** Patients with stage I FL who have been treated with radiation therapy and have subsequently relapsed are eligible.

2.1.1.3 No prior systemic treatment for FL with chemotherapy, targeted small molecule therapy, or monoclonal antibody therapy prior to the first dose of copanlisib treatment. Patients may have received prior radiation therapy only; radiation therapy must have been completed >12 weeks prior to the first dose of copanlisib. **NOTE:** Prior short-term (≤7 days) use of corticosteroids for acute medical complications related to sites of FL involvement is permitted.

2.1.1.4 Patients must meet standard criteria for initiation of systemic therapy as evidenced by presence of one of the following:

- Development of symptomatic enlarged lymph nodes or spleen
- Development of B symptoms (fever, night sweats, weight loss) or severe pruritus
- Development of significant serous pleural or pericardial effusions (small effusions seen only on CT scans are not indications for systemic therapy)

- Development of bone marrow failure as a result of involvement by FL and not attributable to other causes; this would be manifest as a Hgb < 9 g/dl, absolute neutrophil count < 1 x 10^9/L, or platelet count < 75 x 10^9/L
- Critical organ involvement, organ compression (e.g., ureteric obstruction or epidural compression), or significant risk of future organ compressions
- Increase in the size of lymph nodes on CT scans indicating progression of disease from previous CT scans

2.1.1.5 Adequate tissue from diagnostic biopsy; formalin fixed tissue block or 20 slides of tumor sample (archival or fresh) must be available for performance of correlative studies

2.1.1.6 Be ≥18 years of age on day of signing informed consent

NOTE: Because no dosing or adverse event data are currently available on the use of (copanlisib) in patients <18 years of age, children are excluded from this study

2.1.1.7 ECOG performance status 0-2 (see **Appendix A**)

2.1.1.8 Adequate organ function as evidenced by the following laboratory parameters:

• Absolute neutrophil count (ANC)	≥ 1,500 /mm^3 (unless due to involvement by lymphoma or benign ethnic neutropenia)
• Platelets	≥ 75,000 / mcL (unless due to involvement by lymphoma; transfusions not permitted)
• Hemoglobin	≥ 8 g/dL (transfusions permitted)
• Renal function	Glomerular filtration rate (GFR) ≥ 40 mL/min/1.73 m2 as estimated by the Modification of Diet in Renal Disease (MDRD) abbreviated formula. If not on target, a 24-hour urine creatinine clearance can be used to directly measure.
• Serum total bilirubin	≤ 1.5 X ULN **OR** (< 3 x ULN for patients with Gilbert syndrome, patients with cholestasis due to compressive adenopathies of the hepatic hilum or documented liver involvement or with biliary obstruction due to lymphoma)
• AST (SGOT) and ALT (SGPT)	≤ 2.5 x ULN (≤ 5 x ULN for patients with liver involvement by lymphoma)
• Lipase	≤ 1.5 x ULN

2.1.1.9 Women of childbearing potential (WOCBP) and men must agree to use effective contraception when sexually active. This applies for the time period between signing of the informed consent form and for at least 1 month after the last dose of copanlisib and 12 months after the last dose of rituximab, whichever is later, for WOCBP and for men after the last administration of study treatment.

NOTE: A woman is considered of childbearing potential, (i.e., fertile), following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include but are not limited to hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no

menses for continuous 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. The investigator or a designated associate is requested to advise the patient how to achieve highly effective birth control (failure rate of less than 1%), e.g., intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomized partner and sexual abstinence. The use of condoms by male patients is required unless the female partner is permanently sterile. See **Appendix B** for complete details of acceptable contraceptive methods.

2.1.1.10 Ability of patient to understand and the willingness to sign a written informed consent document

2.1.2 Exclusion Criteria

2.1.2.1 Known lymphomatous involvement of the central nervous system

2.1.2.2 History of any known primary or acquired immunodeficiency syndrome (e.g., HIV)

2.1.2.3 CMV PCR positive at baseline

2.1.2.4 Hepatitis B surface antigen (HbsAg) or core antibody (HbcAb) positive with a positive Hep B DNA Quantitative, HBV Viral Load result.

NOTE: Subjects with positive hepatitis B serology (HbsAg or HbcAb) may be enrolled onto the study but they must have a negative Hep B DNA Quantitative, HBV Viral Load result before enrollment. See Section **3.4.2** for more information.

2.1.2.5 Uncontrolled intercurrent illness including, but not limited to the following that may limit interpretation of results or that could increase risk to the patient at the discretion of the investigator:

- Active autoimmune disease that has required systemic treatment in the past 12 months (i.e., with use of disease modifying agents, corticosteroids or immunosuppressive drugs). **NOTE:** Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
- History of (non-infectious) pneumonitis that required steroids, evidence of interstitial lung disease or active, non-infectious pneumonitis.
- Active hepatitis C infection. **NOTE:** Subjects who are hepatitis C antibody positive will need to have a negative HCV PCR result before enrollment. Those with a positive PCR for hepatitis C are excluded.
- Congestive heart failure > New York Heart Association (NYHA) class 2
- Unstable angina
- Myocardial infarction in the past 6 months
- Uncontrolled hypertension despite optimal medical management
- Arterial thromboembolic events such as cerebrovascular accident (including transient ischemic attacks), in prior 3 months
- Uncontrolled Type I or II diabetes despite optimal medical management
- Any second malignancy that requires active systemic therapy

- Known mental or physical illness that would interfere with cooperation with the requirements of the trial or confound the results or interpretation of the results of the trial and, in the opinion of the treating investigator, would make the patient inappropriate for entry into the study.
- Severe hepatic impairment (Child-Pugh C)

2.1.2.6 Requirement to continue on any of the medications that are excluded (see Section **4.3**)

2.1.2.7 Organ compromise that, in the opinion of the PI, necessitates immediate cytoreductive therapy

2.1.2.8 Pregnant or breast-feeding patients. Women of childbearing potential must have a serum pregnancy test performed a maximum of 7 days before start of treatment, and a negative result must be documented before start of treatment

2.1.2.9 Major surgical procedure or significant traumatic injury (as judged by the investigator) within 28 days before start of treatment, or have not recovered from major side effects, open biopsy within 7 days before start of treatment

2.1.3 Recruitment Strategies

Study participants will be recruited from the population of patients screened in the lymphoid malignancies clinic of the National Institutes of Health. Our research team currently has an active protocol to study the clonal evolution in FL that enrolls patients with untreated FL who are appropriate for observation as well as those who require immediate treatment. In addition, we participate in a locoregional consortium of eight academic institutions within the Mid-Atlantic region (Mid-Atlantic Lymphoma Research Consortium) that shares information regarding active clinical protocols and aims to enhance patient recruitment across the region. Our patients for this study will therefore consist of those from the FL clonal evolution study, referrals from outside physicians, and patient self-referrals. In addition, this study will be posted on NIH websites and NIH social media forums.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

A waiver of consent for these activities has been requested in Section **12.4.1**.

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the study consent. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent (unless otherwise noted).

NOTE: Assessments and procedures to confirm study eligibility should be completed within 28 days prior to registration (unless otherwise noted). See also the Study Calendar (Section **3.8**).

2.2.2.1 Clinical Evaluations

- Disease history, including: diagnosis, prior radiation treatment (if applicable), and significant prior/ongoing side effects and symptoms
- Complete medical history, including: all active conditions considered to be clinically significant by the treating investigator
- Physical examination, including: height (screening only), weight, vital signs (i.e., temperature, pulse, respiratory rate, and blood pressure); review of concomitant medications and symptoms/side effects; and, assessment of performance status using the ECOG scale

2.2.2.2 Laboratory Evaluations

- CBC with differential
- Chemistry panels (as noted) or specific analyte required for eligibility, including: Creatinine (i.e., or Acute Care Panel); Albumin (i.e., or Mineral Panel), ALT, AST, total and direct (if required) bilirubin (i.e., or Hepatic Panel); serum lipase, and 24-hour urine creatinine clearance (if needed to measure CrCl)
- Coagulation panel: PT/INR and aPTT
- LDH
- Urinalysis (with microscopic examination if abnormal)
- Hepatitis B surface antigen (HBsAg), Hepatitis B core antibody (HbcAb), Hepatitis C antibody (HCV) [qualitative]) (within 3 months allowed)
    - Hep B DNA Quantitative, HBV Viral Load for subjects who are HBsAg or HbcAb positive.
- CMV PCR and CMV IgM and IgG
- HIV antibody (within 3 months allowed)
- Hemoglobin A1C
- Urine and/or serum HCG in women of childbearing potential (within 7 days prior to initiation of study therapy)

2.2.2.3 Imaging Studies

NOTE: Results from NIH only.

- CT chest, abdomen and pelvis or MRI
- ^{18}F-FDG PET/CT

2.2.3 Other Procedures

- Pathologic review/confirmation of diagnosis by Laboratory of Pathology, NCI (no time limit). A tissue sample is required for this evaluation; if archival sample is not available, a fresh tumor biopsy will be obtained.
- Flow cytometry will be performed on peripheral blood for both diagnostic and staging purposes (only NIH results accepted; NCI Laboratory of Pathology)

- Bone marrow aspiration with flow cytometry (optional at screening at PI discretion) and biopsy (results from outside NIH accepted; within 12 months)

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at:

https://nih.sharepoint.com/sites/NCI-CCR-OCD-Communications/SitePages/OEC-Administrative---Clinical-Research-(ADCR).aspx?Mode=Edit.

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of an eligibility criterion (e.g., laboratory factor) may be rescreened if other eligibility are still met (e.g., prior treatment requirements, etc.).

2.3.2 Treatment Assignment Procedures

NOTE: For NCI CCR registration purposes only.

2.3.2.1 Cohorts

Number	Name	Description
1	Untreated FL	Untreated FL Stage II-IV or stage I FL who have been treated with radiation therapy and have subsequently relapsed (up to 65 patients)

2.3.2.2 Arms

Number	Name	Description
1	Experimental treatment	Window of treatment with Copanlisib 60mg via IV for a single 28-day cycle, once weekly for the first 3 weeks and then a 1-week break followed by induction therapy with copanlisib and rituximab. Induction therapy will be 6 cycles (28 days) of: copanlisib dose and administration same as window, rituximab 375mg/m^2 via IV, once weekly for the first 4 weeks during cycle 1, subsequent cycles (cycles 2-6), rituximab will be dosed only once on day 1 of the cycle. Further treatment with additional cycles of copanlisib + rituximab will be response adapted.

2.3.2.3 Arm Assignment

This is a single arm , non-randomized study (i.e., patients in Cohort 1 are directly assigned to Arm 1).

2.4 BASELINE EVALUATION

The baseline evaluations should be performed within 14 days prior to the first dose of copanlisib unless otherwise noted; tests performed as part of screening do not need to be repeated if they were performed within the specified window. See the Study Calendar (Section **3.8**) for details.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

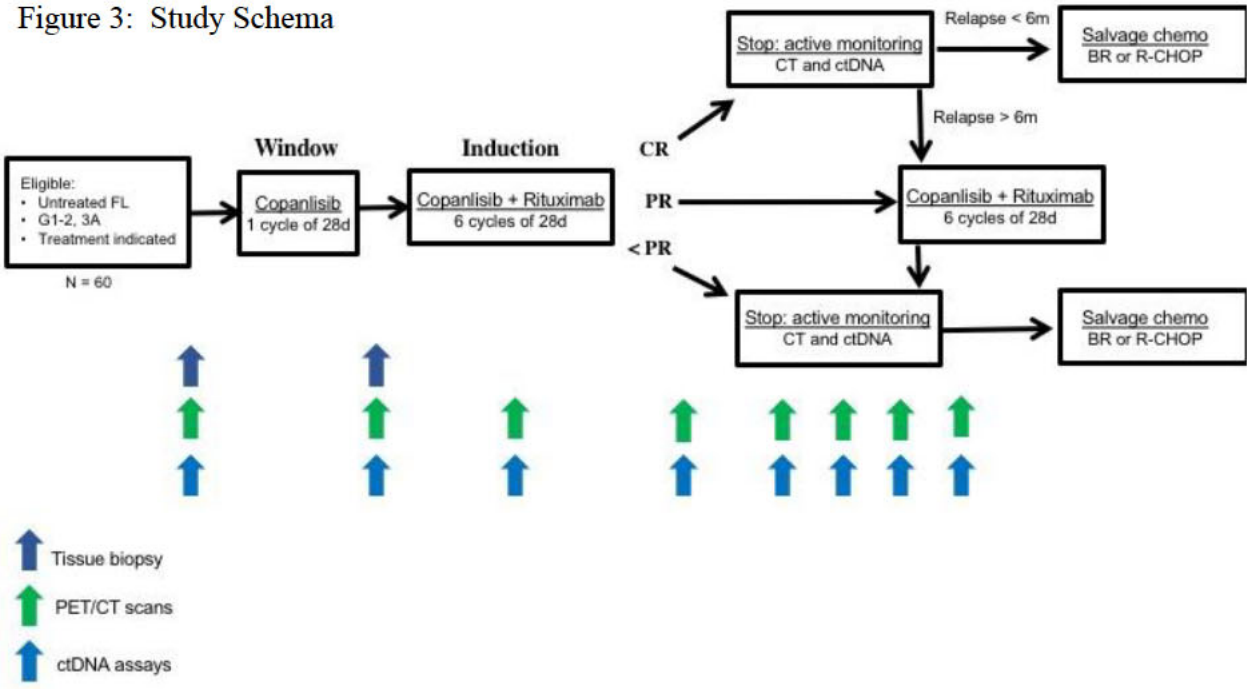
This is a non-randomized, open-label, single arm, single institution phase 2 study of copanlisib and rituximab in patients with untreated FL. Duration of therapy with copanlisib and rituximab will be response-adapted based on response to first 6 cycles of induction therapy.

Patients who achieve a complete response (CR) to induction therapy will stop treatment and undergo active surveillance with periodic CT scans and assays for ctDNA for up to 5 years. If disease relapses within 6 months from finishing induction therapy, these patients will be treated with salvage chemotherapy. If disease relapses greater than 6 months from finishing induction therapy, these patients will be treated with a second 6 cycles of (re-)induction therapy.

Patients who achieve a partial response (PR) to induction therapy will be treated with an additional 6 cycles of copanlisib and rituximab at a reduced (i.e., maintenance) schedule. After 6 cycles of maintenance therapy, these patients will stop treatment and undergo active surveillance with periodic CT scans and assays for ctDNA for up to 5 years. At the time of disease progression or relapse, these patients will be treated with salvage chemotherapy.

Patients who do not achieve at least a partial response (PR) to induction therapy will stop therapy and undergo active surveillance with periodic CT scans and assays for ctDNA for up to 5 years. At the time of disease progression, these patients will be treated with salvage chemotherapy.

Figure 3: Study Schema



3.2 DRUG ADMINISTRATION

3.2.1 Drug Administration – Window and Induction Therapy

The treatment cycle (i.e., "Cycle 0") for the window of copanlisib monotherapy will be 28 days.

Each cycle of treatment during induction therapy is 28 days. Patients will be treated for up to 6 cycles of induction therapy with copanlisib and rituximab (i.e., Cycles 1-6). A window between cycles of -3/+7 due to scheduling or other administrative reasons is allowed; additional delays may apply based on toxicities.

Treatment will generally be given on an outpatient basis; however, may be given as an inpatient for matters of convenience or investigator preference in case of additional monitoring.

3.2.2 Copanlisib

All patients will receive copanlisib at a fixed dose of 60mg administered via IV in Cycle 0 and for Cycles 1-6. Dosing is not based upon subject weight. Infusions may be done peripherally or via central venous access device.

Copanlisib will be administered as a 60-minute infusion. Every effort should be made to target the infusion timing as close to 60 minutes as possible; however, a preferred window of -5/+10 minutes to account for variability of infusion pumps is permitted. On infusion days, patient's blood pressure will be monitored at 0 h (pre-dose), 30 (±10) min (mid-infusion), 60 (±10) min (end of infusion) (See Section **3.3.6**).

No premedications are required for copanlisib.

Copanlisib dosing is weekly for the first 3 weeks of each cycle (i.e., Days 1, 8, and 15) followed by a 1-week break (i.e., no infusion on Day 22).

3.2.3 Rituximab

Patients will receive rituximab at 375mg/m^2 administered via IV in Cycles 1-6. Infusions may be done peripherally or via central venous access device. Rituximab will be administered per current standard of practice, including premedications (see Section **14.2.5**).

Rituximab dosing is weekly for the first 4 weeks (i.e., Days 1, 8, 15, and 22) during Cycle 1. With subsequent cycles (i.e., Cycles 2-6), rituximab will be dosed only once on Day 1.

3.2.4 Drug Administration – Maintenance Therapy

Patients who achieve a PR to the first 6 cycles of induction therapy with copanlisib and rituximab, but do not meet criteria for CR, can be treated with an additional 6 cycles of copanlisib and rituximab with a modified schedule of maintenance therapy (i.e., Cycles 7-12).

Patients will receive copanlisib at the same dose as during induction therapy; however, dosing is every other week during each 28-day cycle (i.e., Days 1 and 15) with each dose followed by a 1-week break (i.e., no infusion on Day 8 or 22).

Patients will also receive rituximab at the same dose as during induction therapy; however, dosing is only on Day 1 of each maintenance therapy cycle.

3.2.5 Drug Administration – Salvage Chemotherapy Regimens

Patients who relapse or progress after completing study therapy with copanlisib and rituximab (i.e., patients who do not achieve at least a PR to induction therapy, those who relapse after CR within 6 months from initial induction therapy, and those patients who progress or relapse after

maintenance or re-induction therapy) will be offered treatment at the NCI with standard salvage chemotherapy regimens as deemed most appropriate per the most recent National Comprehensive Cancer Network (NCCN) guidelines. These patients will be considered to have completed protocol therapy and will be followed for PFS and OS. During treatment with salvage chemotherapy, adverse events will not be collected and recorded, unless considered at least possibly related to the copanlisib and/or rituximab. Patients must meet conventional definitions of disease relapse or progression as per the investigator and reappearance of ctDNA alone is not considered disease relapse.

