NCI Protocol #: Not Applicable

DF/HCC Protocol #: 16-281

TITLE: A phase II study of the CHK1 inhibitor LY2606368 in patients with advanced solid tumors exhibiting replicative stress or homologous recombination repair deficiency

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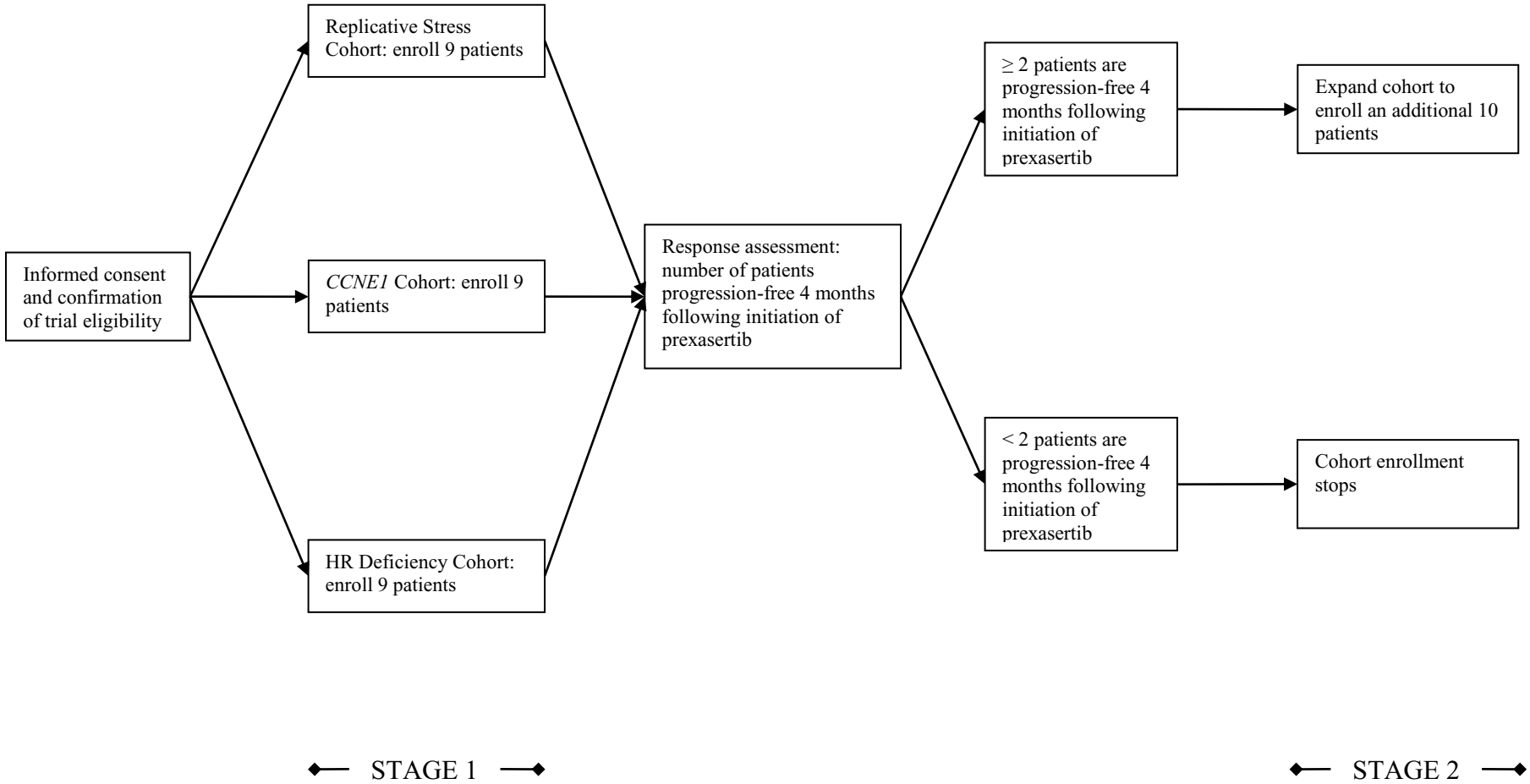
Agent: LY2606368/Prexasertib – Eli Lilly and Company

IND #: 130,512
IND Sponsor: Investigator-Initiated Study, Geoffrey Shapiro MD, PhD

Protocol Type / Version # / Version Date: Revised, Version #5, 26 January 2018

SCHEMA


Informed consent and confirmation of trial eligibility
Replicative Stress Cohort: enroll 9 patients
CCNE1 Cohort: enroll 9 patients
HR Deficiency Cohort: enroll 9 patients
Response assessment: number of patients progression-free 4 months following initiation of prexasertib
≥ 2 patients are progression-free 4 months following initiation of prexasertib
Expand cohort to enroll an additional 10 patients
< 2 patients are progression-free 4 months following initiation of prexasertib
Cohort enrollment stops
STAGE 1
STAGE 2

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1. OBJECTIVES

1.1 Study Design

This is an open label, phase II, three-arm study exploring the anti-tumor activity of the checkpoint kinase 1 (CHK1) inhibitor prexasertib (LY2606368) in patients with advanced solid tumors exhibiting replicative stress, a homologous repair (HR) deficiency, or *CCNE1* amplification.

1.2 Primary Objectives

1) To evaluate the activity of the CHK1 inhibitor prexasertib in patients with tumors exhibiting either replication stress, HR deficiency, or *CCNE1* amplification; specifically, to determine the proportion of patients alive and progression-free at 4 months in these cohorts.

1.3 Secondary Objectives

1) To assess toxicity following CHK1 inhibition among participants in these cohorts.
2) To examine the objective response and overall survival rate following CHK1 inhibition in these patient cohorts.

1.4 Exploratory Objectives

1) Pre-treatment biopsies will be obtained from patients in the first stage of the study in both the replicative stress and HR deficiency arms, and will be subjected to whole exome sequencing (WES) to explore potential genomic determinants of sensitivity and resistance to CHK1 inhibition.
2) Cell-free DNA (cfDNA) will be collected from patients on study and analyzed for mutations associated with the replicative stress and HR deficiency pathways.

2. BACKGROUND

2.1 LY2606368 (Prexasertib)

Please refer to the prexasertib investigator's brochure (IB) for comprehensive background information. LY2606368 monomesylate monohydrate (LY2606368 or prexasertib) is an adenosine triphosphate (ATP)-competitive selective inhibitor of CHK1. Prexasertib is being developed as a treatment for patients with advanced cancer and may have utility both as a single agent and in combination with deoxyribonucleic acid (DNA)-damaging agents or targeted

agents.

CHK1 is a multifunctional protein kinase and regulator of cell-cycle progression[1]. During cell cycle progression, the integrity of DNA is continuously monitored, with DNA damage or perturbation of DNA metabolism activating cell cycle checkpoints. Activation of these checkpoints leads to cell cycle arrest in order to provide the cell with the time necessary to repair DNA damage and complete DNA replication prior to progressing to mitosis. CHK1 is a key component of the checkpoint response and whose activation in response to DNA damage (e.g. from cytotoxic chemotherapeutic agents like cisplatin) arrests the cell in the S or G2 phase of the cell cycle. Inhibition of CHK1 leads to abrogation of cell cycle checkpoints, thereby preventing DNA repair and resulting in premature entry into mitosis[2]. Following early entry into mitosis, the cell is rapidly killed by mitotic catastrophe attributable to failed chromosomal segregation due to a damaged or incompletely replicated genome.