Standard chemotherapy regimens for FL include rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) or bendamustine and rituximab (BR) for up to 6 cycles. R-CHOP is typically administered every 21 days while BR is typically administered every 28 days.

After salvage chemotherapy, follow-up for overall survival will continue as per the Study Calendar (Section **3.8**).

3.2.6 Supportive Medications

3.2.6.1 Pneumocystis Jiroveci Prophylaxis

All subjects should receive prophylaxis for Pneumocystis Jiroveci during study therapy administration. Trimethoprim/sulfamethoxazole 1 DS tablet by mouth on Monday, Wednesday, and Friday is the preferred schedule.

Prophylaxis will begin Cycle 0, Day 1 (Day 1 of the copanlisib window; +1/-1 day) and will be stopped upon completion of study therapy. Continued administration of prophylaxis beyond completion of study therapy will only be performed if deemed clinically necessary (e.g., based on inadequate immune reconstitution).

Subjects allergic to either component may receive inhaled pentamidine 300 mg once a month or other standard treatments.

3.2.6.2 Antiemetics

Prophylactic anti-emetics may be administered according to standard practice. The routine use of standard antiemetics, including 5-HT3 blockers, such as granisetron, ondansetron, or an equivalent agent, is allowed as needed. The use of corticosteroids as antiemetics prior to copanlisib administration will be not allowed.

3.2.6.3 Hepatitis B Reactivation Treatment

In the event that patients develop reactivation of Hepatitis B without any evidence of active Hepatitis B infection, then medications such as entecavir will be administered according to local medical standards.

3.3 DOSE MODIFICATIONS

Adverse events may occur shortly after the first dose or several months after the last dose of treatment. Copanlisib must be withheld for all drug-related toxicities considered severe or life-threatening AEs as per below. For all AEs/SAEs, the treating investigator may use discretion with regards to dose holds, modifications, and supportive care. It is recognized that attribution of causality of any AE to the test drug specifically may be difficult. However, in previous trials, certain toxicities were seen only in relation to copanlisib, e.g., transient increases in glucose and

blood pressure. Based on this knowledge the investigator may decide on the necessary dose modifications as per **Table 1**.

Table 1: **Dose levels of copanlisib**

Dose level 1 (starting dose):	60 mg of copanlisib
Dose level -1:	45 mg of copanlisib
Dose level -2:	30 mg of copanlisib

After having fully recovered from toxicity and in the absence of any criteria for further dose reduction or study drug discontinuation, re-escalation from dose level -2 to dose level -1, or from dose level -1 to dose level 1 will be allowed at the investigator's discretion, with the exception of non-infectious pneumonitis (NIP). Patients who do not tolerate copanlisib dose of 30 mg must discontinue study treatment permanently.

Dosing interruptions are permitted in the case of medical and/or surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Patients should be placed back on study therapy within 3 weeks of the scheduled interruption, whenever possible. The reason for interruption should be documented in the medical/research records. These interruptions will not be considered protocol deviations.

3.3.1 Unacceptable Toxicity – Withdrawal Criteria

- CTCAE Grade 4 hypertension
- CTCAE Grade 4 dermatologic toxicity
- CTCAE Grade ≥ 3 non-infectious pneumonitis
- Persistent post-infusion hyperglycemia despite optimal glucose lowering therapy in consultation with a diabetologist or endocrinologist
- Drug-induced pancreatitis
- Development of a new malignancy. New malignancy will be reported as an SAE.
- Severe allergic reaction to study drug (such as CTCAE Grade 3 or 4 hypersensitivity reaction).

3.3.2 Hematologic toxicity

Dose modifications and treatment interruptions for copanlisib must be done according to the guidelines in **Table 2**. The investigator may judge a more conservative dose modification appropriate. Therefore, if these guidelines are not followed, the rationale for other measures is to be documented in detail in the patient's medical record.

Table 2: **Dose modification of copanlisib/for hematological toxicity**

Hematological toxicity (any of the following)	Study treatment action (for any toxicity)
• CTCAE Grade ≥ 3 thrombocytopenia (platelet < 50,000/mm^3) • Febrile neutropenia [a] • CTCAE Grade ≥ 3 neutropenia (ANC < 1000/mm^3) • INR or PTT CTCAE Grade ≥3 with bleeding [b] • CTCAE Grade ≥ 3 anemia (Hb < 8 g/dL)	Delay infusion until criteria displayed in Section 3.5.1 are met.[d] Patient can be treated at one dose level lower at the investigator's discretion.[c] If more dose reductions are required than allowed per protocol, discontinue copanlisib permanently. The lowest dose level is 30 mg.

ANC = absolute neutrophil count; CTCAE = Common Terminology Criteria of Adverse Events; Hb = hemoglobin; INR = international normalized ratio; PTT = partial thromboplastin time

a: These patients should recover from neutropenia, without fever

b: International normalized ratio (INR) and partial thromboplastin time (PTT) should have returned to ≤ 1.5 and ≤ 1.5 x ULN, respectively, with no signs of bleeding

c: After having fully recovered from toxicity and in the absence of any criteria for further dose reduction or study drug discontinuation, re-escalation to dose level -1 or 1 is allowed at the investigator's discretion

d: Treatment with transfusion or growth factors is allowed at the investigator's discretion

Neutropenia and febrile neutropenia are listed in the current version of the IB as expected adverse events. If the guidelines given in **Table 2** are not followed, the rationale for other measures is to be documented in detail in the patient's medical record.

3.3.3 Non-hematologic toxicity

Dose modification recommendations for non-hematologic toxicities attributable to copanlisib other than glucose increases (Section **3.3.7**), dermatologic toxicity (Section **3.3.4**), non-infectious pneumonitis (Section **3.3.5**), and blood pressure increases (Section **3.3.6**) are outlined below in **Table 3**. The investigator may judge a more conservative dose modification appropriate. Therefore, if these guidelines are not followed, the rationale for other measures is to be documented in detail in the patient's medical record.

Table 3: **Dose modification of copanlisib for non-hematological toxicity***

Toxicity [a]	Occurrence	Study Drug Action	
		For current course of therapy	For next course of therapy
Grade 1-2	Any appearance	No change	No change
Grade 3[b]	1st appearance	Delay until Grade ≤ 2	No change
	2nd appearance	Delay until Grade ≤ 2	Decrease by one dose level [c]
	3rd appearance	Delay until Grade ≤ 2	Decrease by one dose level [c]
	4th appearance	Permanently discontinue	–
Grade 4	Any appearance	Permanently discontinue	–
Toxicity requiring delay for > 21 days		Permanently discontinue	–

* except glucose increases, dermatologic toxicity, non-infectious pneumonitis and blood pressure increases
a: Toxicities according to CTCAE
b: Despite maximum supportive therapy
c: Not applicable for 30 mg dose level
A delay >28 days in study drug administration due to toxicities will cause permanent discontinuation of study treatment. Except in cases of delays due to reactivation of CMV where delyas could be up to 2 months
Copanlisib must be discontinued if the lowest dose level of 30 mg is not tolerated.
After having fully recovered from toxicity and in the absence of any criteria for further dose reduction or study drug discontinuation, re-escalation to dose level -1 or 1 will be allowed at the investigator's discretion.

3.3.4 Dermatologic toxicity

The guidelines for dose modifications in cases of dermatologic toxicity are outlined in **Table 4** below (dose modification of copanlisib for dermatological toxicity). If these guidelines are not followed, the rationale for other measures will be documented in detail in the patient's medical record.

Table 4: **Dose modification recommendations of copanlisib for dermatologic toxicity**

		Study Drug Action	
Toxicity [a]	Occurrence	For current course of therapy	For next course of therapy
Grade 1	Any appearance	No change	No change
Grade 2 [b]	1st appearance	Interruption until Grade ≤ 1	No change
	2nd appearance	Interruption until Grade ≤ 1	Decrease by one dose level [c]
	3rd appearance	Interruption until Grade ≤ 1	Decrease by one dose level [c]
	4th appearance	Permanent discontinuation	–
Grade 3 [b]	1st appearance	Interruption until Grade ≤ 1	Decrease by one dose level [c]
	2nd appearance	Interruption until Grade ≤ 1	Decrease by one dose level [c]
	3rd appearance	Permanent discontinuation	–
Grade 4	1st appearance	Permanent discontinuation	–

a: Toxicities according to CTCAE
b: Despite maximum supportive therapy
c: Not applicable for 30 mg dose level
The lowest dose level is 30 mg; if a patient is already on the 30 mg dose level study drug and meets criteria for further decrease of dose, study drug will be discontinued permanently.

3.3.5 Non-infectious pneumonitis (NIP)

In the event of suspected NIP of any grade, copanlisib must be interrupted and a diagnostic examination of a patient experiencing pulmonary symptoms should be conducted. If NIP is the

final diagnosis, copanlisib must be permanently discontinued if CTCAE grade ≥3; for CTCAE grade 2 treat till resolution or ≤ CTCAE 1 and resume copanlisib at a reduced dose of 45mg. If grade 2 NIP recurs, discontinue copanlisib (see **Table 5**). Pneumonitis is to be reported as such only in the event of NIP. Treat NIP with systemic steroids at the discretion of treating investigator. The investigator is requested to differentiate between non-infectious pneumonitis (NIP), and infectious pneumonitis (viral, bacterial, fungal), aspiration pneumonitis, or other pneumonitis clearly not due to a potential hypersensitivity reaction to the copanlisib infusion; and provide the basis for his/her assessment that it is infectious or other, as appropriate.

Table 5: **Dose adjustment in cases of non-infectious pneumonitis (NIP)**

Suspected or confirmed NIP per CTCAE	Action Taken	Re-treatment dose after recovery
Grade 1	No Change	NA
Grade 2	Dose Interruption Until recovery to ≤ grade 1	Decrease dose to the next lowest dose level [a]
Grade 2 second occurrence	Permanent Discontinuation	NA
Grade 3	Permanent Discontinuation	NA
Grade 4	Permanent Discontinuation	NA

3.3.6 Hypertension

No dose of copanlisib should be given if blood pressure is ≥ 150/90 mmHg. Antihypertensive medication may be given to control the increased blood pressure. Dosing can proceed on the scheduled day if there are at least 2 consecutive measurements <150/90 mmHg, about 5-10 (+5) minutes apart. Otherwise dosing must be delayed (see **Table 6**).

If drug-related hypertension (post-dose blood pressure of CTCAE Grade 3 or ≥ 160/100 mmHg) is not manageable with optimal antihypertensive treatment, the dose for the subsequent copanlisib administrations may be reduced by 1 or 2 dose levels at the investigator's discretion. Patients with a post-dose blood pressure that may have life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis) must permanently discontinue the study drug.

Table 6: **Dose modification of copanlisib for hypertension**

Toxicity (CTCAE)	Study drug action	Recommendation
Pre-dose measurements: BP ⩾ 150/90 mmHg	No dose should be given until recovery to < 150/90 mmHg.	Consider BP lowering medication. Dosing can proceed on the scheduled day if after at least 2 consecutive BP measurements about 5-10 (+5) minutes apart returns to < 150/90 mmHg. If BP does not return to < 150/90 mmHg, delay dosing until next visit.
During infusion: CTCAE hypertension of grade 3 or ⩾ 160/100 mmHg	Infusion can be interrupted and administration of BP lowering therapy should be initiated.	Infusion may be resumed when BP has returned to < 150/90 mmHg at the investigator's discretion or skipped. Subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion.[b]
All other times: • Drug-related CTCAE hypertension of grade 3 or ⩾ 160/100 mmHg [a]	–	Administration of BP lowering therapy should be initiated according to local standard of care. Additional measurements to be performed as clinically indicated until recovery to < 150/90 mmHg. Subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion.[b]
• CTCAE hypertension of grade 4	Permanent discontinuation	–

CTCAE = Common Terminology Criteria for Adverse Events; BP = Blood pressure
[a]: Not manageable despite optimal antihypertensive treatment.
[b]: The lowest dose level is 30mg.

It is important that patients with pre-existing hypertension adhere to their regular medication schedule and take their usual doses on the days of study drug infusion.

The management of acute blood pressure increases following copanlisib will need to be individualized for each patient, but experience in Phase I has suggested the benefit of dihydropyridine calcium channel blockers (i.e. amlodipine, felodipine). Nitrates should also be considered. Verapamil and diltiazem (non-dihydropyridine calcium channel blockers and moderate inhibitors of CYP 3A4) should be used with caution due to a potential CYP 3A4 interaction. In general, it is advisable for investigators to be prepared, so that antihypertensive medication is readily available in case of need.

In the event of the occurrence of hypertension ≥ 150/90 mmHg during infusion of copanlisib at any cycle, antihypertensive treatment is suggested as indicated (**Table 6**). In the event of the occurrence of CTCAE Grade 3 hypertension during infusion of copanlisib, the infusion should be interrupted, and antihypertensive treatment administered. Infusion can be resumed when blood pressure has returned to <150/90 mmHg.

Blood pressure will be measured every 5 – 10 minutes prior to each copanlisib dose (no more than 4 measurements) until there are two consecutive results < 150/90 mmHg. If blood pressure is ≥ 150/90 mmHg, the investigator can consider a medical intervention or delaying the infusion of study drug. The patient should rest for 5-10 minutes before blood pressure is recorded.

On infusion days, blood pressure will be measured at 0 h (pre-dose), 30 (±10) min after infusion starts (mid-infusion), and 60 (±10) min (end of infusion).

3.3.7 Blood glucose

Because of its inhibitory effect on the PI3K α-isoform, which is implicated in insulin metabolism, copanlisib infusions could be associated with a temporary increase in blood glucose. Addition of a meal in close proximity to copanlisib infusion may exacerbate glucose increase. It is recommended that timing and content of caloric intake on infusion days is monitored by the investigators. For patients with type 1 or type 2 diabetes, consultation with an endocrinologist will be done prior to first infusion of copanlisib. The investigator will review the glucose profile during and post the copanlisib infusions; see **Table 7** for requirements.

Table 7: **Fasting requirements and pre-dose glucose levels**

Period	Fasting ≥ 8 h required before first glucose measurement	Pre-dose glucose levels	Fasting required before study drug infusion
Day 1 of cycle 0	Yes	<160 mg/dL (8.9 mmol/L)	Yes[b]
Day 1 of subsequent cycles	No	<160 mg/dL (fasting) (8.9 mmol/L) < 200 mg/dL (11.1 mmol/L) (non-fasting or random)	Conditional [a,b,c]
Days 8 and 15 of each cycle	No	<160 mg/dL (8.9 mmol/L) (fasting) <200 mg/dL(11.1 mmol/L) (non-fasting or random)	Conditional [a,b,c]

a. The decision regarding meal timing and fasting can be made by the investigator based on glucose response patterns during prior treatment days (see text below regarding meal timing on infusion days for further details).

b. Diabetic patients who take insulin treatment at any cycle visit:

Timing and content of caloric intake on infusion days will be managed by the investigator. Consultation with treating physician or diabetes/endocrinologist is advised.

c. A small, light low glycemic index meal may be taken at least 4 hours before the start of the copanlisib infusion for patients who have their infusions scheduled at a later hour, or due to their age or medical condition when fasting prior to infusion is not viable.

Timing and content of caloric intake on infusion days will be managed by the investigator. Consultation with treating physician or diabetes/endocrinology physician is advised. Post-infusion glucose monitoring will be added in all patients with pre-existing diabetes mellitus and patients who have random non-fasting glucose levels exceeding 200mg/dL.

The investigator may manage the timing of post infusion meals based on the glucose profile during prior infusion(s) to minimize glucose increases. This is in addition to glucose lowering medication. Patients may require home glucose monitoring if hyperglycemia is persistent. The specific type of monitoring will be individualized with consultation from an endocrinologist, but may include pre-meal and post-meal monitoring of blood glucose monitoring.

All glucose measurements, oral glucose lowering medication and/or insulin administration, if applicable, and meal timing will be collected as part of the clinical source documentation.

NOTE: Caloric intake and timing recommendations for diabetic patients who require insulin treatment prior to the infusion at any cycle visit should be managed by the investigator based on consultation with treating physician or diabetes/endocrinologist physician.

3.3.7.1 Glucose measurements

Cycle 0 Day 1:

Fasting is required (8-hour minimum) only on Cycle 0 Day 1 before the first glucose measurement on date of infusion. A low glycemic index meal may be taken 2 hours post-infusion unless the patient needs to have a low glycemic meal and is unable to fast for this period of time, then glucose test should be taken prior to meal intake.

All subsequent infusions after Cycle 0 Day 1:

The decision regarding meal timing can be made by the investigator based on glucose response patterns during prior treatment days.

NOTE: If patient needs to have a low glycemic meal prior to the infusion, then glucose test should be taken prior to the meal and/or at 1-2 hours after the meal.

After Cycle 0 Day 1, a low glycemic index meal may be taken at least 4 h before the start of the study drug infusion for patients who have their infusions scheduled at a later treatment time or due to their age or medical condition when fasting prior to infusion is not viable.

3.3.7.2 Glucose monitoring

Treatment Cycle 0, Day 1

The following assessments should be performed on Cycle 0, Day 1 before receiving study treatment unless otherwise specified.

- On Cycle 0 Day 1, patients should be fasting for at least 8 hours prior to the pre-dose glucose measurement. For details on fasting requirements and glucose measurement during Cycle 0 Day 1 please refer to **Table 7**.
- Blood glucose will be measured prior to infusion either with serum chemistry on the day of infusion or an immediate fingerstick glucose check within 30 minutes pre-dose and subsequently, if clinically indicated at the discretion of the investigator. Patients should be educated on the signs and symptoms of hyperglycemia, such as frequent urination, increased thirst, blurred vision, headaches and difficulty concentrating and must report these to the investigator or their physician immediately. It is recommended for all patients on Cycle 0 Day 1 to measure glucose levels post-infusion at 3, 5, 6 and 8 hours (study specific measurements).

All subsequent infusions after Cycle 0 Day 1

If hyperglycemia noted less than 250 mg/dL on Cycle 0 Day 1, at the discretion of the investigator, post-infusion glucose monitoring may or may not be performed. Pre-infusion glucose test prior to each subsequent infusion either with serum chemistry or an immediate fingerstick glucose check within 30 minutes pre-dose is required to ensure fasting levels less than 160 mg/dL or random/non-fasting glucose levels less than 200 mg/dL.

Glucose monitoring at home

- For all patients (who experienced persistent glucose >250 mg/dL (13.9 mmol/L) or received anti-diabetic treatment on the day of infusion), fasting glucose should be measured

the next day. If glucose reading is < 100 mg/dL (5.5 mmol/L) (non-diabetic) or < 160 mg/dL (8.9 mmol/L) (diabetic) record and stop further glucose measurements until the next copanlisib infusion. If glucose is > 100 mg/dL (5.5 mmol/L) (non-diabetic) or > 160 mg/dL(8.9 mmol/L) (diabetic), the study nurse or investigator should be informed. If the patient has known diabetes and already monitors his/her blood glucose as part of routine diabetes care, the routine measurements should not be replaced by the study-specific measurements. In this situation, patients should add the study-specific measurements to their routine.

- In non-diabetic patients who experience persisting glucose > 250 mg/dL (13.9mmol/L) or who require treatment to maintain optimal glucose levels; consultation with endocrinologist is recommended. These non-diabetic patients will be trained how to measure their capillary blood glucose levels at home. If applicable, patients will be provided with glucose meter and supplies (lancets, test strips and diary) to register measured values and record oral glucose lowering medication and/or insulin administration.

3.3.7.3 Management of infusion-related hyperglycemia

Mild to moderate asymptomatic increases of blood glucose may occur with copanlisib infusion, and with larger increases potentially occurring post-prandially. Please refer to table below for dose modifications and management of hyperglycemia.

Glucose Increase	Assessment	Management
Asymptomatic glucose increases ≤ 250mg/dL (13.9 mmol/L)	Does not generally require treatment with glucose lowering medication.	Oral Hydration
Asymptomatic glucose increase > 250 mg/dl (13.9 mmol/L)	Repeat glucose testing. If glucose value is decreasing, glucose levels may be followed without glucose lowering medication treatment if hydration status is normal as clinically assessed Consultation with endocrinologist is recommended	Oral and or IV Hydration as appropriate
Symptomatic or persisting glucose increase > 250mg/dL (13.9 mmol/L)	Hydration status should be clinically assessed. If clinical assessment is consistent with dehydration, fluids should be given as clinically appropriate (orally or IV). Laboratory test confirming increase should be repeated. If the repeated glucose value is persistent and/or patient is symptomatic and/or the hydration status indicates the need for hydration, glucose lowering medication should be administered. Prompt input from an endocrinologist should be obtained.	Keep well hydrated rapid/ short acting insulin may be given if glucose persisting at > 250 mg/dL (13.9 mmol/L), and the patient is symptomatic Rapid/short acting insulin according to the institution sliding scale coverage of glucose persisting at > 250 mg/dL (13.9 mmol/L) is recommended, with oral or IV hydration as clinically appropriate
Asymptomatic Glucose increase > 500mg/dL	Will require dose reduction for subsequent treatment infusion.	Keep well hydrated rapid/ short acting insulin may be

Glucose Increase	Assessment	Management
	Repeat glucose testing. If glucose value is decreasing, glucose levels may be followed without glucose lowering medication treatment if hydration status is normal as clinically assessed Consultation with endocrinologist is recommended	given if glucose persisting at > 250 mg/dL (13.9 mmol/L), and the patient is symptomatic For insulin naïve patients, monitor for signs of hypoglycemia 3 hours post insulin administration due to risk for hypoglycemic events.

3.3.7.4 Management of persistent infusion-related hyperglycemia on subsequent days

Criteria	Recommendation	Suggested Treatment
Persistent glucose > 200 mg/dL (11.1 mmol/L) (non-fasting) or >160 mg/dL (8.9 mmol/L) (fasting) post infusion	Oral Glucose Lowering Medication, and consultation with endocrinologist recommended	The use of sulphonylurea/metaglinides, insulin secretagogues medications to manage increased glucose levels post drug infusions is not recommended Treatment with glucose lowering medication suggested according the local standards of practice

3.4 On Study Assessments/Evaluations

Upon successful enrollment, and following completion of the Screening/Baseline visits, patients will begin treatment with copanlisib in the single agent window (days -28 to -1).

Beginning with Cycle 1 of combined copanlisib and rituximab, pre-treatment assessments may be performed up to 3 days prior to Day 1 of a cycle (7 days for imaging), except where otherwise noted. The results from all procedures/tests must be reviewed prior to initiation of each cycle of treatment for consideration of dose modifications.

Copanlisib and rituximab will continue until disease progression, unacceptable treatment-related toxicity, or other reasons outlined in Section **3.10**.

Refer to the Study Calendar (Section **3.8**) for all tests and procedures to be conducted on study/during treatment.

3.4.1 Cytomegalovirus (CMV) Reactivation Monitoring and Considerations

CMV reactivation can occur in patients treated with copanlisib and is manifested by positive CMV PCR without evidence of active CMV infection or organ involvement. CMV screening will be performed in all patients before study enrollment. This will include CMV IgM and IgG status as well as baseline CMV PCR.

Patients with a positive CMV PCR at screening will not be enrolled onto the study per exclusion criteria (Section **2.1.2.3**). All patients will have CMV PCR drawn before each new cycle, and each surveillance visit until 6 months after therapy is complete (see Study Calendar, Section **3.8**). If a surveillance CMV PCR becomes positive during therapy, then they will be assessed for evidence of organ involvement. If no organ involvement, patients can continue on study therapy at the discretion of the PI and will be managed according to local standards including use of anti-CMV medications such as valganciclovir. If patients develop CMV infection associated with organ involvement, they will be treated with anti-CMV medications and removed from study therapy.

3.4.2 Hepatitis B Reactivation Monitoring and Considerations

HBV reactivation, in some cases resulting in fulminant hepatitis, hepatic failure and death can occur in patients treated with anti-CD20 antibodies including rituximab. HBV screening will be performed in all patients before study enrollment. At a minimum this will include HBsAg and HBcAb status. These can be complemented with other appropriate markers as per local guidelines. Patients with active Hepatitis B disease will not be enrolled onto the study per exclusion criteria (Section **2.1.2.4**). Patients with positive Hepatitis B serology, such as HbsAg or HBcAb, but without evidence of active Hepatitis B disease, may be enrolled and will be monitored and managed following local medical standards to prevent hepatitis reactivation; including prophylaxis with medications such as entecavir as well as periodic monitoring of hepatitis DNA.

If HBV reactivation occurs without any evidence on active Hepatitis B infection and normal liver associated enzymes, patients may continue on study treatment if, at the discretion of the investigator, they are receiving clinical benefit. These patients will be treated with medications such as entecavir to prevent liver injury.

Patients with a positive Hepatitis B serology, but without evidence of active Hepatitis B disease, will be monitored for Hepatitis B DNA via a Hep B DNA Quantitative, HBV Viral Load before every new cycle. Please see Study Calendar (Section **3.8)**.

3.5 TREATMENT CONSIDERATIONS/EXCEPTIONS

3.5.1 Laboratory Criteria For Initiating A Cycle of Copanlisib

In order to initiate a subsequent cycle of treatment with copanlisib, patients must meet minimum requirements for laboratory criteria as indicated below:

• Glucose	< 200 mg/dL (non-fasting)
• Hemoglobin	≥ 8 g/dL [a]
• ANC	≥ 1,000/mm^3
• Platelets	≥ 75,000/mm^3
• ALT	<2.5 x ULN [b]
• AST	<2.5 x ULN [c]
• Total bilirubin	within normal limits [d]
• GFR (MDRD)	≥ 40 mL/min/1.73 m^2

a. If hemoglobin is < 8 g/dL but ≥ 6 g/dL on the day of planned study drug administration it is permissible to give the study drug dose as scheduled and transfuse within 48 hours after the dose, if the patient is hemodynamically stable and in opinion of investigator benefits outweigh risks.
b. < 5 x ULN in patients with documented liver involvement by lymphoma or with biliary obstruction due to lymphoma.
c. < 5 x ULN in patients with documented liver involvement by lymphoma or with biliary obstruction due to lymphoma.
d. <3 x ULN in patients with Gilbert syndrome, patients with cholestasis due to compressive adenopathies of the hepatic hilum or documented liver involvement by lymphoma.

A blood count will be performed and assessed prior to study drug infusion on Day 8 and 15 of each cycle. On Days 8 and 15, the dose of copanlisib will be administered if, on the day of scheduled dosing, the laboratory test criteria for hemoglobin, ANC and platelets are met.

Doses scheduled for Days 8 and 15 may be delayed by up to 2 days. A delay of more than 2 days will be considered a missed dose. Missed doses will not be replaced. The minimum interval needed between two infusions of study drug is 5 days.

3.5.2 Discontinuation of Study Therapy

For details regarding formal stopping rules for futility and unacceptable toxicities see Section **10.2.**

3.5.2.1 Post 6 Cycles of Induction Therapy and Achieve a Complete Response

Patients who complete 6 cycles of induction therapy with copanlisib and rituximab and achieve a CR as defined by conventional Lugano response criteria will stop therapy and initiate active surveillance with CT scans and assays for ctDNA for up to 5 years as per Section **3.1**. These patients will not be treated with maintenance therapy as per Section **3.2.4**. Patients who achieve CR and relapse during active surveillance at least 6 months after induction therapy may be eligible for re-induction therapy as per Section **3.5.3**. If disease relapses within 6 months of finishing induction therapy, these patients may be treated with salvage chemotherapy as per Section **3.2.5**.

3.5.2.2 Post 12 Cycles of Induction Therapy

Patients who complete 6 cycles of induction therapy with copanlisib and rituximab as well as 6 cycles of maintenance therapy with copanlisib and rituximab will stop therapy and initiate active surveillance with CT scans and assays for ctDNA for up to 5 years as per Section **3.1**. At the time of disease progression, these patients may be treated with salvage chemotherapy as per Section **3.2.5**.

3.5.2.3 Post 6 Cycles of Induction Therapy and Do Not Achieve a Partial Response

Patients who complete 6 cycles of induction therapy with copanlisib and rituximab and do not achieve at least a PR (best response stable disease or progressive disease) as defined by conventional Lugano response criteria will stop therapy and initiate active surveillance with CT scans and assays for ctDNA for up to 5 years as per Section **3.1**. These patients will not be treated with maintenance therapy. At the time of disease progression, these patients may be treated with salvage chemotherapy as per Section **3.2.5**.

3.5.3 Criteria For Therapy With A Second (Re-) Induction Therapy

Patients who stop therapy with copanlisib and rituximab after achievement of CR may be eligible for a second course of induction therapy (re-induction) with the same drug administration schedule as outlined in Section **3.2** (Induction Therapy schedule) if they relapse after stopping study treatment. This retreatment is termed the Second Induction Phase of this study and is only available if the study remains open and the subject meets the following conditions:

- Stopped initial treatment with copanlisib and rituximab after attaining an investigator-determined confirmed CR after 6 cycles of induction
- At least 6 months have passed since the last dose of copanlisib
- Did not receive any anti-cancer treatment since the last dose of copanlisib
- No evidence of histologic transformation to diffuse large B-cell lymphoma
- Has a performance status of 0-2 on the ECOG Performance Scale
- Demonstrates adequate organ function as detailed in Section **2.1.1.8**
- Female subject of childbearing potential should have a negative serum or urine pregnancy test within 72 hours prior to receiving retreatment with study medication.

- Female subject of childbearing potential, as defined in Section **2.1.1.9**, should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity. This applies for the time period between signing of the informed consent form and for at least 1 month after the last dose of copanlisib and 12 months after the last dose of rituximab, whichever is later, after the last dose of study medication (**Appendix B**).
  - Male subject should agree to use an adequate method of contraception. This applies for the time period between signing of the informed consent form and for at least 1 month after the last dose of copanlisib and 12 months after the last dose of rituximab, whichever is later, after the last dose of study therapy.
- Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might interfere with the subject's participation for the full duration of the trial or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

Patients who restart treatment will be retreated at the same dose and dose interval as when they last received copanlisib and rituximab. Treatment will be administered for an additional 6 cycles.

After discontinuing the Second Course/Retreatment Phase, patients should return to the site for a Safety Follow-up Visit (Section **3.7.1**) and then proceed to the Follow-Up Period of the study (Sections **3.7.2** and **3.7.3**).

3.6 POST-TREATMENT EVALUATIONS

Patients will be evaluated at the end of study treatment (+/-2 weeks), about 30 days following the last dose of treatment (+/-7 days), and post-treatment prior to (or at) disease progression. See Study Calendar (Section **3.8**) for additional information. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30-day safety follow-up visit must occur before the first dose of the new therapy.

Unless otherwise noted, follow-up will occur at the following time points: every 3 months (+/- 2 weeks) for two years after completion of induction therapy, every 6 months for years 2-5 (+/- 4 weeks), and then annually thereafter (+/- 6 weeks) at the discretion of the investigator. Any other evaluations and tests should be performed as clinically indicated.

Upon disease progression or initiation of other anti-cancer therapy, contact will be for survival only until the subject is off study (i.e., every 3 months [+/-4 weeks]); unless otherwise clinically indicated. See Study Calendar (Section **3.8**) for additional information. Any adverse events which are present at the time of discontinuation should be followed in accordance with the safety requirements.

3.7 FOLLOW-UP EVALUATIONS

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30-day Safety Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

3.7.1 30-Day Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes

first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Patients with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 30 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded. Patients who are eligible for retreatment with copanlisib and rituximab (as described in Section **3.5.3**) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

3.7.2 Follow-Up Visits – Prior to Disease Progression

Patients who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed as detailed in Section **3.6** Scans will be done at least every 6 months to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with copanlisib and rituximab as detailed in Section **3.5.3**. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Patients who are eligible to receive retreatment with copanlisib and rituximab according to the criteria in Section **3.5.3** will move from the follow-up phase to the Second Course Phase when they experience disease progression.

3.7.3 Follow-Up Visits – Survival/Post-Disease Progression

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted at least every 3 months (+/-4 weeks) to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first (see Section **3.8**, Study Calendar).

3.8 STUDY CALENDAR

Procedure	**Screening**	**Baseline**	**Copanlisib Window (Cycle 0)**	**Induction Therapy Every Cycle**			**Maintenance Therapy Every Cycle**		**Disease Evaluations**		**End of Treatment**	**Follow-Up**		
			D -28 to -1	D1	D8	D15	D1	D15	Cycles 3 & 9	Cycles 6 & 12		Safety (Day 30)	Follow-Up (Prior to PD)	Survival (Post-PD/ Other Therapy)
Scheduling Window (Days):	-28 to -1[1]		-14	-3	±1	±1	-3	±1	Last 7 days of the cycle		Treatment discon./PD[2]	+7	Every 3 or 6 months[3]	Every 3 months[4]
Confirmation of Diagnosis	X													
Physical Exam[5]	X	X	X	X			X				X	X	X	
ECOG PS	X	X	X	X			X				X	X	X	
CBC with Differential	X	X	X[6]	X	X	X	X	X			X	X	X	
Chemistry Panels[7]	X	X	X	X	X	X	X	X			X	X	X	
Lipase, Hemoglobin A1C	X													
LDH	X	X	X	X			X				X	X	X	
Total protein		X	X	X			X				X	X	X	
PT/INR and aPTT	X		X	X			X				X	X	X	
Urinalysis	X	X	X	X			X				X			
Pregnancy Test (urine/serum; WOCBP) [8]	X	X	X	X			X							
Hepatitis and HIV Testing	X													
Hep B DNA Quantitative, HBV Viral Load[9]	X		X	X			X					X	X[10]	
CMV PCR and CMV IgM and IgG[11]	X		X	X			X						X	
Beta-2 microglobulin, SPEP, Serum free light chains, Quantitative serum IG levels		X												
Lymphocyte Phenotype (T, B, NK cell subsets)		X		X							X		X	
Peripheral Blood Flow Cytometry[12]		X	X							X	X		X (PD)	
Bone Marrow Aspiration/Biopsy[13]	X									X			X	
CT Scans[14]	X	X	X						X	X	X		X	
^{18}F-FDG-PET/CT Scan[15]	X	X	(Pre-C1D1)							X	X (PD)		X (PD)	
Electrocardigram (ECG)[19]		X	X						X	X				
Treatment[16]			X	X			X							

Procedure	Screening	Baseline	Copanlisib Window (Cycle 0)	Induction Therapy Every Cycle			Maintenance Therapy Every Cycle		Disease Evaluations		End of Treatment	Follow-Up		
			D -28 to -1	D1	D8	D15	D1	D15	Cycles 3 & 9	Cycles 6 & 12		Safety (Day 30)	Follow-Up (Prior to PD)	Survival (Post-PD/ Other Therapy)
Scheduling Window (Days):	-28 to -1[1]		-14	-3	±1	±1	-3	±1	Last 7 days of the cycle		Treatment discon./PD[2]	+7	Every 3 or 6 months[3]	Every 3 months[4]
Symptoms/Adverse Events Assessment, Concomitant Medication Review	X	X		X	X	X	X	X		X	X	X		
Research Tissues (archival/fresh biopsy)[17]		X	X						X (PD)		X (PD)			
Research Saliva/Buccal (baseline only), Research Blood Samples[18]		X	X	X					X	X	X		X	
Survival Status														X

NOTE: Additional assessments may be done as clinically indicated. Patients eligible for re-treatment (Section **3.5.3**) should resume procedures and follow-up per the Induction Therapy schedule above; then, move to repeated End of Treatment and Follow-Up assessments as indicated. Research samples will not be repeated/collected in patients who initiate re-treatment on protocol.