In addition to its role in cell cycle checkpoint control, CHK1 contributes to the DNA damage response through its role in coordinating DNA repair. Specifically, CHK1 is essential for HR repair of DNA double strand breaks (DSBs) [3] and CHK1 phosphorylates FANCE and FANCD2[4,5], proteins involved in the Fanconi Anemia DNA repair pathway, which serves to remove DNA cross-links induced by DNA damaging agents like cisplatin. As such, CHK1 inhibition may increase the activity of DNA-damaging cytotoxic chemotherapeutics by interfering with DNA repair. CHK1 also plays a role in the correction of erroneous kinetochore-microtubule attachments and optimal spindle checkpoint signaling, suggesting that CHK1 inhibition may enhance chromosome mis-segregation and increase the activity of anti-mitotic drugs[6].

CHK1 also phosphorylates multiple downstream targets that regulate DNA replication, chromosome alignment, spindle checkpoints, and exit from cytokinesis[7]. In normal cell cycle progression, CHK1 prevents the activation of late-stage origins until near the end of S phase and stabilizes active replication forks. When CHK1 is inhibited, the number of active replication origins increases, resulting in stalled replication forks and causing DNA strand breakage and replication catastrophe[8-12]. Cells deficient in CHK1 have increased spontaneous chromosome mis-segregation and lose the ability to recruit the spindle checkpoint protein BUBR1 to kinetochores and fail to activate the spindle checkpoint in response to misaligned chromosomes[13]. This finding suggests that CHK1 is essential for stable attachment of mitotic spindles to metaphase chromosomes. It is therefore postulated that CHK1 inhibition alone can generate DNA damage and mitotic catastrophe.

As of 13 January 2017, there were 7 Eli Lilly-sponsored studies assessing prexasertib. In these studies, prexasertib has been administered to 260 patients as monotherapy and to 113 patients in combination with other treatments. Please refer to the investigator's brochure (IB) for full information. The first-in-human phase I study, I4D-MC-JTJA (JTJA), evaluated monotherapy prexasertib in patients with advanced cancer. Study I4D-MC-JTJF (JTJF) evaluated prexasertib in combination with chemotherapy or targeted agents in patients with advanced cancer.

2.1.1 Prexasertib Pre-Clinical Studies

2.1.1.1 In Vitro Biology

Prexasertib is a potent ATP-competitive inhibitor of CHK1. In an *in vitro* enzyme assay, prexasertib inhibits the activity of CHK1 with an IC_{50} of 1 nM and an inhibition binding constant (K_i) of 0.5 nM. The compound is a selective protein kinase inhibitor demonstrating an IC_{50} of less than 100 nM against only 8 out of 223 kinases in the Upstate Biotechnology kinase screening panel. Only CHK2 and the p90S6 kinases (RSKs) were inhibited by prexasertib at an IC_{50} of less than 10 nM.

As a single agent, prexasertib has potent *in vitro* effects on cells which reflects the essential protective role that CHK1 plays in DNA replication. In this regard, CHK1 functions as a negative regulator of replication origin activation; specifically, keeping late replication origins silent until late S phase. When CHK1 protein is depleted, more replication origins are activated in early S phase than the replication apparatus can tolerate, resulting in slowed and arrested DNA replication forks and DNA double-strand breakage. Chemical inhibition of CHK1 with prexasertib results in a similar outcome, namely, an initial accumulation of cells in S-phase, extensive DNA damage, premature entry into mitosis with unrepaired DNA, and induction of apoptosis. Prexasertib's activity is dependent upon inhibition of CHK1 and subsequent increase in CDC25A activation of CDK2, which results in an increase in the number of replication forks while reducing their stability. Following prexasertib treatment, cells demonstrate a rapid appearance of terminal deoxynucleotidyl transferase nick end labeling (TUNEL) and pH2AX-positive double-stranded DNA breaks in the S-phase cell population. The majority of treated mitotic nuclei consist of extensively fragmented chromosomes, indicating that prexasertib causes replication catastrophe[12]. Additionally, treatment with prexasertib causes a cellular phenotype identical to that reported in knockdown experiments utilizing CHK1 selective ribonucleic acid (RNA) interference (RNAi)[14]; HeLa cells treated with prexasertib show a clear defect in chromatin condensation and failure of the mitotic spindle to attach to chromosomes. Taken together, these results indicate that *in vitro*, prexasertib as a single agent behaves mechanistically as a DNA-damaging agent, a checkpoint inhibitor, an inhibitor of DNA replication and mitosis, and as an inducer of replication catastrophe.

The broad anti-proliferative effects of single agent prexasertib were demonstrated in various tissue culture studies whereby growth inhibition or clonogenic survival in soft agar served as phenotypic endpoints. Specifically, the evaluation of prexasertib in 395 cell lines derived from multiple cancer types and various histologies showed widespread anti-proliferative effects, with growth of 64% of these lines inhibited at IC_{50} concentrations of 50 nM or below.

Similarly, a subset of the 49 cell lines derived from squamous cell cancers (SCC) have been evaluated for response to prexasertib and these studies showed that 61% of these lines had IC_{50} values of 50 nM or below. Related studies evaluating clonogenic survival in soft agar showed that prexasertib potently inhibited survival in 44 of 54 (81%) models derived from orthotopically grown human tumors representing 16 different tumor types. The mean IC_{50} values determined in the 44 most sensitive models ranged from 2 to 33 nM.

2.1.1.2 Prexasertib Pharmacokinetic/Pharmacodynamic Models

An indirect response pharmacokinetic (PK)/pharmacodynamic (PD) model was developed in

order to link prexasertib plasma concentrations with the inhibition of phosphorylated CHK1 (pCHK1) and tumor growth response in Calu-6 xenografts following IV administration of prexasertib (monotherapy). The IC_{50} determined from the nonclinical single agent PK/PD model is 14.1 ng/mL. The PK/PD model predicts that in Calu-6 xenograft tumor models, an average pCHK1 inhibition of 49.7% (90% confidence interval [CI]: 45-54.7%) and 70.5% (90% CI: 67.5-74.5%) over the first 72-hours following prexasertib administration is correlated with the minimum and maximum tumor responses, respectively. The minimum and maximum tumor responses are defined as the smallest and largest statistically significant tumor regressions, respectively, when compared to controls.

The predicted human systemic prexasertib exposure that correlates with the minimum and maximum clinical tumor response with prexasertib monotherapy is an area under the plasma concentration-time curve from 0 – 72 hours ($AUC_{(0-72)}$) of 664 ng•hr/mL (90% CI: 353-1236 ng•hr/mL) and 1896 ng•hr/mL (90% CI: 1008-3533 ng•hr/mL), respectively.

2.1.1.3 Pre-Clinical Pharmacokinetics and Toxicokinetics

The plasma toxicokinetics of prexasertib were evaluated in Sprague-Dawley rats and beagles following single and repeated intravenous (IV) infusions administered as daily doses on 3 consecutive days per week for 3 weeks. In both rats and dogs, increases in exposures were approximately dose-proportional following single and repeat doses. No plasma accumulation was noted in either rats or dogs, and there were no sex-related differences in PKs.

Pharmacokinetic parameters of prexasertib were estimated in rats and dogs following a single IV administration of [^{14}C]LY2606368 formulated in Captisol. Prexasertib was rapidly cleared in both species and had high volumes of distribution suggesting good tissue distribution. The major metabolic routes associated with the clearance of prexasertib were based on oxidative deamination and O-demethylation in dogs and on O-demethylation in rats.

Radiolabeled studies show that hepatobiliary excretion plays a major role in the elimination of prexasertib in both rats and dogs. In rats, drug-derived radioactivity crossed both the blood brain barrier and the blood–testis barrier but at very low levels. Overall, radioactivity was selectively associated with melanin-containing tissues in rats.

2.1.1.4 Single Dose Pharmacokinetics

The PK of prexasertib and prexasertib-derived radioactivity were evaluated in Sprague-Dawley rats and beagles following an IV administration of [^{14}C]LY2606368 and summarized in **Table 1** below:

Table 1: Summary of Prexasertib Derived Radioactivity Pharmacokinetic Parameters in Rat and Dog Plasma

Species (Sex)	Sprague-Dawley Rats (Male)[a]		Beagle Dogs (Male)[b]	
Parameter	LY2606368	LY2606368-Derived Radioactivity	LY2606368 (SD)	LY2606368-Derived Radioactivity (SD)
C_{max}[c]	674	1800	644 (31)	929 (30)
$AUC_{(0-\infty)}$[d]	1480	9160	953 (84)	49200 (8000)[e]
$t_{1/2}$ (hr)	2.12	17.8	3.37 (1.54)	197 (48)[f]
V_d (L/kg)	13.6	17.1	9.85 (2.2)	21.8 (2.4)
CL (mL/min/kg)	113	18.2	69 (6.7)	1.35 (0.22)

Abbreviations: $AUC_{(0-\infty)}$ = area under the plasma concentration-time curve from the start of the infusion to infinity; C_{max} = maximum observed plasma concentration; CL = clearance; SD = standard deviation; $t_{1/2}$ = half-life; V_d = volume of distribution.

a 10 mg/kg of [^{14}C]LY2606368, 1-hour infusion, n = 3 per time point, sparse sampling in rats.

b 4 mg/kg of [^{14}C]LY2606368, 1-hour infusion, n = 3 per time point.

c ng equivalents/g or ng/mL.

d ng equivalents·hour/g or ng·hour/mL.

e A significant portion of this AUC was based on extrapolated area – last data point was at 120 hours.

f Samples were collected up to 120 hours only; therefore, this value needs to be interpreted with caution.

Concentrations of prexasertib declined rapidly in a monoexponential fashion following a single 1-hour IV infusion of [^{14}C]LY2606368 in both rats and dogs. At C_{max}, prexasertib accounted for approximately 37% and 69% of the plasma radioactivity in rats and dogs, respectively. However, the exposure ($AUC_{(0-\infty)}$) of prexasertib accounted for approximately 16% and 2% of the radioactivity exposure in plasma in rats and dogs, respectively, indicating the presence of circulating metabolites. Total radioactivity in plasma was followed by a biexponential decline with a steep alpha phase observed during the first 8 hours post start of infusion, and a relatively longer terminal elimination half-life ($t_{1/2}$) for the residual low levels of radioactivity. Metabolites appear to contribute to the longer $t_{1/2}$ of radioactivity when compared with that of prexasertib.

2.1.1.5 Multi-Dose Toxicokinetics

Prexasertib was administered as a 1-hour IV infusion to male and female Sprague-Dawley rats in doses ranging from 1 to 40 mg/kg/day on 3 consecutive days per week for 3 weeks (dosed on days 1, 2, 3, 8, 9, 10, 15, 16, and 17). Toxicokinetic parameters were calculated and are summarized in **Table 2** below:

Table 2: Toxicokinetic Parameters of Prexasertib in Rats Following a Single and Repeated 1-Hour IV Infusion Administered as Daily Doses on 3 Consecutive Days per Week for 3 Weeks

	Administered Dose of LY2606368 (mg/kg)							
Parameter	1		4		10		40	
Sex	M	F	M	F	M	F	M	F
Day 1								
C_{max} (ng/mL)	204	265	661	602	1855	1520	5245	6120
$AUC_{(0-24)}$ (ng·hr/mL)	330	319	1166	998	3405	2415	11,365	11,294
Day 17								
C_{max} (ng/mL)	209	144	729	513	1810	1317	7337	5543
$AUC_{(0-24)}$ (ng·hr/mL)	418	245	1245	1150	3636	2085	16,748	12,018

Abbreviations: $AUC_{(0-24)}$ = area under the plasma concentration-time curve from time 0 to 24 hours or last quantifiable time point; C_{max} = maximum observed plasma concentration; F = female; M = male.

Prexasertib was also administered as a 1-hour IV infusion to male and female beagles in doses ranging from 1 to 10 mg/kg/day and as a 2-hour IV infusion at a dose of 40 mg/kg/day on 3 consecutive days per week for 3 weeks (dosed on days 1, 2, 3, 8, 9, 10, 15, 16, and 17). Toxicokinetic parameters were calculated and are summarized in **Table 3**:

Table 3: Mean ± SD Toxicokinetic Parameters of Prexasertib in Dogs Following a Single and Repeated IV Infusion Administered on 3 Consecutive Days per Week for 3 Weeks

	Administered Dose of LY2606368 (mg/kg)							
Parameter	**1**		**4**		**10**		**40**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 1								
C_{max} (ng/mL)	118	227	438	496	1464[b]	1298[b]	7270	6950
C_{max} SD	11	209	33	33	252	78	5400	630
$AUC_{(0-24)}$ (ng·hr/mL)	213	262	833	1011	2918[b]	3005[b]	13,059	14,667
$AUC_{(0-24)}$ SD	12	84	68	177	471	200	10611	2281
Day 2[a]								
C_{max} (ng/mL)	n/a	n/a	n/a	n/a	n/a	n/a	5420[b]	5862[b]
C_{max} SD	n/a	n/a	n/a	n/a	n/a	n/a	725	1156
$AUC_{(0-24)}$ (ng·hr/mL)	n/a	n/a	n/a	n/a	n/a	n/a	15,285[b]	17,287[b]
$AUC_{(0-24)}$ SD	n/a	n/a	n/a	n/a	n/a	n/a	1694	1669
Day 10[a]								
C_{max} (ng/mL)	n/a	n/a	n/a	n/a	n/a	n/a	3013[c]	3238[c]
C_{max} SD	n/a	n/a	n/a	n/a	n/a	n/a	437	736
$AUC_{(0-24)}$ (ng·hr/mL)	n/a	n/a	n/a	n/a	n/a	n/a	10,723[c]	12,270[c]
$AUC_{(0-24)}$ SD	n/a	n/a	n/a	n/a	n/a	n/a	938	954
Day 17								
Cmax (ng/mL)	131	123	525	641	1432[b]	1317	n/a	n/a
C_{max} SD	12	14	56	15	48	6	n/a	n/a
$AUC_{(0-24)}$ (ng·hr/mL)	237	224	915	1124	2767[b]	2834	n/a	n/a
$AUC_{(0-24)}$ SD	19	10	99	121	323	314	n/a	n/a

Abbreviations: $AUC_{(0-24)}$ = area under the plasma concentration-time curve from time 0 to 24 hours or last quantifiable time point; C_{max} = maximum observed plasma concentration; F = female; M = male; n/a = not applicable; SD = standard deviation.

All data points represent n = 3 except where noted.

[a] Day 2 and Day 10 data represent a 2-hour infusion and only applies to the 40-mg/kg dose group.

[b] n = 5.

[c] n = 4.

For both rats and dogs, systemic exposures to prexasertib were generally similar for male and female animals on days 1 and 17. No accumulation of prexasertib in plasma was observed following multiple doses. Increases in maximum observed plasma concentration (C_{max}) and AUC from time 0 to 24 hours post-dose [$AUC_{(0-24)}$] were approximately dose-proportional on days 1 and 17.

2.1.1.6 Pre-Clinical Enzyme Induction

No indications of *in vivo* CYP induction were observed during the 3-week repeat-dose studies in rats and dogs. A slight decrease (ranging from approximately 1% to 24% in rats and 4% to 29% in dogs) in total CYP content was observed in prexasertib-treated groups compared with the vehicle-treated group.

2.1.1.7 Prexasertib Distribution

Radioactivity was extensively distributed in tissues and organs following a 1-hour IV infusion of [^{14}C]LY2606368 to pigmented male and female and non-pigmented male rats. In most tissues, peak radioactivity concentrations were observed at the end of infusion in both male and female pigmented rats. In pigmented male rats, the tissues showing the highest peak concentrations were the eye uveal tract, adrenal gland, pancreas, stomach mucosa, kidney medulla, and myocardium. In pigmented female rats, the tissues showing the highest peak concentrations were adrenal gland, pancreas, liver, stomach mucosa, exorbital lacrimal gland, and eye uveal tract.