1 Screening and Baseline evaluations should be performed within 28 days prior to enrollment and dosing, respectively, with the following exceptions: Confirmation of diagnosis (no time limit); HIV antibody, Hepatitis B surface antigen and Hepatitis C antibody (within 3 months); Bone marrow aspiration with flow cytometry (optional) (within 12 months). **NOTE:** Any screening tests performed within the specified time frame for baseline do not need to be repeated.

2 To be done at treatment discontinuation (+/- 2 weeks) or may coincide with the safety follow-up visit. If treatment is discontinued for a reason other than disease progression, assessments should be repeated at the time of progression. If subject to initiate new anti-cancer therapy assessments should occur before the first dose of the new therapy. See Section 3.2.5 for additional information on salvage chemotherapy.

3 Follow-up to occur about every 3 months (+/- 2 weeks) for first 2 years after induction therapy, every 6 months for years 2-5 (+/- 4 weeks), and then annually (+/- 6 weeks) at discretion of investigator. Imaging to be done every 6 months, as per Section 3.7.2. Any other evaluations and tests should be performed as clinically indicated.

4 After disease progression or initiation of new anti-cancer therapy, contact for survival about every 3 months (+/- 4 weeks). See Section 3.7.

5 Physical exams to include history, vitals, weight, and height (screening only).

6 A blood count will be performed and assessed prior to study drug infusion on Day 8 and 15 of each cycle. On Days 8 and 15, the dose of copanlisib will be administered if, on the day of scheduled dosing, the laboratory test criteria for hemoglobin, ANC and platelets are met.

7 Chemistry panels include: Acute care, Hepatic, and Mineral.

8 Pregnancy test for WOCBP required within 7 days of D1 in cycle 0 and within 7 days of D1 of each cycle .

9 Hep B DNA Quantitative, HBV Viral Load testing will be done on subjects who test positive for Hepatitis serology (HbsAg/HbcAb) at screening. For subjects who test positive (at Screening) for hepatitis B serology but have a negative Hep B DNA Quantitative, HBV Viral Load, on-going testing will be done before the first day of each cycle.

10 Hep B DNA Quantitative, HBV Viral Load testing will be done during follow-up visits for the first 6 months after therapy on subjects who tested positive for Heptatitis serology (HbsAg/HbcAb) at screening.

11 CMV PCR will be performed at baseline and at the start of every cycle. CMV IgM and IgG will be done at baseline only. CMV PCR testing will be done during follow-up visits for the first 6 months after therapy.

12 Peripheral blood flow cytometry for diagnostic and staging purposes; repeat in follow-up to assess disease status and response as indicated.

[13] Bone marrow aspiration with flow cytometry and biopsy (within 12 months prior to starting treatment) if clinically indicated; repeat in follow-up to confirm response or progression.

[14] CT scans (preferred) of chest, abdomen and pelvis at baseline; may be adjusted to assess additional known sites of disease, as needed. Scans performed after cycles 3 and 6 (last 7 days of each cycle) of both induction and maintenance, as applicable. Repeat also within last 7 days of copanlisib window. MRIs may be used instead of CT scans as necessary.

[15] PET scans to be performed after cycles 6 and 12 (last 7 days of each cycle) of both induction and maintenance, as applicable. Repeat also within last 7 days of copanlisib window.

[16] Induction therapy consists of up to 6 cycles of copanlisib and rituximab. For patients who achieve PR to Induction Therapy, but not CR, treat with up to an additional 6 cycles of Copanlisib+Rituximab at reduced schedule. Please refer to Section **3.2** for all treatment days/cycles for copanlisib and rituximab.

[17] If adequate archival tissue at baseline, fresh tumor biopsy is optional. Optional "on-treatment" tumor biopsies will be performed at disease progression in all subjects (see Section **5**).

[18] Samples for correlative research blood and saliva (preferred)/buccal swab samples to be collected as indicated in Section **5**.

[19] All participants will undergo ECG at baseline. Participants with prolonged QTc at baseline, participants with congenital prolonged QT syndrome, and participants chronically on medications listed in Section **4.2** will have ECG monitoring after copanlisib window, and every 3 cycles thereafter.

3.9 COST AND COMPENSATION

3.9.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by their insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.9.2 Compensation

No compensation is provided for participants on this study.

3.9.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.10 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all patients complete a safety visit approximately 30 days following the last dose of study therapy. Additional safety visits and follow-up will continue as per Section **3.6**.

Criteria for Removal from Protocol Therapy

- Confirmed radiographic disease progression
- Unacceptable toxicity as listed in Section **3.3.1**
- Intercurrent illness that prevents further administration of treatment
- Requirement for use of prohibited therapies as listed in Section **4.3**
- Pregnancy
- Subject's requests to be withdrawn from protocol therapy
- Noncompliance with trial treatment or procedure requirements such as a delay in test drug administration due to toxicities for > 28 days
- Investigator's decision to withdraw the subject
- Study is cancelled for any reason

3.10.1 Off-Study Criteria

- Completion of study follow-up period
- Subject requests to be withdrawn from study
- Subject is lost to follow-up
- Death
- Study is cancelled for any reason
- Screen failure

3.10.2 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for three (3) consecutive scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required.

4.1 ACCEPTABLE/PERMITTED MEDICATIONS

All treatments that the investigator considers necessary for a subject's welfare, including the use of growth factors support (e.g., filgastrim injections) may be administered at the discretion of the investigator in keeping with the community standards of medical care. Palliative and supportive care for the other disease-related symptoms and for toxicity associated with treatment will be offered to all patients in this trial.

All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded.

4.2 MEDICATIONS TO BE USED WITH CAUTION

Patients who are chronically on the following medications that can prolong QT interval will have ECG performed after copanlisib window treatment (C0) and every 3 cycles thereafter until the end of treatment.

- Antiarrhythmics: Amiodarone, Dofetilide, Disopyramide, Flecainide, Procainamide, Quinidine, Sotalol.
- Antibiotics: Erythromycin, Gatifloxacin, Grepafloxacin, Levofloxacin, Pentamidine, Moxifloxacin, Sparfloxacin

- Anticonvulsants: Fosphenytoin, Felbamate
- Antidepressants: Venlafaxine
- Antidiarrheals: Octreotide
- Antiemetics: Dolasetron, Droperidol, Domperidone, Palonosetron
- Antipsychotics: Chlorpromazine, Haloperidol, Mesoridazine, Pimozide, Quetiapine, Risperidone, Thioridazine, Ziprasidone
- Antivirals: Foscarnet, Amantadine
- Anti-asthmatics: Salmeterol
- Calcium Channel Blockers: Nicardipine, Bepridil, Isradipine
- Migraine medications: Naratriptan, Sumatriptan, Zolmitriptan
- Narcotics: Methadone

4.3 PROHIBITED MEDICATIONS

Patients are prohibited from receiving the following therapies during treatment on this trial. Patients who, in the assessment by the investigator, require the use of any of the following treatments for clinical management should be removed from the trial. Patients may receive other medications that the investigator deems to be medically necessary.

- Other therapy for the disease under study not specified in this protocol, unless specifically noted as permitted
- Investigational agents other than copanlisib (chemotherapy or biologic agents)
- Radiation therapy
- Corticosteroid therapy at a daily dose higher than 15 mg prednisone or equivalent is not allowed, unless for one of the following exceptions:
    - as a premedication prior to rituximab infusion
    - as a premedication prior to a radiology scan as per local standard practice
    - as treatment for a treatment-emergent toxicity

  Previous corticosteroid therapy must be stopped or reduced to the allowed dose 7 days before performing the screening PET-CT and/or CT/MRI, whichever is performed first, and again prior to the first study drug administration. If a patient is on chronic corticosteroid therapy, corticosteroids should be de-escalated to the maximum allowed dose after the patient has signed the IC. Patients may be using topical or inhaled corticosteroids.
- Immunosuppressive therapy
- Anti-arrhythmic therapy (beta blockers or digoxin are permitted)
- Herbal preparations/medications. These include but are not limited to St. John's wort, Kava, ephedra (ma huang), ginkgo biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, and ginseng. Patients should stop using these herbal medications 7 days prior to first dose of study drug
- CYP3A4 inhibitors and inducers. Copanlisib is primarily metabolized by CYP3A4. Therefore, concomitant use of strong inhibitors of CYP3A4 and strong inducers of CYP3A are not permitted. Moderate inhibitors should be used with caution.

See below table of common medications. Please refer also to the following listing/ website: https://drug-interactions.medicine.iu.edu/main-table

Category	Drug name
Strong CYP3A Inhibitors	Voriconazole, boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir/ritonavir, eltegravir/ritonavir, grapefruit juice, indinavir/ritonavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, saquinavir/ritonavir, telaprevir, telithromycin, tipranavir/ritonavir, troleandomycin,
Moderate CYP3A Inhibitors	Grapefruit, grapefruit hybrids, pummelos, star-fruit, and Seville oranges (citrus paradisi fruit juice)
Strong CYP3A Inducers	Avasimibe, carbamazepine, mitotane, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin), St. John's wort (hypericum perforatum)
CYP3A Substrates with NTI	Alfentanil, apixaban (doses >2.5 mg only), aprepitant, astemizole, cisapride, cyclosporine, diergotamine, dihydroergotamine, ergotamine, fentanyl, lovastatin, nicardipine, nisoldipine, pimozide, quinidine, rivaroxaban, simvastatin, sirolimus, tacrolimus, terfenadine, thioridazine,

4.4 Other Considerations (Copanlisib IB)

4.4.1 Diet

Patients must fast for at least 8 hours prior to the first dose of copanlisib. After the first dose, patients should maintain a normal diet unless modifications are required to manage an AE such as elevated blood glucose, diarrhea, nausea or vomiting.

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 Summary

An important correlative focus of this study is to utilize multiplatform genomic analyses of tumor biopsies to identify biomarkers that predict response of resistance to copanlisib. To achieve this goal, tumor biopsies will be collected in all patients prior to therapy and optional tumor biopsies will be performed on patients with accessible sites of lymphoma involvement after the copanlisib window (on-treatment biopsy) as well as at the time of disease progression. These optional biopsies will be studied by the Staudt lab using integrative analyses to further characterize mechanisms of both intrinsic and acquired resistance to copanlisib.

The molecular annotation will include a combination of gene-expression profiling, RNA sequencing, whole-exome sequencing, and identification of copy number abnormalities via array comparative genomic hybridization in the Staudt lab. Specific aims of these molecular correlates will be based on published literature regarding gene expression and molecular signatures that identify subsets of patients who are considered high-risk for early treatment failure when given standard therapy. If we observe clinical responses in this group of high-risk patients, the signatures can be further refined based on our clinical-genetic correlations to develop a new signature of response, including the potential for mutations in the PI3K pathway.

Lastly, we aim to identify mechanisms of therapeutic resistance through comparative genomic analysis of circulating tumor DNA and of biopsies taken before treatment and at time of clinical progression.

Sample	Collection Details	Time Points	Supervising Laboratory/ Investigator^
Blood Samples			
Pharmacokinetics (Copanlisib PK)	• 1 x 4 mL Lithium Heparin (green) – *place sample on ice after collection*	• C0D1 – Within 5 minutes post-end of infusion (EOI) of copanlisib (preferred; up to 15 minutes post-EOI permitted)	BPC/ NEBA
Pharmacogenetics	• 1 x 6 mL EDTA	• Post-cycle 3	BPC
ctDNA/cfDNA, Plasma banking	• 1 x 10 mL Streck/BCT	• Baseline • During copanlisib window on days -22,-15 and -8, and post-window/pre-C1D1* • Each response assessment • End of Treatment/ Disease Progression (PD) • Follow-up (prior to PD)	BPC
PBMCs/ Immune subsets	• 2 x 8 mL CPTs (sodium citrate)	• Baseline • Post-window/pre-C1D1 • Post-cycles 3, 6 and 12, as applicable/received • Follow-up (prior to PD): 6 month and 12 month • PD	BPC
Other Samples			
Archival	FFPE (block or slides); biopsy is required if archival is not adequate or unavailable	• Baseline only	BPC/ NCI LP
Tumor Biopsy	Excision (single/multiple nodes) or core (4-6 passes); placed in formalin/FFPE and media per routine practice	• Baseline • Post-window/pre-C1D1* • PD	
Germline DNA	Blood, Buccal swab, or Saliva (preferred)	• Baseline	BPC
Flow cytometry (peripheral blood)	2 x 10 mL NaHep (sodium heparin)	• Baseline • Post-window/pre-C1D1 • Post-cycles 6 and 12, as applicable/received • PD	NCI LP

Sample	Collection Details	Time Points	Supervising Laboratory/ Investigator^
*Copanlisib window blood sample sample collection days may be adjusted based upon subject's schedule at discretion of the PI without being a protocol deviation. The biopsy will ideally occur in the week off (i.e., week 4 prior to C1D1); however, may occur at any time during the window at the discretion of the PI. ^The location of specimen processing or analysis may be adjusted with the permission of the PI or laboratory investigator **NOTE:** • Tubes/media may be adjusted at the time of collection based upon materials available and/or to ensure the best viable samples are collected for planned routine and/or research analysis at the time of procedure. • All blood/tubes – *with the exception of PK samples/tubes* – should remain at ambient temperature after collection and processed on the day of collection, do not place samples on ice.			

5.2 BIOSPECMEN COLLECTION

5.2.1 Summary

The planned analyses described below may be done on leftover and/or shared sample portions from the respective laboratories, as needed. In addition to the prospectively collected samples below, leftover portions of samples sent for routine laboratory testing (e.g., plasma from CBC/hematologies) may also be retrieved for research tests prior to being discarded. The planned prospective analyses are identified below; laboratories may share resources or collaborate on analyses, if appropriate (e.g., isolation/analysis of DNA not prospectively planned by one lab may be incorporated if needed during the planned analyses).

Portions of all samples may be banked for future research analyses; prospective consent will be obtained during the informed consent process.

The blood drawing limits for research purposes are as follows:

- For adult subjects: The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

5.2.2 Blood Samples – Samples Biospecimen Processing Core - BPC

For questions, please contact the BPC, 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number). Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred). For sample pickup, page 102-11964. For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov. The samples will be processed, barcoded, and stored in the BPC until requested by the investigator.

5.2.2.1 Peripheral Blood Mononuclear Cells (PBMCs)

- Collect blood (approx. 6mL or 1 teaspoon) in one lavender-top EDTA tube
- Put samples immediately on wet ice and refrigerate
- PBMCs will be isolated per routine laboratory techniques
- Extra samples may be requested if the original sample has insufficient DNA for analysis. The additional sample can range from one to two tubes of blood (approximately 1-2 teaspoons total) or an extra saliva sample.

- Genomic DNA will be extracted from peripheral blood mononuclear cells using the QIAmp DNA blood mini-kit (Qiagen, Inc, Valencia, CA). This method has been previously utilized for the isolation of genomic DNA in the BPC 13 and has resulted in DNA yields of approximately 50-400 ng/0.5ml of serum (Price, DK. personal communication).
- Saliva DNA will be collected using Salviette/Oragene® kit for saliva. The samples will be processed and DNA extracted/isolated per kit instructions and established techniques.
- Analyses to be performed: DNA will be analyzed on a Pharmacoscan (ThermoFisher Scientific) genotyping platform that tests for 4,627 genetic variations in 1,191 drug disposition genes, including cytochrome P450s (CYPs), Glutathione transferases (GSTs), sulfotransferases (SULTs), as well as genes involved in facilitation of drug transporters, global regulation of drug metabolizing/transporting proteins, drug binding proteins, and drug targets.

5.2.2.2 Circulating Tumor DNA (ctDNA) and plasma banking

- Collect blood in cell-free DNA (e.g., Streck BCT/collection tubes) gently invert the tubes 8-10 times immediately after collection.
- Plasma will be isolated and frozen at -80ºC until analysis (e.g., centrifuged at 1800 x g for 10 minutes at room temperature; plasma transferred/frozen in aliquots of 1.5-2 mL each).

5.2.2.3 Pharmacokinetics (PK) Sampling

PK sampling will be performed in all patients for copanlisib, its metabolite M-1 and other metabolites as follows:

- Collect blood in specified tubes (e.g., 4 mL vacutainer tubes containing lithium-heparin)
- Immediately after collection, tubes must be gently inverted several times to mix with anticoagulant, and placed on ice.
- Within 10-15 after collection, plasma will be isolated (e.g., 1000 g for 10 minutes at 4°C), transferred to a cryovial (e.g., 2mL screw cap polypropylene cryovial), and frozen upright at -60C to -80C within 60 minutes after the blood draw.
- Samples will be stored until shipment/transfer for analysis to NEBA.

5.2.2.4 Peripheral Blood Mononuclear Cells (PBMCs)

- Collect blood in Cell Preparation Tubes with sodium citrate (blue/black speckled top); gently invert the tubes 8-10 times immediately after collection.
- PBMCs will be isolated per routine laboratory techniques.

5.2.3 Blood Samples – NCI Laboratory of Pathology

The blood samples for flow cytometry should be sent to the NCI Laboratory of Pathology; please contact NCI Laboratory of Pathology for questions and for notification of samples.

5.2.3.1 Flow cytometry

- Collect blood, bone marrow, and/or lymph node samples in NaHep (sodium heparin) tubes; gently invert the tubes 8-10 times immediately after collection.
- Send samples to the NCI Laboratory of Pathology for specialized flow cytometry processing and analysis.
- Studies to be performed on these samples include: multi-parameter flow cytometry to determine the percentage of aberrant lymphoma cells. Reagents to be used include but are

not limited to characteristic B-cell markers including: CD10, CD19, CD20, CD21, CD22, CD23, Bcl-2, Bcl-6, kappa and lambda light chain as well as various T-cell markers including: CD3, CD5, CD4 and CD8.

5.2.4 Tissue Samples

5.2.4.1 Archival tissue

Archival block(s) or slides (i.e., at least 20 unstained slides, 5-microns) are required at baseline; these may also be required in follow-up in case of future routine procedures or in case additional tissue is needed even in the event of optional tumor biopsy.