Radioactivity concentrations in central nervous system tissues protected by the blood–brain barrier (ie, cerebellum, cerebrum, medulla, and spinal cord) were low, measurable only in brain olfactory lobe (males) and spinal cord (females) up to 2 hours and 1 hour post start of infusion, respectively. Low levels of [^{14}C]LY2606368-related radioactivity were observed in testes of pigmented and non-pigmented male rats, suggesting that low levels of drug-derived radioactivity crossed the blood–testis barrier. Furthermore, [^{14}C]LY2606368-related radioactivity was selectively associated with melanin-containing tissues.

There was some association of [^{14}C]LY2606368-related radioactivity with the cellular component of blood in dogs (blood to plasma [B:P] ratio ≥1 up to 8 hours post-dose) and a minimal association to the cellular component of blood in rats (B:P ratio <1 up to 24 hours post-dose).

2.1.1.8 Pre-Clinical Metabolism

Metabolism of prexasertib has been evaluated in both male beagle dogs and male Sprague-Dawley rats following a single 1-hour IV administration of [^{14}C]LY2606368. Overall, prexasertib and a total of 15 metabolites were detected in dogs and 13 metabolites were detected in rats.

In dogs, prexasertib was the major drug-related entity circulating in the plasma up to the 4-hour time point, and radiolabeled thiocyanate was the predominant circulating entity at all subsequent time points up to 120 hours, and is the likely contributor for the long $t_{1/2}$ illustrated in **Table 1**. Oxidative and glucuronide metabolites were also observed in plasma at the early sampling times. Oxidative metabolites along with prexasertib were the predominant excreted entities in both feces and urine. Oxidative deamination of the propylamine side chain and O-demethylation to form the corresponding phenol appear to be predominantly responsible for clearance of prexasertib. Overall, oxidative metabolism was the path of clearance of prexasertib followed by smaller contributions from renal and biliary excretion.

In rats, no quantifiable radioactive peaks were reported at or beyond the 4-hour time point, demonstrating rapid clearance of both prexasertib and related radioactivity from plasma. A glucuronide metabolite of prexasertib was the predominant drug-related circulating entity followed by prexasertib. Oxidative metabolites along with prexasertib were the major excreted entities in feces, urine, and bile. Additionally, glucuronides and acetylated metabolites were detected in both urine and bile. Formation of the O-demethylated phenol appears to be predominantly responsible for the clearance of prexasertib. Overall, oxidative metabolism and glucuronidation seems to be the major routes responsible for the clearance of prexasertib

followed by smaller contributions from renal and biliary excretion in rats.

2.1.1.9 Pre-Clinical Excretion

Following a single IV infusion in dogs, approximately 72.9% and 16.3% of the administered radioactivity was recovered in feces and urine, respectively. Radioactivity was eliminated rapidly, with approximately 84% of the administered dose recovered in the initial 48 hours post start of infusion. However, the remaining radioactivity was eliminated slowly, with measurable radioactive concentrations in excreta at all the collection intervals through 168 hours post start of infusion.

In rats with intact bile ducts, mean recoveries in feces and urine accounted for 58.6% and 34.1% of the administered dose, respectively. Approximately 85% of the dose was eliminated in the first 24 hours post start of infusion. In bile duct–cannulated rats, radioactivity recoveries in bile and urine represented approximately 45% and 37% of the administered radioactivity, respectively.

The data show that hepatobiliary excretion plays a major role in the elimination of prexasertib both in rats and in dogs.

2.1.1.10 Summary of Pre-Clinical Safety Pharmacology and Toxicology

To support human clinical studies, the toxicity profile of prexasertib has been characterized in rats and dogs through repeat-dose toxicology, safety pharmacology, and genetic toxicology studies. These studies demonstrate a toxicity profile that is considered to be monitorable and reversible.

In repeat-dose toxicity studies in rats and dogs, treatment-related findings associated with prexasertib were generally similar to those of a cytotoxic chemotherapeutic agent, with dose-limiting effects primarily related to bone marrow suppression and gastrointestinal (GI) injury. These effects were dose dependent, with hematologic effects also being shown to be schedule-dependent. In rats, treatment-related neutropenia was schedule-dependent, with more severe effects associated with 3 consecutive days compared with a single dose. On the basis of these collective studies in animals, the dose-limiting toxicity in humans was expected to be one or more of the following: myelosuppression, GI toxicity, tachycardia, and/or hypotension.

Results from the nonclinical toxicology, safety pharmacology, and genetic toxicology studies for prexasertib demonstrate a toxicity profile that is considered to be monitorable and reversible.

2.1.2 Prexasertib (LY2606368) Human Studies

As of 13 January 2017, 373 patients have been exposed to prexasertib. Please refer to the IB for complete information.

2.1.2.1 Pharmacokinetics

Prexasertib systemic exposure increased in a dose-dependent manner with consistent PK profiles across the dose range 10 to 130 mg/m^2 after single-dose and multiple-dose administration across 2 different schedules of administration in cancer patients. A population PK analysis has been conducted across all doses and days of administration in Cycles 1 and 2 (N=146). The population PK analysis determined that a linear, 3-compartment PK model best described the prexasertib PK profile. The population median CL was 82.2 L/hr, with a volume of distribution at steady-state (V_{ss}) of 1276 L, and median terminal half-life of 27 hours. The population model–predicted post-hoc individual CL values after single-dose and multiple-dose administration from both schedules of administration in Cycles 1 and 2 were consistent across the dose range 10 to 130 mg/m^2, indicating dose-independent and time-independent PK behavior. The interpatient variability varied across PK parameters of the model (percentage coefficient of variation [CV%]=27% to 73%). Please refer to the IB for complete information.

2.1.2.2 Pharmacodynamics

Blood, hair, and tissue samples were collected and analyzed for various biomarker assessments in a Lilly-sponsored trial of prexasertib. These assessments included circulating plasma DNA concentrations, circulating tumor cells, phosphohistone 2AX, and cytokeratin 18. However, due to variability in the clinical pharmacodynamic (PD) data and the corresponding lack of statistically significant changes, these assessments were not used to inform dose selection. Because the magnitude and duration of pCHK1 inhibition associated with the recommended phase 2 dose of 105 mg/m^2 in humans is not known, human PD profile simulations after administration of 105 mg/m^2 were generated by linking the human population PK model to the murine single-agent PK/PD indirect response model (that is, they assume a similar PD response in humans as that characterized in murine xenografts). Simulation results of the predicted median human percentage pCHK1 profile demonstrate that the average percentage pCHK1 inhibition over the first 72 hours of Cycle 1 after 105 mg/m^2 is 41.7% (90% CI: 25.0% to 59.5%). The predicted average percentage pCHK1 inhibition over the first 72 hours following administration of 105 mg/m^2 once every 14 days approaches the average percentage pCHK1 inhibition of 49.7% (90% CI: 45.0% to 54.7%) over the first 72 hours following prexasertib administration described for the minimum tumor response in nonclinical xenograft models. However, the predictive capability of this approach has limitations given the differences between animal models and humans (e.g., protein binding, tumor size/location, target expression, immune system, and prior therapies in humans). As a result, the specific magnitude and duration of pCHK1 inhibition required for optimal clinical activity are currently not known.

2.1.2.3 Clinical Metabolism

Exploratory analysis (non-radiolabeled) of plasma and urine samples collected from 3 patients following the administration of a single 50 mg/m^2 dose of prexasertib showed the presence of prexasertib as the major entity (prexasertib appeared as the peak with the most intense mass spectrometric response) in both plasma as well as urine, along with multiple minor oxidative and glucuronide metabolite peaks.

2.1.2.4 Clinical Drug Interactions

The potential for drug interactions was evaluated in human liver microsomes. Prexasertib (LY2606368) exhibited *in vitro* potential for competitive inhibition of CYP1A2 (K_i = 1.6 μM = 767 ng/mL) and of CYP2D6 (K_i = 2.8 μM = 1343 ng/mL). There was no inhibition of CYP2C8, CYP2C9, CYP2C19, CYP2B6, or CYP3A4 over a range of concentrations (0.103 - 25 μM). Furthermore, potential time-plus nicotinamide adenine dinucleotide phosphate (NADPH)-dependent inhibition by prexasertib was not observed for any CYPs over the concentration range tested (0.103 to 25 μM).

Simulations of human PK profiles that were performed using a dynamic physiologically based pharmacokinetic (PBPK) model (SimCYP®), suggest that the potential for a clinical drug interaction is minimal. More specifically, simulations of drug-drug interaction (DDI) studies with prexasertib and sensitive CYP2D6 probe substrates (desipramine and dextromethorphan) did not predict any clinically significant DDIs with either substrate. The increase in CYP2D6 substrate AUC and C_{max} ratios were < 1.2 for both substrates at the recommended prexasertib phase II dose of 105 mg/m^2, when administered either as a single dose or following daily administration over three consecutive days.

Similarly, human simulations of DDI studies with prexasertib and sensitive CYP1A2 probe substrates (phenacetin and theophylline) did not predict any clinically significant DDIs with either one of the substrates. The increase in CYP1A2 substrate AUC and C_{max} ratios were < 1.2 for both substrates at the highest proposed prexasertib phase II dose of 105 mg/m^2, when administered either as a single dose or following daily administration over three consecutive days.

The effect of prexasertib on CYP mRNA levels was evaluated using cryopreserved human hepatocytes from a single donor. There was no induction of CYP1A2, CYP2B6 or CYP3A4 mRNA observed at concentrations corresponding to current clinical C_{max} concentrations. However, concentration dependent down regulation of CYP3A4 mRNA was observed *in vitro* with no loss or change in CYP3A4 activity. The mechanism of this modulation and its clinical relevance has not been evaluated. The likelihood of this finding manifesting *in vivo* is further minimized given the bi-weekly dosing regimen.

The relative contributions of microsomal CYP-mediated clearance of prexasertib was studied *in vitro* using a panel of recombinant human cytochrome P450s (rCYPs): rCYP1A2, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C19, rCYP2D6, rCYP2E1, rCYP2J2, rCYP3A4, and rCYP3A5. Based on a substrate depletion approach, CYP1A2, CYP2D6, and CYP2J2 are responsible for 88%, 9.6% and 2.8% of hepatic CYP-mediated clearance of prexasertib, respectively, with other CYPs not appearing to be involved in the metabolism of prexasertib. Although CYP1A2 seems to be the major CYP involved in the *in vitro* clearance of prexasertib, the relative contributions of other non-CYP elimination pathways as well as the *in vivo* clearance pathways have not been evaluated.

Overall, *in vitro* data showed that prexasertib was 63.1 to 65.3% protein-bound in human plasma over the concentration range of 0.5 to 50 μM and 48.8 to 54.6% protein-bound in human liver

microsomes over the concentration range of 1 to 10 μM.

2.1.2.5 Clinical Safety Evaluation of Prexasertib

Please refer to the prexasertib IB for complete safety background information. Neutropenia has been the most frequent and severe toxicity after treatment with 105 mg/m^2 prexasertib every 14 days. Grade ≥4 decreases in neutrophils deemed related to prexasertib treatment have been observed in 57.7% of patients. In general, neutrophil counts reach nadir on approximately Day 8, and Grade 4 decreases are often transient (e.g., less than 5 days). Febrile neutropenia has been reported in 10.8% of patients, but no patients have discontinued or died because of febrile neutropenia. Related Grade ≥4 decreases in platelets have been observed in 5.2% of patients, and 3 patients (1.4%) had clinically significant bleeding (epistaxis and hematochezia) in the setting of thrombocytopenia. No patients experienced Grade ≥4 anemia deemed related to prexasertib treatment.

Non-hematologic toxicity occurs at a lower frequency than hematologic toxicity and has been predominantly Grade 1 or 2 in severity. Fatigue and nausea are the only non-hematologic AEs that are considered to be related to study drug treatment occurring in more than 10% of patients. The non-hematologic treatment-emergent AEs (TEAEs) occurring in greater than 5% of patients treated at 105 mg/m^2 every 14 days and deemed by the investigators to be related to prexasertib treatment were fatigue (25.8%), nausea (11.7%), decreased appetite (8.5%), diarrhea (7.0%), and headache (5.6%).

2.1.2.6 Serious Adverse Events

Cumulatively, 360 serious adverse events (SAEs) have been reported in 193 patients in 6 Lilly-sponsored and 3 Investigator-sponsored monotherapy or combination studies up to 14 January 2017. In the 373 patients treated in the Lilly-sponsored studies, 289 SAEs in 159 patients have been reported. Out of 289 SAEs, 117 events were assessed as being possibly related to study drug treatment. The majority of drug-related events (76 events [65%]) were hematologic toxicities including neutropenia, leukopenia, anemia, febrile neutropenia, thrombocytopenia, or pancytopenia. Of the remaining related events, 14 were complications occurring in the setting of hematologic toxicity such as infections (10 events), bleeding events (3 events), or pyrexia (1 event).

2.1.2.7 Deaths

As of 13 January 2017, no patients had died due to adverse events (AEs) deemed related to study treatment. Please refer to the IB for comprehensive prexasertib background information.

2.2 Study Disease

In cancer cells, uncontrolled cell growth and sustained proliferative signaling can result in replication stress. Replication stress is associated with several genomic alterations that drive unbridled S phase entry, resulting in error prone replication, nucleotide or replication factor

depletion and the slowing or stalling of replication forks [15-17]. These genomic alterations include *MYC* amplification [15], cyclin E gene (*CCNE1*) amplification [18], and loss of the retinoblastoma protein (*Rb*) [19]. Additionally, mutations in the E3 ubiquitin ligase *FBXW7* can stabilize MYC, cyclin E, and other replication stress-inducing oncoproteins [20,21].

MYC amplification is involved broadly in many cancers; *MYC* over-expression results in increased proliferation, cellular survival and angiogenesis, promoting tumor growth[22]. High levels of *MYC* have been linked to both aggressive prostate and triple-negative breast cancer (TNBC)[23]. *CCNE1* amplification is the most frequent amplified gene in ovarian serous carcinomas and is correlated with poor prognosis and primary treatment resistance. *CCNE1* amplification is also associated with disease progression and poor clinical outcome in breast, bladder, and colorectal carcinoma[24]. *Rb* loss is associated with approximately 40% of TNBCs that harbor a p53 mutation (estimated to occur in > 80% of all TNBC patients)[25].

In addition to replication stress, uncontrolled cell cycle progression in cancer cells results in double strand DNA breaks. HR repair is essential for repair of this type of DNA damage and the maintenance of genomic stability and deficiency in HR repair is implicated in tumorigenesis. Mutations in HR associated genes have been identified in a number of cancers[26-30]. Germline or somatic mutations in HR genes, including *BRCA 1/2*, *RAD51C*, *RAD51D*, and *ATM*, are present in nearly 1/3 of all ovarian carcinomas (including both serous and non-serous histologies). Germline mutations among this group are frequently associated with familial forms of breast and pancreatic cancers[30,31].