5.2.4.2 Lymph node excision or core needle biopsy procedure

Lymph node excision or core needle biopsy will be performed per routine standard of care, by Surgery Consultants or Interventional Radiology, as appropriate. A procedure-specific consent form will be signed by the patient prior to the procedure. Every attempt will be made to perform excisional lymph node biopsies to obtain the best quality tissue for translational investigation. Consideration of alternative biopsy methods (e.g., core needle biopsy) will only be made if follow-up excisional biopsy is not possible/safe or patient is unwilling to undergo repeat excisional lymph node biopsy.

In the event that a surgical biopsy procedure is performed, more than one lymph node and at more than one anatomic site may be collected, provided the additional procedures do not present unacceptable risk to the patient. In the event of core needle biopsy, these are obtained typically by using a 16-18G needle at the discretion of the provider performing the procedure. Conscious sedation or general anesthesia may be used, if warranted, and the use and risks are acceptable to the patient.

Potential site(s) of biopsy include, but are not limited to: bone marrow lesions, bony lesions, extramedullary disease/masses, and lymph nodes. The type of procedure to be done and manner in which it will proceed (e.g., excision/core, single vs. multiple sites of biopsy) will be discussed with the patient prior to the biopsy procedure. The patient will be reminded that all sampling for research is voluntary.

5.2.4.3 Sample handling/processing

When performed, excisions or core biopsies will be placed in sterile collection/core cylinder tubes with appropriate media (e.g., formalin or sterile saline); gently invert/inspect tubes with media 8-10 times immediately after collection to ensure the core(s) is completed immersed in the media.Tissue samples will be handled/processed as below prior to planned analyses, as appropriate:

- Any required routine review for histopathologic confirmation of diagnosis and/or grade will occur per standard of care (e.g., H&E, immunohistochemistry), if required.
- Formalin samples will be fixed and paraffin-embedded per routine techniques.

5.2.5 Other Samples

5.2.5.1 Germline DNA

Germline DNA will be collected by blood, buccal swab, and/or saliva samples (preferred). These will ideally be collected at baseline; however, may be collected at any point on study based on supplies. Standardized, commercial collection kits or tubes will be used (e.g., 1, 5-10 mL K_2EDTA tube for blood; Isohelix SK-1 for buccal swabs; Salviette/Oragene® for saliva). In the

case of buccal swabs, two (2) samples may be collected in order to ensure adequate DNA collection.

The samples will be processed and DNA extracted/isolated per kit instructions and established techniques. These will also be handled by the BPC lab (see Section **5.2.2** for contact information).

5.3 BIOMARKER AND RESEARCH METHODS

The technology platforms that are able to interrogate genomic structure and function are constantly in flux; therefore, the exact nature of the methodologies that will be employed will be assessed at the time that the samples are collected and ready for analysis.

Note: Platforms and procedures may be adjusted based upon current technology and/or collaborations in place at the time of actual analyses.

The following are technologies that are currently in use for each planned analysis:

5.3.1 Tumor Profiling

Immunohistochemical (IHC) analyses, including FISH for BCL2, will be done on every patient's baseline tumor biopsy. The routine IHC panel for diagnosis of FL will be performed on tumor tissue samples, including but not necessarily limited to CD3, CD5, CD10, CD20, CD21, CD23, BCL2, BCL6, MUM1 and MIB-1.

5.3.2 Microenvironment Tissue Profiling

IHC analyses will also take place on tumor tissue samples to assessment for contribution of the tumor microenvironment. IHC that will be performed may include but are not necessarily limited to CD4, CD8, CD11c, CD21, CD23, CD25, C35, CD68, CD163, PD-L2, PD-1, FOXP3, FOXP1, CXCR4, CXCR5, CXCL13, IRF-8, IgD, ICOS, and ICOS-L.

5.3.3 Immune Subset Analysis

Peripheral blood mononuclear cells (PBMC) will be assessed using multiparameter flow cytometry for immune subsets including but not necessarily limited to CD8+ T-cells, CD4+Foxp3- T-cells, Tregs, T_{ex}, Th1, Th2 and Th17+ CD4+ T-cells, monocyte subsets, MDSC subsets. Assessment may include functional markers, i.e. PD-1, Tim-3, CTLA-4, PD-L1, HLA-DR, Ki67 and/or CD40.

5.3.4 DNA/RNA Sequencing

Genomic DNA and total RNA will be extracted from tumor samples using a Qiagen All-prep kit. For individual target genes that are recurrently mutated in FL, classical Sanger sequencing will be performed on PCR amplicons, using primers surrounding the known sites of mutation. To broadly assess mutations, next generation sequencing (e.g., on an Illumina HiSeq 2000 platform) will be employed, using a paired end sequencing strategy of libraries constructed from tumor DNA. DNA will either be sequenced in its entirety from a whole genome library or will be first enriched for exonic sequences using the Agilent Sure Select system, aiming for 30X or 100X average coverage per base, respectively. The sequence fragments will be mapped back to the genome using the BWA algorithm. Of sequences overlapping a particular base pair in the genome, the percent mutant calls greater that 20% with a minimum of 25X coverage will be considered as an arbitrary threshold for single nucleotide variants (SNVs). SNVs that are not present in the matched normal sample will be considered candidate somatic mutations.

A related technology, RNA-Seq, utilizes RNA from the tumor specimen to create a cDNA library for high-throughput sequencing. RNA-seq will be performed using Illumina kits followed by high-

throughput sequencing on an Illumina HighSeq 2000 machine. The cutoffs for coverage and percent mutant calls mentioned above will also be used to identify putative SNVs. RNA sequencing will also be used to read out digital gene expression across the genome as described.

Recent advances in genomic technologies enable GEP at the single cell level, a distinct advantage over conventional GEP which cannot always distinguish tumor vs non-tumor gene expression(60, 61). Single-cell approaches allow identification of the evolution of rare populations of resistant tumor cells, as well as identification of TME cells critical for the survival of the tumor. The Center for Cancer Research (CCR) has recently opened a single cell analysis core facility within the CCR Genomics Core. This facility has the ability to take purified viably frozen cells banked from patient biopsies and prepare them, using well-validated 10X Genomics technology, for single-cell RNA sequencing. This core is directly integrated with the NCI Sequencing core facility to provide high-quality, deep-sequencing of the single cell RNA-SEQ samples, as well as 'first-pass' data processing and analysis. Data will then be transferred to lymphoma researchers and bio-informaticians in the Staudt lab for further analysis of gene expression patterns and cellular population dynamics.

5.3.5 DNA Copy Number Analysis

Array comparative genomic hybridization (e.g., on Agilent 240K or Affy SNP 6.0 microarrays) will be used to assess DNA copy number alterations as described, in tumor DNA to yield somatically acquired regions of copy number gain and loss.

5.3.6 Pharmacokinetics (PK)

Coded (linked) samples (Section **5.4.2**) will be sent to a contracted laboratory at the request of Bayer for analysis of copanlisib pharmacokinetics: NorthEast Bioanalytical Laboratories, LLC (NEBA) under the direction of Vipin Agarwal, Ph.D., MBA.

NEBA will run subject samples for PK analysis in batches of approximately 30 samples each; with the intent for reanalysis of samples due to out of calibration range concentration (i.e., secondary to large difference in parent and metabolite concentrations).

The laboratory contact information is as follows:

NorthEast Bioanalytical Laboratories LLC
925 Sherman Avenue
Hamden,CT 06514

5.3.7 Detecting Minimal Residual Disease (MRD)/ circulating tumor DNA (ctDNA)

5.3.7.1 Rationale for MRD assessment

Detecting Minimal Residual Disease (MRD) can be a powerful tool to monitor patients' response to treatment and early detection of relapse. It is of research interest to determine if circulating tumor DNA before, during, or after therapy is predictive of long-term disease-free survival. Foresight Diagnostics will assess whether circulating tumor DNA from the Human Material can be used as biomarkers that correlate with disease-free survival. Foresight Diagnostics will utilize their proprietary Phased variant Enrichment and Detection Sequencing (PhasED-Seq) which leverages multiple somatic mutations within individual DNA fragments to improve sensitivity of minimal residual disease (MRD) in patients with B-cell lymphomas beyond what can be achieved with other methods. Data from experiments conducted by

Foresight Diagnostics using the human material will be provided to NCI and such data provided by Foresight Diagnostics to NCI may be used by NCI for any purpose.

5.3.7.2 Samples to be sent to Foresight Diagnostics

Bloods from the storage/future use samples collected from select patients at the following time points may be sent, if available:

- Baseline
- Post-window/pre-C1D1
- Each response assessment
- Follow-up surveillance

5.3.7.3 Sample collection and processing

Portions of the other samples collected, including blood (serum, plasma and/or buffy coat), frozen or formalin fixed and paraffin embedded (FFPE) human tissue, and data from select patients will be sent.

5.3.7.4 Shipping information

Only coded (linked) samples will be shared as described (see Section **5.4.2)** for process of coding, and sample request instructions. The samples and data will sent in batches to ForeSight Diagnosticsat the address listed below.

ForeSight Diagnostics (c/o Jake Chabon)
12635 E. Montview Blvd Suite 224
Aurora CO 80045

5.3.8 CCP-32 assay

De-identified FFPE (Formalin-fixed, paraffin-embedded) tissue from up to 60 FL patients enrolled on the copanlisib-rituximab study will be analyzed using the CCP-32 assay to identify patients in the poor prognosis group. This data will be correlated with other prognostic markers in FL including FLIPI, genetic profile of the tumor, baseline levels of circulating tumor DNA, and TME signatures defined by RNA (Ribonucleic Acid) sequencing. The clinical outcomes (rates of CR, duration of CR, and PFS).

5.3.8.1 General Handling Considerations

FFPE tissues are not considered to be infectious, but gloves are still recommended whenhandling all study materials.

Study specimens are FFPE tissue from previously diagnosed FL. Study specimens are being catalogued and will undergo a pathology QC at banking site. By shipping samples to MDAZL, the banking site attests that all inclusion/exclusion criteria from the study are satisfied.

Handling conditions (based on MDAZL policies/procedures)

- Stability studies performed in MDAZL have yielded reproducible results of nCounter assays from unstained tissue sections stored at ambient laboratory conditions for up to 52 months.
- USS should be transported at room temperature. Cool packs are recommended to be utilized during the months of April through October.

- A shipping manifest listing all slides (H&E and USS) must be included with each shipment, verified by two people at both the shipping site and MDAZL. The template document and training for its use will be provided by MDAZL.
- MDAZL considers both physical and electronic signatures as valid per Mayo Clinic Policy.

5.3.8.2 Test Requirements:

- Minimum of 60% tumor content by area, which may be achieved by macrodissection if necessary. This review may be performed at either the banking site or MDAZL (Molecular Diagnostics - Arizona Laboratory) as needed.
- A standard H&E (Hematoxylin and Eosin) slide must be cut in proximity with the section(s) used for analysis for QC (Quality Control) to be valid.
- USS from the diagnostic block, cut between 5-10 microns in thickness, mounted on positively charged slides.
    - The number of USS to be used for RNA extraction per sample is approximated by the following table, which assumes slides are at 5 microns thickness. Thicker sections (up to 10 microns) or more than the recommended number of slides may be sent and/or used at the discretion of pathologists or technologists.
    - If during review it is discovered that insufficient sections are available for a specimen, an alternate block may be sourced, the specimen may be excluded from the study, or the specimen may be included in the study with the consensus of study leadership.

Table 8 Unstained Tissue Slide Input Requirements

Tumor Surface Area	Number of Slides @ 5µ thickness	
	Minimum Required #	**Recommended #**
4 mm^2	5	10
5–11 mm^2	4	8
12–24 mm^2	3	6
25–99 mm^2	2	4
≥ 100 mm^2	1	2

5.3.8.3 Tissue Review Procedure

Tissue review will be performed by a board-certified hematopathologist and trained laboratory staff at either site:

- H&E slides will be routed to a study hematopathologist using established protocols. It is recommended to use a standard H&E recut procedure to ensure the H&E has reasonable proximity to the USS.
- Hematopathologist or designee will use established protocols to review the H&E for tumor content and area. A batch tissue review form may be utilized to record tumor percentage, area, and number of slides to be cut. The template may be provided by MDAZL.
- Hematopathologist or designee utilizes **Table 8** to determine the appropriate number of USS to request per established protocols. If the minimum slide number criterion cannot

be met, the specimen may either be excluded from the study or included at the discretion and consensus of the study leadership.

- Proceed to Shipping and Receiving Procedure.

5.3.8.4 Shipping and Receiving Procedure

The following is to be performed by study staff at both banking and receiving locations:

- USS will be cut according to the specifications in the General Handling section and prepared for shipment, using banking site-specific procedures. It is recommended to protect the slides well from movement during shipping to reduce the risk of breakage.
- Two people at the banking site shall confirm the contents of all sample shipments prior to shipment by signing a printed copy of the sample manifest. If performed at the banking site, a copy of the pathology QC form should also accompany the shipment. The manifest will include a statement verifying that the patient and samples have met the inclusion criteria and do not meet any of the exclusion criteria.
- The banking site will communicate with testing site the details of the batch shipment. This may include the shipment tracking number, an electronic copy of the sample manifest, and any other relevant documentation.
- The shipment will be sent overnight. Slides should be shipped at ambient temperature, and cool packs are recommended to be utilized between the months of April through October.
- Two staff members at MDAZL will verify the contents of the shipment against the shipping manifest, sign, and retain a copy of the manifest. A copy of the signed manifest may be sent to the banking site if desired.

  Two people are required to independently verify package contents, including number of slides, specimen IDs, and condition in which specimens are received. If there are any discrepancies or problems, the banking site will be notified, and resolution confirmed by MDAZL.
- MDAZL will store the original manifest with signatures. An electronic copy will also be retained.
- Slides will be stored at ambient laboratory conditions until they are used for the study. Unused slides may be returned to the banking site upon completion of the study if requested.
- MDAZL will proceed with nucleic acid isolation and the nCounter assay on qualified specimens per established protocols.

5.3.8.5 Shipping Address

Unstained slides as well as one H&E should be shipped to:

Colleen Ramsower
Mayo Clinic Arizona
13400 E. Shea Blvd
CRB 1-250 MDAZL
Scottsdale, AZ 85259
Phone: 480-301-4934

An email to notify that a shipment has been sent including the tracking number should be sent to Ramsower.Colleen@mayo.edu.

5.3.9 Other Analyses

Other analyses include the following:

- Cell analysis and histological (e.g., H&E), immunohistochemical review and analysis per standard and established research techniques (e.g., FISH for BCL2 translocation, and other IHC analyses in blood and tissue).
- cfDNA/ctDNA for liquid genotyping as a non-invasive dynamic monitoring of disease as well as monitoring for individual molecular aberrations that herald progression or disease transformation; specifically, amplification and sequencing of the VDJ segment of the immunoglobulin receptor is planned

5.3.10 Future Use

Any blood, tissue, or other products or portions leftover from other analyses will be stored for future research.

5.4 SAMPLE STORAGE, TRACKING AND DISPOSITION

5.4.1 General

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting patients will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

If the patient withdraws consent his/her data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

5.4.2 Clinical Pharmacology Program/ Biospecimen Processing Core (BPC)

5.4.2.1 Sample Data Collection

All samples sent to the Biospecimen Processing Core (BPC) of the Clinical Pharmacology Program will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined BPC lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by the BPC. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. Data will be recorded for each sample (e.g., patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location). Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.4.2.2 Sample Storage

Barcoded samples are stored in barcoded boxes in a locked freezers at appropriate temperatures (e.g., -20ºC to -80ºC) according to stability requirements. These freezers are located onsite in the BPC and offsite.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.4.3 Hematopathology Section of Laboratory of Pathology (Tissue samples)

Archival and/or freshly collected and processed tumor tissue may be stored in the Hematopathology Section of Laboratory of Pathology until ready for planned and/or future research assays if the patient has agreed to allowing specimens to be used in future research studies. IRB approval will be obtained before using any samples to conduct studies that are not described within this protocol. Samples will be stored under conditions appropriate to the type of sample and processing (e.g., ambient or frozen).

Tissue that is given to the technician will be assigned an accession number (HP#) in the HP Case Log book; sample tracking also takes place with a FileMaker Pro data base called HP Patient Information and Specimen Inventory. A Patient background sheet may be filled out and filed with any accompanying paperwork, with final reports and any supplemental reports that follow added as completed.

5.5 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.5.1 Description of the scope of genetic/genomic analysis

The research correlates for this study are expected to include DNA/RNA sequencing of tumors, including circulating tumor (ct) DNA. In addition, whole exome sequencing may include evaluation for known lymphoma mutations. For any genetic studies performed, the results will be deposited in a database such as dbGaP per NIH requirements. Although there is controlled access to such a database, such a submission carries theoretical risks of revealing the identity of the subject. This is discussed in the consent.

5.5.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

Confidentiality for genetic samples will be maintained as described (Section **5.4**). In addition, a Certificate of Confidentiality has been obtained for this study.

5.5.3 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for this study are defined as disorders appearing in the American College of

Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis.

5.5.4 Genetic Counseling

Subjects will be contacted with a request to provide a sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH to have genetic education and counseling to explain this result; at the time of any such event(s), these activities will be funded by the NCI/CCR in consideration of the specific circumstances. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

5.6 Exploratory Analysis of Early [^{18}F]-FDG-PET/CT and CT Scans

An exploratory objective of this study is to identify a mutational or gene-expression signature that predicts early clinical response to copanlisib monotherapy. The post-copanlisib window early imaging assessment will be done to assess early response to copanlisib. The post-copanlisib window early imaging assessment will entail one [^{18}F]-FDG PET/CT and one CT scan. The [^{18}F]-FDG PET/CT scan will entail the injection of 10mCi dose for all ages and body sizes.

Response will be calculated as described in Section **6.3**.