2.3 Replicative Stress and HR Deficiency Cohort Rationale

Inhibitors of CHK1 have been shown to sensitize cells to DNA damaging agents and have been under development in combination with chemotherapy[2]. However, there is recent interest in defining the monotherapy vulnerabilities of these agents. Emerging data suggest that when undergoing severe replication stress, cells require CHK1 for stabilizing replication forks[32-35] and to promote homologous recombination (HR) repair via the phosphorylation and activation of HR associated proteins (e.g., *RAD51* and Fanconi anemia proteins) for repair of DNA strand breaks during replication[3,36-38], therefore inhibition of CHK1 may be lethal to these cells. In cells with HR deficiency, there is increased intrinsic DNA damage that activates the ATR-CHK1 pathway for checkpoint control. Inhibition of CHK1 in HR deficient cells will disrupt checkpoint control, allowing for continued cell cycle progression without DNA repair and resulting in mitotic catastrophe. Inhibition of CHK1 activity itself also leads to replication stress, with an increase in DNA replication initiation and an associated increase in RPA protein, single-stranded DNA formation, and DNA strand breaks[9,39]. HR-deficient cells cannot faithfully repair such strand breaks, resulting in cell death. In addition, it has been shown that severe replication stress and HR repair deficiency are synthetically lethal[40]. These observations suggest that cancer cells under replication stress or those with HR deficiency may be susceptible to CHK1 inhibitor monothera
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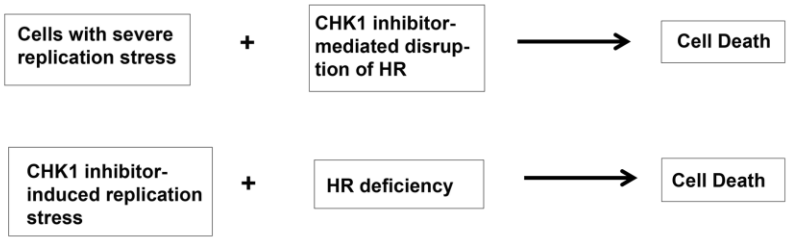


Clinical and preclinical studies support this hypothesis that CHK1 inhibition may be clinically relevant in patients with tumors exhibiting replication stress or HR deficiency.

In a retrospective analyses performed during assessment of prexasertib in anal cell carcinoma and head and neck cancer populations, patients with tumors harboring *FBXW7* mutations achieved partial response or stable disease (Lilly Data on File). Additionally, breast, ovarian and endometrial cancer cell lines harboring *CCNE1* amplification have been found to be sensitive to prexasertib, with the most sensitive cell line (KLE endometrial cancer) harboring both *CCNE1* amplification and *FBXW7* mutation. Taken together, these data suggest that cells with replication stress will have vulnerability to CHK1 inhibitor monotherapy, with CHK1 inhibition leading to destabilization of replication forks and disrupted HR, events critical to the survival of cells that with severe replication stress.

In the clinical setting, patients with anal and head and neck carcinomas harboring HR deficiency associated mutations (e.g., mutations in *BRCA1/2, MRE11,* or FANCC) were among those responding to treatment. Prior pre-clinical work performed in the D'Andrea laboratory examined the effect of CHK1 inhibition in model systems exhibiting defects in the Fanconi Anemia (FA) DNA repair pathway (which includes *BRCA1/2*). These studies found that FA-deficient cell lines were hypersensitive to CHK1 inhibition by siRNAs targeting CHK1 and pharmacologic inhibition by the CHK1 inhibitors Gö6976 and UCN-01[41]. An siRNA screen designed to identify genes synthetically lethal with CHK1 inhibition was also carried out, identifying genes required for FA pathway function. These findings were confirmed *in vivo*, with whole zebrafish embryos depleted for FANCD2 demonstrating hypersensitivity to Gö6976. There was also an increased accumulation of DNA strand and chromosomal breaks when CHK1 was inactivated in FA-deficient cell lines. These results suggest that CHK1 and the FA pathway mutually compensate in maintaining genome integrity.

2.4 *CCNE1* Cohort Rationale

As of 5 December 2017, a total of 15 participants have been enrolled to the trial. Ten participants were enrolled to the replicative stress cohort during the first enrollment stage (one participant was replaced after failing to complete the first cycle of dosing). Of these ten, one participant with *CCNE1* amplified cholangiocarcinoma exhibited stable disease per RECIST 1.1 criteria and has successfully initiated his 14th cycle of therapy. The patient's tumor was found to harbor 113 copies of *CCNE1*.

No other participants met the 4 month progression-free target for expansion, and the replicative stress cohort is now closed to enrollment. Only one other participant with *CCNE1* amplification was enrolled to the cohort, a patient with esophageal adenocarcinoma with 25 copies of *CCNE1*.

This participant had a mixed response on their first restaging scan but was ultimately determined to have progressed. The remaining participants had *MYC* amplifications (n=4), *FBXW7* mutations (n=3), and a *SETD2* deletion (n=1).

CCNE1 plays a key role in cell proliferation by binding to cyclin dependent kinases (CDKs), chiefly CDK2, in late G1 phase of the cell cycle[42]. Cyclin E1/CDK2 facilitates G1/S phase transition through phosphorylation of downstream targets including Rb protein. In normal cells, cyclin E1 accumulates at the G1/S phase boundary and is degraded as the cell passes through S phase. This periodicity is regulated by cell-cycle dependent transcription and post-translational protein turnover by ubiquitin-dependent proteolysis. Cyclin E1/CDK2 is frequently dysregulated in cancer, including ovarian, breast, colorectal, and bladder cancers, where overexpression of cyclin E is associated with a poor prognosis[43-47].

In ovarian cancer, high level CCNE1 expression is associated with increased tumor grade[48]. Dr. David Bowtell's group at Peter MacCallum Cancer Center[49,50] and others[48,51-53] have shown that *CCNE1* amplification and overexpression are associated with reduced overall survival in high grade serous ovarian cancers (HGSOC). Preclinical work performed in the Center for DNA Damage and DNA Repair (CDDR) at Dana-Farber Cancer Institute has confirmed that prexasertib exhibits monotherapy anti-tumor activity across both ovarian cell lines (**Figure 1**) and patient-derived xenograft (PDX) models that exhibit increased *CCNE1* copy number (**Figure 2**) (unpublished data). The Cancer Genome Atlas (TCGA) project reported that *CCNE1* amplification is mutually exclusive with *BRCA1/2* germline mutations, suggesting that patients with *CCNE1* amplification are less likely to benefit from treatments such as poly (ADP-ribose) polymerase (PARP) inhibitors and will require novel therapeutic approaches[40,47].

The observed clinical response to therapy combined with the preclinical data gives justification to examine prexasertib in a small cohort of *CCNE1* amplified patients. Although increased *CCNE1* copy number can lead to increased cyclin E protein expression, this is not a universal finding. As **Figure 3** demonstrates, we can expect that 50% of tumors will have high cyclin E protein levels in the presence of any degree of *CCNE1* amplification (unpublished data). Our collaborators have further evaluated the *CCNE1* amplified group to see if a certain degree of gene amplification correlates with high cyclin E protein expression. Based on this data, we are proposing to establish 6-fold *CCNE1* amplification as the minimum required to be enrolled in the *CCNE1* amplified cohort as it will give the highest probability of treating tumors with high cyclin E protein expression, but will allow consideration of borderline participants following consultation with the overall principal investigator.

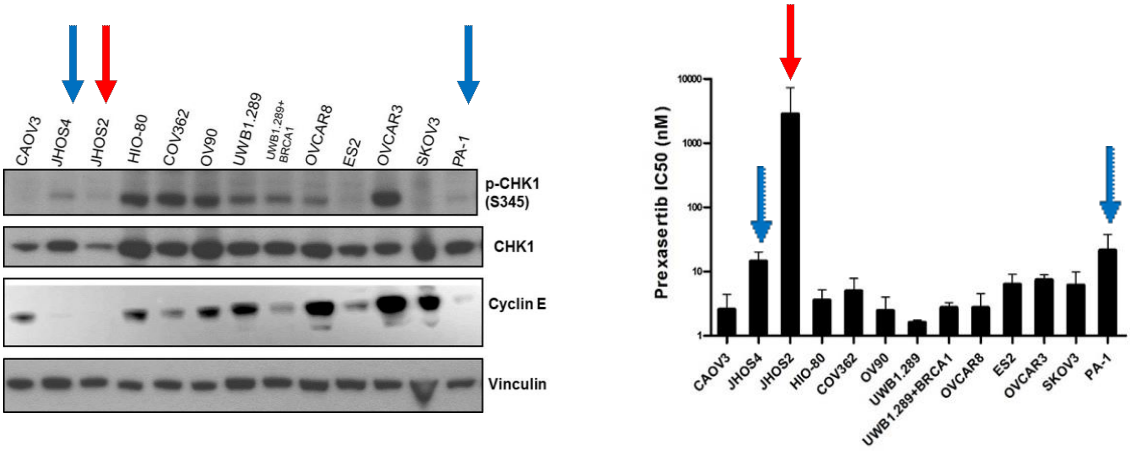


Figure 1: Cyclin E protein levels are low in prexasertib-resistant ovarian cancer cell lines. Cells exposed to prexasertib or DMSO for 72 hours in 96 well plates and survival was determined using CellTiter Glo reagent. IC_{50} values for prexasertib were high in ovarian cancer cell lines with low levels of cyclin E proteins. *Shapiro and D'Andrea, CDDR, unpublished data.*

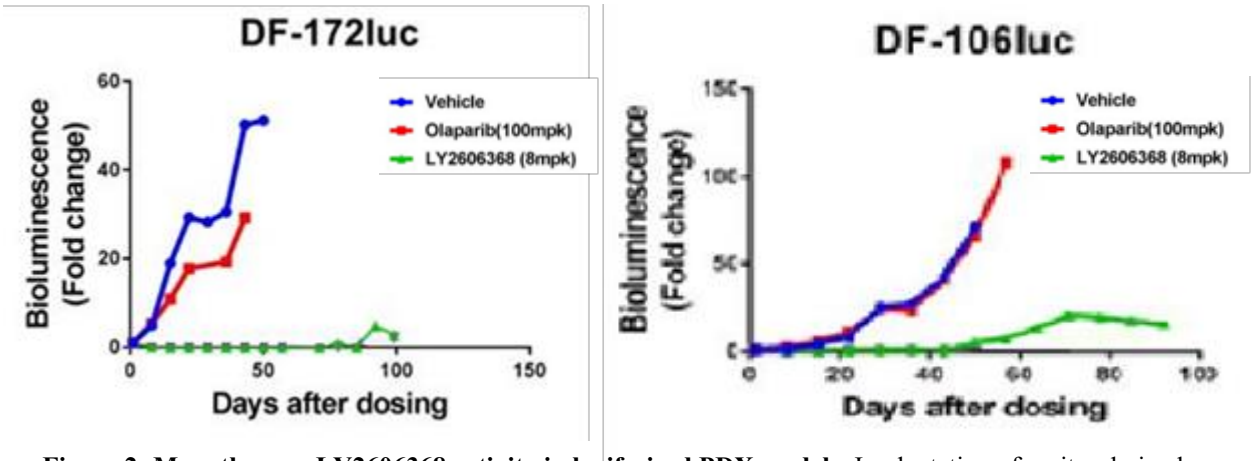


Figure 2: Monotherapy LY2606368 activity in luciferized PDX models. Implantation of ascites derived cells, ~5 x 10^6 for each of the PDX types in NSG mice, 5 mice per each PDX. **DF-172luc:** mixed serous and endometroid histologic subtype post 2 prior lines of chemotherapy, with high cyclin E copy number, and *TP53, RET,* and *RAD51C* mutations. **DF-106luc:** HGSOC histologic subtype post 1 prior line of chemotherapy, with high cyclin E copy number, *TP53* mutation, and *CDKN2A* loss. *Shapiro and D'Andrea, CDDR, unpublished data.*

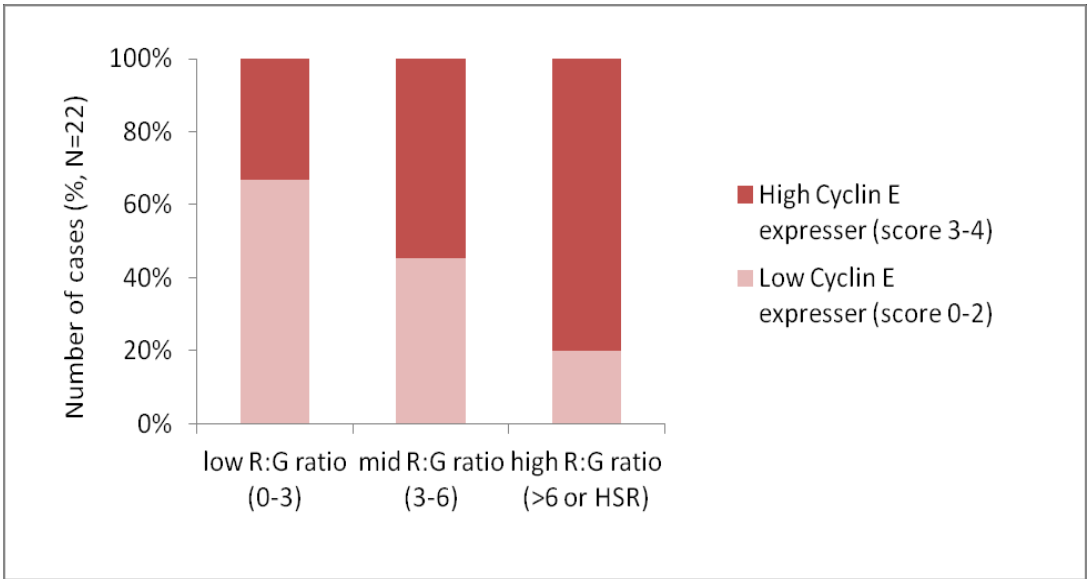


Figure 3: Six-fold *CCNE1* amplification is associated with high cyclin E expression within tumor cells. Degree of cyclin E protein expression for non-amplified, moderately amplified and highly amplified *CCNE1*. 80% of tumors with 6-fold or greater *CCNE1* gene amplification were highly expressed cyclin E protein, compared with 25% and 50% of tumors with low or moderate amplification respectively.

2.5 Correlative Studies Background

Use of targeted therapies in patients with genomically complicated tumors presents a variety of confounding variables that could contribute to success or failure of the therapy. Correlative studies are designed to both enable better understanding of the possible reasons that prexasertib (LY2606368) may prove to be effective or ineffective in individual patients and to enable us to develop more effective targeting strategies in the future.

2.5.1 Collection of Fresh Tumor Tissue – Replicative Stress and HR Cohorts

In order to retrospectively explore the determinants of response and resistance to prexasertib, a mandatory pre-treatment fresh tumor biopsy will be obtained from the patients during Stage 1 of the 2-stage trial. The nine biopsies from both cohorts will be sufficient to demonstrate meaningful results, however will allow patients who are not amenable to biopsy or for whom a biopsy would pose significant risk to enroll on trial at the later stage.

Analysis of biopsy samples taken at distinct points enables addressing of key questions including the genomic lesions present in samples studied and their potential impact upon response following prexasertib therapy.

It is the anticipation that most patients on this study will have had their tumor genomically characterized by either the OncoPanel test at DFCI/BWH (a custom hybrid capture panel which via next-generation sequencing will identify mutations and copy-number alterations in cancer associated genes) or via another CLIA-certified method at the time of enrollment. A pre-

treatment tumor tissue sample will be subjected to WES at the Broad Institute Center for Cancer Precision Medicine. WES enables a comprehensive analysis of DNA mutation in human tumor samples. Gene expression profiling has been demonstrated to predict disease-free and overall survival in multiple cancers and guide future treatment decisions and biologic discovery[54-56]. WES will be performed to profile the HR proficiency of the tumor, and will involve up to 100 genes of interest in DNA repair in order to obtain the entire HR state. This will allow identification of further genomic alterations that may occur outside of the pre-set enrollment genetic alterations of these patients. The data obtained via WES will enable us to identify secondary alterations that may impact clinical response.