6 DATA COLLECTION AND EVALUATION

6.1 Data Collection

6.1.1 Summary

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first administration of study drug through 30 days post the last administration of study drug. After 30 days, only adverse events which are serious and related to the study intervention need to be recorded.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.1.2 Data Collection/Recording Exceptions

6.1.2.1 Abnormal Laboratory Values

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

6.1.2.2 Hospitalizations

Any cases of planned or prolonged hospitalization are not considered reportable serious adverse events if for the following reasons:

- Technical, practical, or social reasons, in absence of an AE
- Routine treatment or monitoring of the studied indication not associated with any deterioration in condition, including scheduled therapy or standard procedure for the target disease of the study, and those required to allow efficacy measurement for the study
- Diagnostic or elective surgical procedures for preexisting conditions or a procedure that is planned (e.g., planned prior to starting of treatment on study)
- Emergency outpatient treatment or observation that does not result in admission, unless fulfilling other seriousness criteria
- Hospitalizations for administration of IV antibiotics required due to bacterial resistance, and not infection severity, will not be considered SAEs and will not require expedited reporting

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?
I will share human data generated in this research for future research as follows:

_X_ Coded, linked data in an NIH-funded or approved public repository.
_X_ Coded, linked data in another public repository
_X_ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
_X_ Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?
Data will be shared through:

_X_ An NIH-funded or approved public repository. Insert name or names: ClinicalTrials.gov, dbGaP.

_X_ BTRIS (automatic for activities in the Clinical Center)

_X_ Approved outside collaborators under appropriate individual agreements.

_X_ Publication and/or public presentations.

When will the data be shared?

_X_ Before publication.

_X_ At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

Response rate of FL will be assessed according to the International Working Group (IWG) response criteria. In addition, we will also look at overall response rate according to the 5-point Lugano classification for interpreting FDG-PET scans.

When appropriate, for patients with disease involvement of the bone marrow at baseline, repeat assessment will be done to confirm response.

6.3.1 Response Criteria for Follicular Lymphoma –

The following will be used to assess response to induction therapy.

6.3.1.1 International Working Group (IWG) response criteria

The International Working Group (IWG) response criteria will be used (Cheson et al.):

Response Category	Physical Examination	Lymph Nodes	Lymph Node Masses	Bone Marrow
CR	Normal	< 1 cm	< 1 cm	Normal
CRu	Normal	> 1 cm	> 75% decrease	Indeterminate
PR	Normal	Normal	Normal	Positive
	Normal	≥50% decrease	≥50% decrease	Irrelevant
	Decrease in liver/spleen	≥50% decrease	≥50% decrease	Irrelevant
Progression	Enlarging liver/spleen; new sites	New or increased > 50%	New or increased > 50%	Reappearance

6.3.1.2 The Five-Point Scale (5-PS) Deauville criteria

The five-point scale (5-PS) has been validated for use at interim staging and at the end of treatment and was adopted as the preferred reporting method at the First International Workshop

on PET in Lymphoma in Deauville, France (i.e., Deauville criteria), and in several international trials.

The 5-PS scores the most intense uptake in a site of initial disease:

1. if present, as follows: no uptake or no residual uptake (when used at interim)
2. slight uptake, but below blood pool (mediastinum)
3. uptake above mediastinal, but below or equal to uptake in the liver
4. uptake slightly to moderately higher than liver
5. markedly increased uptake or any new lesion (on response evaluation)

6.3.1.3 Lugano Classification of Response

Lugano classification of response criteria with PET (Cheson et al., 2014).

Response and Site	**PET-CT Based Response**	**CT-Based Response**
Complete	**Complete metabolic response**	**Complete radiologic response** *All of the following:*
Lymph nodes and extralymphatic sites	Score 1, 2, or 3* with or without a residual mass on 5PS†	• Target nodes/nodal masses must regress to ≤1.5 cm in LDi • No extralymphatic sites of disease
	NOTE: *It is recognized that in Waldeyer's ring or extranodal sites with high physiologic uptake or with activation within spleen or marrow (e.g., with chemotherapy or myeloid colony-stimulating factors), uptake may be greater than normal mediastinum and/or liver. In this circumstance, complete metabolic response may be inferred if uptake at sites of initial involvement is no greater than surrounding normal tissue even if the tissue has high physiologic uptake.*	
Nonmeasured lesions	Not applicable	Absent
Organ enlargement	Not applicable	Regress to normal
New lesions	None	None
Bone marrow	No evidence of FDG-avid disease in marrow	Normal by morphology; if indeterminate, IHC negative
Partial	**Partial metabolic response**	**Partial response** *All of the following:*
Lymph nodes and extralymphatic sites	Score 4 or 5† with reduced uptake compared with baseline and residual mass(es) of any size *At interim, these findings suggest responding disease.* *At end of treatment, these findings indicate residual disease.*	• ≥50% decrease in SPD of up to 6 target measurable nodes and extranodal sites *When a lesion is too small to measure on CT, assign 5 mm x 5 mm as the default valve; when no longer visible, 0 x 0 mm. For a node >5 mm x 5 mm, but smaller than normal, use actual measurement for calculation.*
Nonmeasured lesions	Not applicable	Absent/normal, regressed, but no increase
Organ enlargement	Not applicable	Spleen must have regressed by >50% in length beyond normal
New lesions	None	None
Bone marrow	Residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow	Not applicable

Response and Site	PET-CT Based Response	CT-Based Response
	in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy or an interval scan.	
No response or stable disease	**No metabolic response**	**Stable disease**
Target nodes/nodal masses, extranodal lesions	Score 4 or 5 with no significant change in FDG uptake from baseline at interim or end of treatment	<50% decrease from baseline in SPD of up to 6 dominant, measurable nodes and extranodal sites; no criteria for progressive disease are met
Nonmeasured lesions	Not applicable	No increase consistent with progression
Organ enlargement	Not applicable	No increase consistent with progression
New lesions	None	None
Bone marrow	No change form baseline	Not applicable
Progressive disease	**Progressive metabolic disease**	**Progressive disease** *Requires at least 1 of the following:*
Individual target nodes/nodal masses	Score 4 or 5 with an increase in intensity of uptake from baseline; *and/or*	An individual node/lesion must be abnormal with: • LDi >1.5 cm, *and* • Increase by ≥ 50% from PPD nadir, *and* • An increase in LDi or SDi from nadir: o 0.5 cm for lesions ≤2 cm o 1.0 cm for lesions >2 cm
Extranodal lesions	New FDG-avid foci consistent with lymphoma at interim or end of treatment assessment	• In the setting of splenomegaly, the splenic length must increase by >50% of the extent of its prior increase beyond baseline (e.g., a 15-cm spleen must increase to >16 cm). If no prior splenomegaly, must increase by at least 2 cm from baseline. • New or recurrent splenomegaly
Nonmeasured lesions	None	New or clear progression of preexisting nonmeasured lesions
New lesions	New FDG-avid foci consistent with lymphoma rather than another etiology (e.g., infection, inflammation). If uncertain regarding etiology of new lesions, biopsy or interval scan may be considered.	• Regrowth of previously resolved lesions • A new node >1.5 cm in any axis • A new extranodal site >1.0 cm in any axis; if <1.0 cm in any axis, its presence must be unequivocal and must be attributable to lymphoma • Assessable disease of any size unequivocally attributable to lymphoma
Bone marrow	New or recurrent FDG-avid foci	New or recurrent involvement

Abbreviations: 5PS, 5-point scale; CT computed tomography; FDG, fluorodeoxyglucose; IHC, immunohistochemistry; LDi, longest transverse diameter of a lesion; MRI, magnetic resonance imaging; PET, positron emission tomography; PPD, cross product of the LDi and perpendicular diameter; SDi, shortest axis perpendicular to the LDi; SPD, sum of the product of the perpendicular diameters for multiple lesions.

Response and Site	PET-CT Based Response	CT-Based Response
*A score of 3 in many patients indicates a good prognosis with standard treatment, especially if at the time of an interim scan. However, in trails involving PET where de-escalation is investigated, it may be preferable to consider a score of 3 as inadequate response (to avoid under treatment). Measured dominant lesions: Up to 6 of the largest dominant nodes, nodal masses, and extranodal lesions selected to be clearly measurable in 2 diameters. Nodes should preferably be from disparate regions of the body and should include, where applicable, mediastinal and retroperitoneal areas. Non-nodal lesions include those in solid organs (e.g., liver spleen, kidneys, and lungs), GI involvement, cutaneous lesions, or those noted on palpation. Nonmeasured lesions: Any disease not selected as measured, dominant disease and truly assessable disease should be considered not measured. These sites include any nodes, nodal masses, and extranodal sites not selected as dominant or measurable or that do not meet the requirements for measurability but are still considered abnormal, as well as truly assessable disease, which is any site of suspected disease that would be difficult to follow quantitatively with measurement, including pleural effusions, ascites, bone lesions, leptomeningeal disease, abdominal masses, and other lesions that cannot be confirmed and followed by imaging. In Waldeyer's ring or in extranodal sites (e.g., GI tract, liver, bone marrow), FDG uptake may be greater than in the mediastinum with complete metabolic response, but should be no higher than surrounding normal physiologic uptake (e.g., with marrow activation as a result of chemotherapy of myeloid growth factors). †PET 5PS: 1, no uptake above background; 2, uptake ≤ mediastinum; 3, uptake > mediastinum but ≤ liver; 4, uptake moderately > liver; 5, uptake markedly higher than liver and/or new lesions; X, new areas of uptake unlikely to be related to lymphoma.		

6.3.2 Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

6.3.3 Duration of Response

The duration of response (DOR) is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started), death, or, in the absence of PD, date of last assessment.

6.3.4 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from the date of study enrollment until time of disease relapse, disease progression, or death, whichever occurs first.

6.4 Toxicity Criteria

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 Definitions

Please refer to definitions provided in Policy 801: Reporting Research Events found at:

https://irbo.nih.gov/hrpp-policy-guidelines/

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at:

https://irbo.nih.gov/hrpp-policy-guidelines/.

NOTE: Only IND Safety Reports that meet the definition of an unanticipated problem or present new information that might affect the willingness of participants to enroll or remain on the study will need to be reported per these policies.

7.2.2 IRB Requirments for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at:

https://irbo.nih.gov/hrpp-policy-guidelines/.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet approximately weekly when patients are being actively treated on the trial to discuss each patient. Decisions about trial continuation will be made based on the efficacy data from prior patients at appropriate time points per the statistical plan.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a

medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 ASSESSMENT OF SAFETY EVENTS

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution (return to baseline or stabilization) of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution (return to baseline or stabilization).

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section **8.4.**

8.3 REPORTING OF SERIOUS ADVERSE EVENTS

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.4**.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted via an electronic SAE reporting system (e.g. HiLIT). In the event of system downtime or issues, SAE reports will be submitted using the CCR SAE Report form to the sponsor at: OSROSafety@mail.nih.gov. CCR SAE report form and instructions can be found at:

https://nih.sharepoint.com/:u:/r/sites/NCI-CCR-OCD-Communications/SitePages/Forms-and-Instructions.aspx?csf=1&web=1&e=uWBXtI.

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 WAIVER OF EXPEDITED REPORTING TO CCR

As death/hospitalization due to disease progression is part of the study objectives (DOR, PFS, OS), and captured as an endpoint in this study, they will not be reported in expedited manner to

the sponsor. However, if there is evidence suggesting a causal relationship between the study drug and the event, report the event in an expedited manner according to section **8.3**

8.5 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

The Principal Investigator is responsible for all of the pharmacovigilance obligations and safety reporting pursuant to the applicable laws and regulations in the country/countries where the study is performed.

Additionally, the Principal Investigator/designee shall immediately, within 24 hours at the latest, report the following to BAYER by fax and/or e-mail (i.e., 973-709-2185 or DrugSafety.GPV.US@bayer.com) all of the following (CCR Safety will follow-up also with final reports within two [2] business days):

- all Serious Adverse Events occurring after start of administration of copanlisib, independent of their causal relationship to the study treatment
- any other relevant safety information including but not limited to:
    - reports of drug exposure via mother / father with and without adverse events (exposure during conception, pregnancy, childbirth and breastfeeding) including their outcome;
    - if linked to a serious adverse event, reports of misuse, abuse, overdose, medication error and other uses outside what is foreseen in the protocol, drug dependency, occupational exposure suspected transmission of an infectious agent, withdrawal syndrome, drug interactions with respect to the copanlisib;
- any communication concerning safety related information to regulatory authorities or ethics committees including but not limited to:
    - Development Safety Update Report for the study;
    - Any other safety related reports, issues and queries that are either raised by or communicated to regulatory authorities or ethics committees (e.g., reportable non-serious cases);
- the following events of special safety interest:
    - Non-infectious pneumonitis (NIP)

      Based on data from lymphoma clinical studies with copanlisib, as soon as there is reasonable suspicion of NIP, the investigator should immediately notify Bayer (within 24 hours) regardless of whether the event is assessed as causally related/not related to the study drug, or as serious/non-serious. The AESI should be entered on an SAE form and if the event is assessed as non-serious, the non-serious assessment should be noted in the form.
    - Pregnancies
        - The investigator must report any pregnancy occurring in a female study patient during her participation in this study. The outcome of the pregnancy should be followed up carefully, and any outcome of the mother and the child at delivery should be reported.

- For a pregnancy in the partner of a male study patient, all efforts will be made to obtain similar information on course and outcome, subject to the partner's consent.
- The investigator should submit them within the same timelines as an SAE.

8.6 REPORTING PREGNANCY

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://nih.sharepoint.com/:u:/r/sites/NCI-CCR-OCD-Communications/SitePages/Forms-and-Instructions.aspx?csf=1&web=1&e=uWBXtI.

8.6.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (**8.1.2**) should be reported as SAEs.

The outcome of all pregnancies should be followed up and documented.

8.6.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm for at least 1 month after the last dose of copanlisib and 12 months after the last dose of rituximab, whichever is later.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies occurring from the date of the first dose until at least 1 month after the last dose of copanlisib and 12 months after the last dose of rituximab, should, if possible, be followed up and documented. Pregnant partners may be offered the opportunity to participate in an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy for safety reporting purposes.

8.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESIS

10.1.1 Primary Endpoint

Complete response (CR) rate after induction therapy, as defined by the proportion of patients who achieve a PET-negative CR in accordance with the 2014 Lugano classification of the International Working Group Criteria for Non-Hodgkin's Lymphoma(62)

10.1.2 Secondary Endpoints

- Safety
- Complete molecular remission rate (CMR)
- Objective tumor response rate (ORR)
- Continuous complete response rate at 30 months (CR30)
- Duration of response (DOR)
- Time to next treatment (TTNT)
- Progression-free survival (PFS)
- Overall survival (OS)

10.1.3 Definitions of Primary and Secondary Endpoints

- Complete response (CR) rate after induction therapy: the proportion of patients who achieve a PET-negative CR in accordance with the 2014 Lugano classification of the International Working Group Criteria for Non-Hodgkin's Lymphoma(62) after induction therapy with copanlisib and rituximab
- Safety: the proportion of patients with adverse events leading to discontinuation of induction therapy with copanlisib and rituximab
- Complete molecular remission (CMR) rate: the proportion of patients who achieve both a complete response and are negative on molecular assays for minimal residual disease after induction therapy with copanlisib and rituximab
- Objective tumor response (ORR) rate: proportion of patients who achieve at least a partial response (PR) to induction therapy with copanlisib and rituximab
- Continuous CR rate at 30 months (CR30): proportion of patients who remain in complete response at 30 months from study enrollment
- Duration of response (DOR): time from first documentation of tumor response to disease progression
- Time to next treatment (TTNT): time from the end of induction therapy with copanlisib and rituximab until initiation of next treatment
- Progression-free survival (PFS): time from study enrollment until disease progression or death from any cause
- Overall survival (OS): time from study enrollment until death from any cause

10.2 SAMPLE SIZE DETERMINATION AND STATISTICAL PLAN

The primary objectives of this trial are to determine if the combination of copanlisib + rituximab is associated with an adequately high complete response rate in patients with previously untreated

follicular lymphoma and to obtain preliminary, pilot information on whether the subset of patients who are identified subsequently as having a high risk signature by gene-expression profiling (GEP) are able to have a modestly high rate of retaining a complete response. As the expected fraction of patients who have a high risk GEP signature is expected to be approximately 25%, the sample size for this study is initially guided by practical considerations regarding the number of patients who may enroll on this trial over a period of 4 years.

The intended sample size for the trial will be 60 evaluable patients, which is the number which may be realistically treated in 4 years at a single institution. As the objective is to obtain a modest number of patients who have a high risk GEP signature, and only 25% of all patients are expected to have this high risk signature, the trial will not plan to end enrollment early unless an unacceptably low proportion of all patients with a complete response (CR) is identified, since enrollment of a large number of patients may be needed to obtain a modest number with the high risk signature.

Since the goal of chemotherapy-free regimens is to develop a cure, then achievement of CR is chosen as the primary endpoint. Based on the published literature, the combination therapy to be evaluated in this trial would be estimated to be associated with at least a 25% CR rate, since copanlisib monotherapy demonstrated a 12% CR rate in cases of relapsed and refractory FL(63). We anticipate that earlier use of copanlisib as well as the combination with rituximab will yield a CR rate of at least 25%. Further, rates of CR that are <25% would be considered uninteresting for further clinical development. At the end of the trial, the overall CR rate will be presented along with 80% and 95% two-sided confidence intervals.

As an early stopping rule for futility, after 30 evaluable patients have had their response status assessed, if there are 4 or fewer patients with a complete response among the 30 evaluable patients (13.3%), the trial will no longer accrue any patients as soon as this can be determined, regardless of the number accrued to the trial, since the one-sided upper 90% confidence interval bound on 4/30 is 24.9%, which is below the anticipated CR rate.

As an early stopping rule for toxicities, if during the initial 20 patients treated on this trial, there are 4 or more patients with grade 3 or higher non-infectious pneumonitis (NIP), then no further patients will be enrolled. If after 20 patients have enrolled, there is a cumulative fraction of patients with grade 3 or higher NIP which exceeds 20%, then no further patients will be accrued to the trial in its present form. Further accrual would only proceed if the dosing of copanlisib were decreased and the trial amended to reduce likely occurrence of this toxicity.

From among all evaluable patients enrolled on the trial, when a high risk GEP signature can be identified as being present or absent in the patients, the CR rate will be determined and this will be used as preliminary information to guide further, more extensive explorations if warranted. If the true probability of a patient having a high risk GEP signature were 25%, then among 60 patients, the probability of having 11 or more patients with this characteristic is 91.4%, the probability of having 12 or more is 85.2%, the probability of having 13 or more is 76.8%, and the probability of having 14 or more is 66.5%. As a result, from among 60 evaluable patients, it is realistic to expect 11 to 14 who have the high risk GEP signature. From among these expected 11 to 14 patients, as well as among the expected 46 to 49 patients without the signature, the CR rate will be reported separately by category along with two-sided 80% and 95% confidence intervals in order to establish in a preliminary, pilot sense if patients who have the high risk signature are able to have a potentially beneficial outcome with treatment, as well as to determine if there is a

prognostic difference between patients according to presence or absence of the signature. In order to allow for a small number of inevaluable patients, the accrual ceiling will be set at 65 patients.

10.3 Populations for Analyses

10.3.1 Evaluable for Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with copanlisib.

10.3.2 Evaluable for Objective Response

Only those patients who have measurable disease present at baseline, have received at least one cycle of induction therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (**NOTE**: Patients who exhibit objective disease progression at any point after receiving at least 1 cycle of induction therapy will also be considered evaluable.)

10.3.3 Evaluable Non-Target Disease Response

Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

10.4 Statistical Analyses

10.4.1 General Approach

The response rate will be determined and reported along with a 95% confidence interval. Other time-to-event outcomes will be reported using Kaplan-Meier curves.

10.4.2 Analysis of the Primary Endpoint

The CR rate will be determined and reported along with a 95% confidence interval.

10.4.3 Analysis of the Secondary Endpoints

The ORR, CMR and the CR30 rate will be determined and reported along with a 95% confidence interval. The duration of response (DOR; beginning at the date clinical response is first identified), time to next treatment (TTNT), progression free survival (PFS), and overall survival (OS) will be estimated using Kaplan-Meier curves with appropriate confidence intervals reported.

10.4.4 Safety Analyses

The type, grade and frequency of toxicities will be reported.

10.4.5 Planned Interim Analyses

As noted in Section **10.2**, the study will be stopped if, after 30 evaluable patients have had their response status assessed, there are 4 or fewer patients with a complete response.

10.4.6 Sub-Group Analyses

None.

10.4.7 Tabulation of Individual Participant Data

None.

10.4.8 Exploratory Analyses

The exploratory objectives such as seeking to identify potential biomarkers that are associated with response, will be assessed using descriptive statistics as well as non-parametric methods such as exact Wilcoxon rank sum tests. The analyses will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENTS

11.1 Cooperative Research and Development Agreement (CRADA)

A CRADA between the National Cancer Institute and Bayer Pharmaceuticals has been fully executed (#03256) 05 August 2019.

12 HUMAN SUBJECTS PROTECTIONS

12.1 Rationale For Subject Selection

Follicular lymphoma affect all races and sexes and only occasionally (~5% of cases) affects adults under the age of 40 years. Overall, we anticipate an even sex distribution. This trial is directed at assessing a novel targeted therapy using copanlisib in patients with untreated FL. Pregnant or nursing mothers are excluded because of the potential teratogenic effects of therapy.

12.2 Participation of Children

Children under the age of 18 will not be eligible for participation on this protocol because FL is rare in young patients, and the inclusion of an occasional young patient will not provide generalizable information that would justify their inclusion on this study.

12.3 Evaluation of Benefits and Risks/Discomforts

Patients may obtain direct benefit from treatment with copanlisib with rituximab. The most common adverse reactions (≥10%) attributable to copanlisib observed in clinical trials were hyperglycemia, hypertension, diarrhea, decreased general strength and energy, neutropenia, nausea, lower respiratory tract infections and thrombocytopenia. Although the potential for serious adverse events exists for these patients, the incidence of them is decidedly lower compared to combination chemotherapy regimens when added to rituximab and management algorithms have been developed. The potential toxicity of copanlisib is reasonable in relation to the potential benefit to this group of patients who have few treatment options.

12.3.1 Risks Related to Study Procedures

12.3.1.1 Blood Collection

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

12.3.1.2 Biopsy Collection – Tumor

The risks of the biopsies include pain, bleeding and infection at the biopsy site. Conscious sedation or general anesthesia may be used for pain management, if warranted, and the use and risks are acceptable to the patient.

12.3.1.3 Biopsy Collection – Bone Marrow

Bone marrow biopsy is minimally invasive and is typically a very safe procedure. Usually the hipbone is numbed with anesthesia. Conscious sedation or general anesthesia may be used, if warranted, and the use and risks are acceptable to the patient. Using a needle, the solid and liquid

portion of bone marrow is taken out. This procedure causes some pain. Very rarely, infection or bleeding may occur at the needle site.

12.3.1.4 Conscious Sedation

The common side effects of conscious sedation include drowsiness, delayed reflexes, hypotension, headache, and nausea. These are generally mild and last no more than a few hours.

12.3.1.5 General Anesthesia

The risks of general anesthesia include decreased breathing rate, aspiration, low heart rate, deacreased heart rate, decreased blood pressure.

12.3.1.6 Imaging

In addition to the radiation risks discussed below, scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, experience a metallic taste, headache, difficulty breathing, hypotension, increased heartrate and swelling. Furthermore, the IV catheter used to administer the contrast may cause bleeding, infection or inflammation of of the skin and vein with pain and swelling. Participants undergoing gadolinium enhanced MRIs may also be at risk for kidney damage. MRIs include the additional risk of damage to hearing.

12.3.1.7 Radiation Exposure

The procedures for performing the [^{18}F]-FDG PET/CT scans, CT scans, and CT-guided biopsies will follow clinical policies, no special procedures apply this additional assessment for research purposes.

In summary, subjects may receive radiation exposure from up to seven CT scans, six [^{18}F]-FDG PET/CT scans, and four CT-guided biopsies inclusive of screening assessments.

The total radiation dose for research purposes will be approximately 19.5 rem. The risk of getting cancer from the radiation exposure in this study is 1.6% and of getting a fatal cancer 0.8%.

12.3.1.8 Other Procedures

There are no physical risks associated with other procedures (e.g., urine or saliva collection, cheek/ buccal swabs). There are minimal risks associated with electrocardiogram (ECG) as it is a relatively safe procedure.

12.4 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (when in person) will be located in a private area (e.g., clinic

consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the subject will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at:

https://nih.sharepoint.com/sites/NCI-CCR-OCD-Communications/SitePages/OEC-Administrative---Clinical-Research-(ADCR).aspx?Mode=Edit.

12.4.1 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in section **2.2.1** may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to IND sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council for Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical sites and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 COPANLISIB (BAY 80-6946, ALIQOPA®) (IND #141280)

Refer to the FDA approved package insert for complete product information.

14.1.1 Source

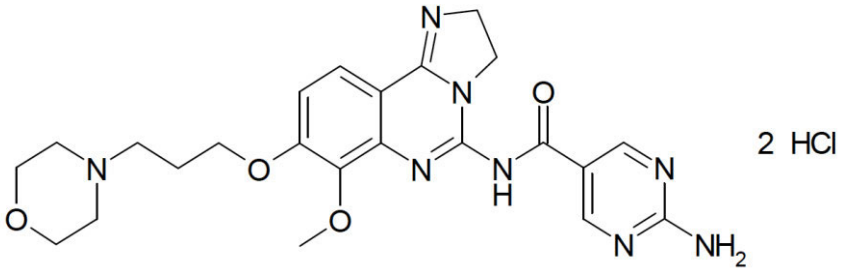
Copanlisib (commercial/marketed supplies) will be provided by Bayer Pharmaceuticals for use by subjects in this clinical trial.

14.1.2 Chemical formula and molecular structure

BAY 80-6946 is the active ingredient (free base) of BAY 84-1236, the dihydrochloride salt, which is used to prepare the freeze dried medicinal product. BAY 84-1236 is isolated either in its pseudopolymorphic form hydrate I (containing 11-17% water) or dried to a water content below 10%. Copanlisib has been assigned as the International Nonproprietary Name (INN) for BAY 80-6946.

Chemical Name (IUPAC):	2-amino-N-[7-methoxy-8-(3-morpholin-4-ylpropoxy)-2,3-dihydroimidazo[1,2-c]quinazolin-5-yl]pyrimidine-5-carboxamide dihydrochloride
BAY number:	BAY 80-6946 (free base); BAY 84-1236 (dihydrochloride salt)

Chemical formula: C23H28N8O4 2HCl
Molecular mass: 553.45 g/mol
Chemical structure:



14.1.3 Mechanism of copanlisib

Copanlisib is a small molecule pan-class 1 PI3K inhibitor with predominant activity against PI3Kα and PI3Kδ isoforms that first demonstrated anti-tumor activity in pre-clinical models characterized by activating genetic aberrations of the PI3K pathway. Copanlisib exhibits potent kinase inhibitory effect on all four isoforms with biochemical IC50 values of 0.5 nM, 0.7 nM, 3.7 nM and 6.4 nM for PI3Kα, PI3Kδ, PI3Kβ and PI3Kγ, respectively. Copanlisib also potently regulates nuclear localization of the forkhead family members resulting in the induction of transcriptional programs that lead to rapid cell death by apoptosis.

14.1.4 Toxicity

The safety data reflect exposure to copanlisib in 168 adults with follicular lymphoma and other hematologic malignancies treated with copanlisib 60 mg or 0.8 mg/kg equivalent in clinical trials. The median duration of treatment was 22 weeks (range 1 to 206 weeks).

Serious adverse reactions were reported in 44 (26%) patients. The most frequent serious adverse reactions that occurred were pneumonia (8%), pneumonitis (5%) and hyperglycemia (5%). The most common adverse reactions (≥20%) were hyperglycemia, diarrhea, decreased general strength and energy, hypertension, leukopenia, neutropenia, nausea, lower respiratory tract infections, and thrombocytopenia. Adverse reactions resulted in dose reduction in 36 (21%) and discontinuation in 27 (16%) patients. The most common reasons for dose reduction were hyperglycemia (7%), neutropenia (5%), and hypertension (5%). The most common reasons for drug discontinuation were pneumonitis (2%) and hyperglycemia (2%).

The tables provide the serious adverse reactions occurring in patients receiving copanlisib Monotherapy (**Table 9**), and the treatment-emergent laboratory abnormalities in ≥10% of patients and ≥4% of Grade ≥3 treated with copanlisib and rituximab (**Table 10**).

Table 9: Serious Adverse Reactions From Multiple Clinical Trials

ADVERSE REACTIONS	Copanlisib N=877		
	All SAR N (%)	Life threatening	Fatal
Metabolism and nutrition disorders			
Hyperglycemia	39 (4.4%)	NA	NA
Blood and lymphatic system disorders			
Febrile neutropenia	13 (1.5%)	NA	NA
Neutropenia (including febrile neutropenia)	14 (1.6%)	NA	NA
Thrombocytopenia	2 (0.2%)	NA	NA
General disorders and administration site conditions			
Fatigue	2 (0.2%)	NA	NA
Gastrointestinal disorders			
Diarrhea	11 (1.3%)	NA	NA
Colitis	5 (0.6%)	NA	NA
Pancreatitis	2 (0.2%)	NA	NA
Vomiting	2 (0.2%)	NA	NA
Vascular disorders			
Hypertension (includes secondary hypertension)	8 (0.9%)	NA	NA
Infection			
Pneumonia	41 (4.7%)	NA	NA
Pneumocytis jirovecii	14 (1.6%)	NA	NA
Lower respiratory infection	5 (0.6%)	NA	NA
Bronchitis	4 (0.5%)	NA	NA
Pneumonia, bacterial	4 (0.5%)	NA	NA
Sepsis	4 (0.5%)	NA	NA
Herpes Zoster	3 (0.3%)	NA	NA
Pneumococcal pneumonia	2 (0.2%)	NA	NA
Pneumonia, viral	2 (0.2%)	NA	NA
Septic shock	2 (0.2%)	NA	NA
Metabolism and nutrition disorders			
Hyperglycemia	39 (4.4%)	NA	NA
Hyponatremia	2 (0.2%)	NA	NA
Pneumonitis	33 (3.8%)	NA	NA
Interstitial lung disease	11 (1.3%)	NA	NA
Skin and Subcutaneous Tissue Disorders			
Dermatitis exfoliative generalised	4 (0.5%)	NA	NA

Table 10: Treatment-emergent Laboratory Abnormalities in ≥10% of Patients and ≥4% of Grade ≥3 Treated with Copanlisib and Rituximab

Laboratory Parameter	Copanlisib with Rituximab N=307		
	Any Grade n (%)	Grade 3 n (%)	Grade 4 n (%)
Hematology abnormalities			
Decreased hemoglobin	209 (68%)	15 (5%)	0
Lymphocyte count decreased	231 (76%)	73 (24%)	13 (4%)
Platelet count decreased	182 (60%)	11 (4%)	3 (1%)
Neutrophil count decreased	226 (74%)	58 (19%)	64 (21%)
Serum chemistry abnormalities			
Hyperglycemia	296 (96%)	193 (63%)	14 (5%)
Hypophosphatemia	127 (42%)	24 (8%)	4 (1%)
Hyponatremia	89 (29%)	16 (5%)	1 (0.1%)
Serum lipase increase	97 (32%)	17 (6%)	3 (1%)

14.1.5 Formulation and Preparation

The following excipients are used to manufacture the medicinal product: mannitol, NaOH, citric acid (except for the 20 mg formulation) and water for injection. The medicinal product is a freeze-dried product containing either 20 mg of copanlisib (equivalent to 23.04 mg BAY 84-1236) or 60 mg of copanlisib (equivalent to 69.12 mg BAY 84-1236) or 80 mg of copanlisib (equivalent to 92.16 mg BAY 84-1236) in a 6 mL injection vial. The IV solution is obtained after reconstitution of the lyophilisate with saline solution. The resulting solution contains the active substance in concentrations as outlined below:

Table 3–1: Reconstitution volumes and resulting copanlisib (BAY 80-6946) concentrations of the 20, 60, and 80 mg formulations

Formulation	Reconstitution volume [mL]	Resulting concentration of BAY 80-6946 [mg/mL]
20 mg	2	10
60 mg	4.4	15
80 mg	4	20

For example, for the 60 mg formulation: reconstitute copanlisib with 4.4 mL of sterile 0.9% NaCl solution leading to a concentration of 15 mg/mL, as follows:

- Withdraw 4.4 mL of sterile 0.9% NaCl solution by using a 5 mL sterile syringe with needle.
- Inject the measured volume through the disinfected stopper surface into the vial of copanlisib.
- Dissolve the lyophilized solid by gently shaking the injection vial for 30 seconds.
- Allow to stand for one minute to let bubbles rise to the surface.
- Check if any undissolved substance is still seen. If yes, repeat the gentle shaking and settling procedure.
- Inspect visually for discoloration and particulate matter. After reconstitution, the solution should be colorless to slightly yellowish

- Once the solution is free of visible particles, withdraw the reconstituted solution for further dilution.
- The reconstituted solution is to be diluted with isotonic sodium chloride (NaCl) solution without falling below a concentration of 0.3 mg/mL copanlisib.

Further dilute the reconstituted solution in 100 mL sterile 0.9% NaCl solution for injection. With a sterile syringe, withdraw the required amount of the reconstituted solution for the desired dosage:

60 mg: Withdraw 4 mL of the reconstituted solution with a sterile syringe.
45 mg: Withdraw 3 mL of the reconstituted solution with a sterile syringe.
30 mg: Withdraw 2 mL of the reconstituted solution with a sterile syringe.

Inject the contents of the syringe into the patient infusion bag of 100 mL sterile 0.9% NaCl solution. Mix the dose well by inverting.

14.1.6 Stability and Storage

The drug product has to be stored between +2°C and +8°C and should not be transported above +30°C. Supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial supplies must be recorded by an authorized person at the trial site. Supplies may not be used for any purpose other than that stated in the protocol.

The diluted solution is physically and chemically stable for 24 hours at room temperature, provided it is not exposed to direct sunlight. However, for microbiologic consideration, diluted solution should be stored between +2°C and +8°C if not administered immediately.

Use reconstituted and diluted copanlisib immediately or store the reconstituted solution in the vial or diluted solution in the infusion bag at 2°C to 8°C (36°F to 46°F) for up to 24 hours before use. Allow the product to adapt to room temperature before use following refrigeration. Avoid exposure of the diluted solution to direct sunlight.

14.1.7 Administration procedures

Copanlisib is administered in a normal saline solution, intravenously, over 1 hour. Administer copanlisib as a single agent, following reconstitution and dilution. Mix only with 0.9% sodium chloride (NaCl) solution. Do not mix or inject copanlisib with other drugs or other diluents. No intravenous glucose preparations should be administered on the days of infusion.

14.2 RITUXIMAB

Refer to the FDA approved package insert for complete product information.

14.2.1 Source

Commercial supplies of rituximab will be purchased by the NIH Clinical Center.

14.2.2 Toxicity

No dose-limiting effects were observed in the Phase I/II studies. Reported adverse events including fever, chills, headache, nausea, vomiting, rhinitis, asthenia, and hypotension, occurred primarily during rituximab infusions and typically responded to an interruption of the infusion and resumption at a slower rate.

Other important events include: fatal infusion reactions, cardiac events, tumor lysis syndrome (TLS), renal events, mucocutaneous reactions, infections, hepatitis B reactivation, progressive multifocal leukoencephalopathy (PML), bowel obstruction and perforation, and immunogenicity.

See the package insert for full information.

14.2.3 Formulation and Preparation

Commercially available in single-use vials containing 10 mL (100 mg) or 50 mL (500 mg) of rituximab solution at a concentration of 10 mg/mL. Rituximab will be diluted with 0.9% Sodium Chloride to prepare a standard product with concentration of 2 mg/ml. Caution should be taken during the preparation of the drug, as shaking can cause aggregation and precipitation of the antibody.

14.2.4 Stability and Storage

Rituximab vials should be stored in a secure refrigerator at 2º to 8°C. After dilution, rituximab is stable at 2-8°C (36-46°F) for 24 hours and for an additional 24 hours at room temperature.

Please refer to the package insert for additional guidance on study drug preparation, handling, and storage.

14.2.5 Administration procedures

A peripheral or central intravenous line will be established. During rituximab infusion, a patient's vital signs (blood pressure, pulse, respiration, temperature) should be monitored according to the standard of care. Medications readily available for the emergency management of anaphylactoid reactions should include: epinephrine (1:1000, 1 mg/mL) for subcutaneous injection, diphenhydramine hydrochloride for intravenous injection, and resuscitation equipment.

Prophylaxis against hypersensitivity and infusion-related reactions associated with rituximab will include acetaminophen 650 mg and diphenhydramine hydrochloride 50-100 mg administered 30-60 minutes prior to starting rituximab.

Rituximab infusions will be administered to patients primarily in an outpatient clinic setting.

- **First dose**:

  The initial dose rate at the time of the first rituximab infusion should be 50mg/hour (25 mL/hr) for the first 30 minutes. If no toxicity is seen, the dose rate may be escalated gradually in 50 mg/hour (25 mL/h) increments at 30 minute intervals to a maximum of 400 mg/hour (maximum rate = 200 mL/h).

- **Second and Subsequent Doses (select the appropriate administration timing):**

  - ***90-minute Administration***

    If the first dose of rituximab is well tolerated, subsequent doses may be administered over 90 minutes with 20% of the total dose given in the first 30 minutes, and remaining 80% of the total dose administered over the subsequent 60 minutes; e.g.:

Two-Step Rate Escalation	Volume to administer (X mL)
1st portion (0 – 30 minutes)	$\frac{\text{Total Dose (mg)}}{2}$ 0.2 = X mL (over 30 min)
2nd portion (30 – 90 minutes)	$\frac{\text{Total Dose (mg)}}{2}$ 0.8 = X mL (over 60 min)

Special Note: The 90-minute infusion scheme is not recommended for patients with clinically significant cardiovascular disease or high circulating lymphocyte counts (≥5000/mcL).

- ***Standard Administration for Second & Subsequent Infusions***

Patients who tolerate initial treatment without experiencing infusion-related adverse effects but for whom the 90-minute infusion scheme during subsequent treatments is considered inappropriate, may receive subsequent rituximab doses at the Standard Rate for Subsequent Infusions, which is as follows:

Begin at an initial rate of 100 mg/hour (50 mL/h) for 30 minutes. If administration is well tolerated, the administration rate may be escalated gradually in 100-mg/hour (50-mL/h) increments at 30-minute intervals to a maximum rate of 400 mg/hour (maximum rate = 200 mL/h).

CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.

15 REFERENCES

1. Anderson JR, Armitage JO, Weisenburger DD. Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. Ann Oncol. 1998;9(7):717-20.
2. Portlock CS, Rosenberg SA. No initial therapy for stage III and IV non-Hodgkin's lymphomas of favorable histologic types. Annals of internal medicine. 1979;90(1):10-3.
3. Horning SJ, Rosenberg SA. The natural history of initially untreated low-grade non-Hodgkin's lymphomas. The New England journal of medicine. 1984;311(23):1471-5.
4. Luminari S, Ferrari A, Manni M, Dondi A, Chiarenza A, Merli F, et al. Long-Term Results of the FOLL05 Trial Comparing R-CVP Versus R-CHOP Versus R-FM for the Initial Treatment of Patients With Advanced-Stage Symptomatic Follicular Lymphoma. J Clin Oncol. 2017:JCO2017741652.
5. Tan D, Horning SJ, Hoppe RT, Levy R, Rosenberg SA, Sigal BM, et al. Improvements in observed and relative survival in follicular grade 1-2 lymphoma during 4 decades: the Stanford University experience. Blood. 2013;122(6):981-7.
6. Solal-Celigny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, et al. Follicular lymphoma international prognostic index. Blood. 2004;104(5):1258-65.
7. Federico M, Bellei M, Marcheselli L, Luminari S, Lopez-Guillermo A, Vitolo U, et al. Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. J Clin Oncol. 2009;27(27):4555-62.
8. Casulo C, Day B, Dawson KL, Zhou X, Flowers CR, Farber CM, et al. Disease characteristics, treatment patterns, and outcomes of follicular lymphoma in patients 40 years of age and younger: an analysis from the National Lymphocare Studydagger. Ann Oncol. 2015;26(11):2311-7.
9. Conconi A, Lobetti-Bodoni C, Montoto S, Lopez-Guillermo A, Coutinho R, Matthews J, et al. Life expectancy of young adults with follicular lymphoma. Ann Oncol. 2015;26(11):2317-22.
10. Casulo C, Byrtek M, Dawson KL, Zhou X, Farber CM, Flowers CR, et al. Early Relapse of Follicular Lymphoma After Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone Defines Patients at High Risk for Death: An Analysis From the National LymphoCare Study. J Clin Oncol. 2015;33(23):2516-22.
11. Casulo C, Le-Rademacher J, Dixon J, Salles G, Hoster E, Herold M, et al. Validation of POD24 As a Robust Early Clinical Endpoint of Poor Survival in Follicular Lymphoma: Results from the Follicular Lymphoma Analysis of Surrogacy Hypothesis (FLASH) Investigation Using Individual Data from 5,453 Patients on 13 Clinical Trials. Blood. 2017;130:412-.
12. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. The New England journal of medicine. 2004;351(21):2159-69.
13. Huet S, Tesson B, Jais JP, Feldman AL, Magnano L, Thomas E, et al. A gene-expression profiling score for prediction of outcome in patients with follicular lymphoma: a retrospective training and validation analysis in three international cohorts. Lancet Oncol. 2018.
14. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015;16(9):1111-22.

15. Jurinovic V, Kridel R, Staiger AM, Szczepanowski M, Horn H, Dreyling MH, et al. Clinicogenetic risk models predict early progression of follicular lymphoma after first-line immunochemotherapy. Blood. 2016;128(8):1112-20.
16. Weigert O, Weinstock DM. The promises and challenges of using gene mutations for patient stratification in follicular lymphoma. Blood. 2017;130(13):1491-8.
17. Kahl BS, Yang DT. Follicular lymphoma: evolving therapeutic strategies. Blood. 2016;127(17):2055-63.
18. Martinelli G, Schmitz SF, Utiger U, Cerny T, Hess U, Bassi S, et al. Long-term follow-up of patients with follicular lymphoma receiving single-agent rituximab at two different schedules in trial SAKK 35/98. J Clin Oncol. 2010;28(29):4480-4.
19. Hiddemann W, Kneba M, Dreyling M, Schmitz N, Lengfelder E, Schmits R, et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood. 2005;106(12):3725-32.
20. Marcus R, Imrie K, Solal-Celigny P, Catalano JV, Dmoszynska A, Raposo JC, et al. Phase III study of R-CVP compared with cyclophosphamide, vincristine, and prednisone alone in patients with previously untreated advanced follicular lymphoma. J Clin Oncol. 2008;26(28):4579-86.
21. Bachy E, Houot R, Morschhauser F, Sonet A, Brice P, Belhadj K, et al. Long-term follow up of the FL2000 study comparing CHVP-interferon to CHVP-interferon plus rituximab in follicular lymphoma. Haematologica. 2013;98(7):1107-14.
22. Rummel MJ, Niederle N, Maschmeyer G, Banat GA, von Grunhagen U, Losem C, et al. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. Lancet. 2013;381(9873):1203-10.
23. Flinn IW, van der Jagt R, Kahl BS, Wood P, Hawkins TE, Macdonald D, et al. Randomized trial of bendamustine-rituximab or R-CHOP/R-CVP in first-line treatment of indolent NHL or MCL: the BRIGHT study. Blood. 2014;123(19):2944-52.
24. Marcus R, Davies A, Ando K, Klapper W, Opat S, Owen C, et al. Obinutuzumab for the First-Line Treatment of Follicular Lymphoma. The New England journal of medicine. 2017;377(14):1331-44.
25. Rummel M, Kaiser U, Balser C, Stauch M, Brugger W, Welslau M, et al. Bendamustine plus rituximab versus fludarabine plus rituximab for patients with relapsed indolent and mantle-cell lymphomas: a multicentre, randomised, open-label, non-inferiority phase 3 trial. Lancet Oncol. 2016;17(1):57-66.
26. Maddocks K, Barr PM, Cheson BD, Little RF, Baizer L, Kahl BS, et al. Recommendations for Clinical Trial Development in Follicular Lymphoma. J Natl Cancer Inst. 2017;109(3).
27. Fowler NH, Davis RE, Rawal S, Nastoupil L, Hagemeister FB, McLaughlin P, et al. Safety and activity of lenalidomide and rituximab in untreated indolent lymphoma: an open-label, phase 2 trial. Lancet Oncol. 2014;15(12):1311-8.
28. Martin P, Jung SH, Pitcher B, Bartlett NL, Blum KA, Shea T, et al. A phase II trial of lenalidomide plus rituximab in previously untreated follicular non-Hodgkin's lymphoma (NHL): CALGB 50803 (Alliance). Ann Oncol. 2017;28(11):2806-12.

29. Shi Q, Flowers CR, Hiddemann W, Marcus R, Herold M, Hagenbeek A, et al. Thirty-Month Complete Response as a Surrogate End Point in First-Line Follicular Lymphoma Therapy: An Individual Patient-Level Analysis of Multiple Randomized Trials. J Clin Oncol. 2016:JCO2016708651.
30. Bergholz JS, Roberts TM, Zhao JJ. Isoform-Selective Phosphatidylinositol 3-Kinase Inhibition in Cancer. J Clin Oncol. 2018;36(13):1339-42.
31. Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K Pathway in Human Disease. Cell. 2017;170(4):605-35.
32. Okkenhaug K, Vanhaesebroeck B. PI3K in lymphocyte development, differentiation and activation. Nat Rev Immunol. 2003;3(4):317-30.
33. Fruman DA, Rommel C. PI3K and cancer: lessons, challenges and opportunities. Nat Rev Drug Discov. 2014;13(2):140-56.
34. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004;304(5670):554.
35. Wang X, Huang H, Young KH. The PTEN tumor suppressor gene and its role in lymphoma pathogenesis. Aging (Albany NY). 2015;7(12):1032-49.
36. Gopal AK, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ, et al. PI3Kdelta inhibition by idelalisib in patients with relapsed indolent lymphoma. The New England journal of medicine. 2014;370(11):1008-18.
37. Gopal AK, Kahl BS, Flowers CR, Martin P, Ansell SM, Abella-Dominicis E, et al. Idelalisib is effective in patients with high-risk follicular lymphoma and early relapse after initial chemoimmunotherapy. Blood. 2017;129(22):3037-9.
38. Liu N, Rowley BR, Bull CO, Schneider C, Haegebarth A, Schatz CA, et al. BAY 80-6946 is a highly selective intravenous PI3K inhibitor with potent p110alpha and p110delta activities in tumor cell lines and xenograft models. Mol Cancer Ther. 2013;12(11):2319-30.
39. Iyengar S, Clear A, Bodor C, Maharaj L, Lee A, Calaminici M, et al. P110alpha-mediated constitutive PI3K signaling limits the efficacy of p110delta-selective inhibition in mantle cell lymphoma, particularly with multiple relapse. Blood. 2013;121(12):2274-84.
40. Paul J, Soujon M, Wengner AM, Zitzmann-Kolbe S, Sturz A, Haike K, et al. Simultaneous Inhibition of PI3Kdelta and PI3Kalpha Induces ABC-DLBCL Regression by Blocking BCR-Dependent and -Independent Activation of NF-kappaB and AKT. Cancer Cell. 2017;31(1):64-78.
41. Patnaik A, Appleman LJ, Tolcher AW, Papadopoulos KP, Beeram M, Rasco DW, et al. First-in-human phase I study of copanlisib (BAY 80-6946), an intravenous pan-class I phosphatidylinositol 3-kinase inhibitor, in patients with advanced solid tumors and non-Hodgkin's lymphomas. Ann Oncol. 2016;27(10):1928-40.
42. Dreyling M, Santoro A, Mollica L, Leppa S, Follows GA, Lenz G, et al. Phosphatidylinositol 3-Kinase Inhibition by Copanlisib in Relapsed or Refractory Indolent Lymphoma. J Clin Oncol. 2017;35(35):3898-905.
43. Mandel P, Metais P. [Not Available]. Comptes rendus des seances de la Societe de biologie et de ses filiales. 1948;142(3-4):241-3.
44. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer research. 2001;61(4):1659-65.
45. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer research. 1977;37(3):646-50.

46. Hohaus S, Giachelia M, Massini G, Mansueto G, Vannata B, Bozzoli V, et al. Cell-free circulating DNA in Hodgkin's and non-Hodgkin's lymphomas. Ann Oncol. 2009;20(8):1408-13.
47. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nature reviews Cancer. 2011;11(6):426-37.
48. Sarkozy C, Huet S, Carlton VE, Fabiani B, Delmer A, Jardin F, et al. The prognostic value of clonal heterogeneity and quantitative assessment of plasma circulating clonal IG-VDJ sequences at diagnosis in patients with follicular lymphoma. Oncotarget. 2017;8(5):8765-74.
49. Roschewski M, Staudt LM, Wilson WH. Dynamic monitoring of circulating tumor DNA in non-Hodgkin lymphoma. Blood. 2016;127(25):3127-32.
50. Ladetto M, Bruggemann M, Monitillo L, Ferrero S, Pepin F, Drandi D, et al. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. Leukemia. 2014;28(6):1299-307.
51. Roschewski M, Dunleavy K, Pittaluga S, Moorhead M, Pepin F, Kong K, et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. Lancet Oncol. 2015;16(5):541-9.
52. Ladetto M, Lobetti-Bodoni C, Mantoan B, Ceccarelli M, Boccomini C, Genuardi E, et al. Persistence of minimal residual disease in bone marrow predicts outcome in follicular lymphomas treated with a rituximab-intensive program. Blood. 2013;122(23):3759-66.
53. Melani C, Roschewski M. Molecular Monitoring of Cell-Free Circulating Tumor DNA in Non-Hodgkin Lymphoma. Oncology (Williston Park). 2016;30(8):731-8, 44.
54. Casulo C, Nastoupil L, Fowler NH, Friedberg JW, Flowers CR. Unmet needs in the first-line treatment of follicular lymphoma. Ann Oncol. 2017;28(9):2094-106.
55. Kridel R, Sehn LH, Gascoyne RD. Pathogenesis of follicular lymphoma. The Journal of clinical investigation. 2012;122(10):3424-31.
56. Scott DW, Gascoyne RD. The tumour microenvironment in B cell lymphomas. Nature reviews Cancer. 2014;14(8):517-34.
57. Fowler N, Nastoupil L, de Vos S, Knapp M, Flinn IW, Chen R, et al. Ibrutinib Plus Rituximab in Treatment-Naive Patients with Follicular Lymphoma: Results from a Multicenter, Phase 2 Study. Blood. 2015;126(23):470-.
58. Salles G, Seymour JF, Offner F, Lopez-Guillermo A, Belada D, Xerri L, et al. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. Lancet. 2011;377(9759):42-51.
59. Luminari S, Ferrari A, Manni M, Dondi A, Chiarenza A, Merli F, et al. Long-Term Results of the FOLL05 Trial Comparing R-CVP Versus R-CHOP Versus R-FM for the Initial Treatment of Patients With Advanced-Stage Symptomatic Follicular Lymphoma. J Clin Oncol. 2018;36(7):689-96.
60. Myklebust JH, Brody J, Kohrt HE, Kolstad A, Czerwinski DK, Walchli S, et al. Distinct patterns of B-cell receptor signaling in non-Hodgkins' lymphomas identified by single cell profiling. Blood. 2016.
61. Andor N, Simonds E, Chen J, Grimes S, Wood C, Czerwinski DK, et al. Massively Parallel Single Cell RNA-Seq of Primary Lymphomas Reveals Distinct Cellular Lineages and Diverse, Intratumoral Transcriptional States. Blood. 2016;128:1090-.
62. Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol. 2014;32(27):3059-68.

63. Dreyling M, Santoro A, Mollica L, Leppa S, Follows GA, Lenz G, et al. Phosphatidylinositol 3-Kinase Inhibition by Copanlisib in Relapsed or Refractory Indolent Lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2017:JCO2017754648.

16 APPENDICES

APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B: GUIDELINES FOR PREGNANCY AND NURSING

Contraception

Copanlisib and/or rituximab may have adverse effects on a fetus in utero. Furthermore, it is not known if copanlisib or rituximab have transient adverse effects on the composition of sperm.

For this trial, male patients will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female patients will be considered of non-reproductive potential if:

1. They are postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.); **OR**
2. They have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening; **OR**
3. They have a congenital or acquired condition that prevents childbearing.

Female and male patients of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 180 days after the last dose of copanlisib or 12 months after the last dose of rituximab, whichever is later, by complying with one of the following:

1. practice abstinence[†] from heterosexual activity; **OR**
2. use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are[‡]:

Single method

Use of one of the following is acceptable:

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method

Requires use of two of the following:

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for patients participating at sites in this country/region.

Patients should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study patients of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period and for at least 1 month after the last dose of copanlisib and 12 months after the last dose of rituximab, whichever is later, for WOCBP and for men. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

Use in Pregnancy

If a subject inadvertently becomes pregnant while on study treatment, the subject will immediately be removed from the study treatment. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to Bayer without delay and within 2 working days to Bayer if the outcome is a serious adverse experience.

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to Bayer. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to Bayer and followed as described above and in Section **8.5**.

Use in Nursing Women

It is unknown whether copanlisib is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, patients who are breast-feeding are not eligible for enrollment.