2.5.2 Collection of Blood – All Cohorts

Cell-free DNA (cfDNA) will be collected and analyzed for mutations associated with the replicative stress and HR deficiency pathways. Levels of tumor DNA will decline longitudinally over time if there is a response to the treatment administered. To further assess this concept, plasma will be collected from participants to evaluate whether circulating free plasma DNA declines with the administration of prexasertib and this information will be compared to clinical outcome.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Baseline evaluations are to be conducted within 14 days prior to start of protocol therapy, with the exception of the informed consent and baseline tumor imaging which may be obtained up to 28 days prior to the start of protocol therapy. Participants must meet the following criteria to be eligible to participate in the study:

3.1.1 Participants must have a pathologically confirmed advanced solid tumor for which standard therapy proven to provide clinical benefit does not exist or is no longer effective.

3.1.2 Participants must have one of the following (confirmed via targeted NextGen Sequencing using the DFCI/BWH OncoPanel or another CLIA-certified method):

- For the replicative stress cohort: *MYC* amplification, *CCNE1* amplification, *Rb* loss, *FBXW7* mutation, or another genomic abnormality indicative of replicative stress as agreed upon with the principal investigator.

  -OR-
- For the HR deficiency cohort: genomic or somatic mutation in *BRCA1*, *BRCA2*, *PALB2*, *RAD51C*, *RAD51D*, *ATR, ATM, CHK2,* the Fanconi anemia pathway genes, or another genomic or somatic mutation in a known HR gene as agreed upon with the principal investigator.

  -OR-
- For enrollment to the *CCNE1* cohort: *CCNE1* amplification of 6-fold or greater.

Patients with borderline amplification levels may be considered following approval from the overall principal investigator.

3.1.3 Participants must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥ 20 mm with conventional techniques or as ≥ 10 mm with spiral CT scan, MRI, or calipers by clinical exam. See **Section 11** for the evaluation of measurable disease.

3.1.4 Age ≥ 18 years. As no dosing or adverse event data are currently available in participants < 18 years of age, children are excluded from this study but will be eligible for future pediatric trials.

3.1.5 ECOG performance status < 2 (see **Appendix A**).

3.1.6 Participants must have adequate organ and marrow function as defined below:

– Absolute neutrophil count	≥ 1.5 K/uL
– Platelet count	≥ 100 K/uL
– Hemoglobin	≥ 9 g/dL (with or without transfusion support)
– Total bilirubin	≤ 1.5 × institutional upper limit of normal (ULN)
– AST(SGOT)/ALT(SGPT)	≤ 2.5 × institutional ULN, unless liver metastases are present and then ≤ 5 × institutional ULN is acceptable
– Serum creatinine	≤ 1.5 × institutional ULN

3.1.7 Participants enrolling to the HR or replicative stress cohorts during Stage 1 must have disease that is amenable to biopsy and be willing to undergo a pre-treatment tumor biopsy.

3.1.8 The potential effects of prexasertib (LY2606368) use during pregnancy and lactation are not known. Nonclinical studies of prexasertib on pregnancy and fetal development have not been performed. To minimize any potential risks, men and women with reproductive potential should use medically approved contraceptive precautions during treatment and for 3 months following the last dose of prexasertib. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and for 3 months after completion of prexasertib administration.

3.1.9 Ability to understand and the willingness to sign a written informed consent document.

3.1.10 QTcF value of ≤ 470 msec on screening electrocardiogram (EKG).

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions will not be eligible to participate

in the study:

3.2.1 Participants who have had chemotherapy, other investigational or biologic therapy, major surgery, or radiotherapy within 3 weeks (6 weeks for nitrosoureas or mitomycin C) prior to the planned first dose of prexasertib (LY2606368) therapy.

3.2.2 Participants who have not recovered to eligibility levels from prior toxicity or adverse events as a result of previous treatment prior to the study.

3.2.3 Participants who have received prior treatment with a CHK1 inhibitor.

3.2.4 Participants who have received prior radiation therapy to > 25% of the bone marrow.

3.2.5 Participants with known untreated brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Participants with a history of brain metastases that have been treated, are no longer taking corticosteroids, and have been stable on imaging for at least one month following the end of treatment are permitted.

3.2.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to prexasertib.

3.2.7 Participants with a personal or family history of long QT syndrome.

3.2.8 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, NYHA Class III/IV heart failure, unstable angina pectoris, cardiac arrhythmia, myocardial infarction within 3 months of enrollment, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.9 Pregnant or breastfeeding females. The potential effects of prexasertib use during pregnancy and breastfeeding are not known and prexasertib has the potential for teratogenic or abortifacient effects.

3.2.10 Known HIV-positive participants are ineligible because these participants are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in HIV-positive participants when indicated.

3.2.11 Participants enrolling to the HR or replicative stress cohort during Stage 1 may not be on anticoagulant therapy unless the treating investigator has deemed it safe to temporarily hold to facilitate the pre-treatment tumor biopsy.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

5. TREATMENT PLAN

5.1 Treatment Regimen

Prexasertib (LY2606368) will be administered on day 1 and day 15 of a 28 day cycle. Prexasertib will be given at the recommended phase II dose of 105 mg/m^2. Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in **Section 7**. Appropriate dose modifications are described in **Section 6**. No other investigational or commercial agents or therapies may be administered with the intent to treat the participant's malignancy, with the exception of palliative radiation therapy to non-target lesions which may be administered with the principal investigator's agreement. Maintenance hormonal therapy for breast or prostate cancer patients will be permitted as long as the therapy was started prior to the initiation of the study medication. Bisphosphonate use is permitted.

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1 Infusion

Patients who completed screening assessments > 72 hours prior to cycle 1 day 1 should

have cycle 1 day 1 laboratory values that re-meet eligibility criteria. If screening assessments were completed ≤ 72 hours prior to cycle 1 day 1, laboratory tests do not need to be repeated on cycle 1 day 1 and the screening laboratory values can be used as the cycle 1 day 1 values.

5.2.2 Subsequent Infusions

Please refer to **Section 6** for toxicity management guidelines.

5.3 Agent Administration

5.3.1 Administration Instructions

Prexasertib (LY2606368) will be given at the recommended phase II dose of 105 mg/m^2 and should be administered IV over 1 hour (+/- 5 minute infusion window) using a central or free flowing peripheral IV line with an in-line, sterile, non-pyrogenic, 0.22-µm filter. Prexasertib should not come in contact with normal saline or Lactated Ringer's solution; the infusion line should be flushed with 5% dextrose injection (D5W) before and after prexasertib administration. The infusion should be completed within 4 hours of priming the infusion set.

Prexasertib should be handled according to standard procedures and precautions consistent with a cytotoxic anticancer drug.

Body surface area (BSA) should be calculated per institutional standards. Dose recalculation for changes in participant weight should be made in accordance with institutional standards.

5.3.2 Hypersensitivity Reactions

Prexasertib mesylate monohydrate is formulated in Captisol® (sulfobutylether-β-cyclodextrin [SBECD]), which has been associated with allergic reactions. The etiology of the hypersensitivity reactions observed with prexasertib mesylate monohydrate is not known.

The patient should be monitored for signs and symptoms indicative of an infusion-related reaction for the duration of the infusion (including nausea, dizziness, chest pain, diaphoresis, flushing, decreased blood pressure, shortness of breath, hypoxia, and tachycardia). In the event of a suspected infusion reaction, institutional guidelines for managing infusion reactions should be followed. For subsequent infusions, premedication with diphenhydramine hydrochloride (dosing per investigator discretion) and/or other premedication as per institutional standards or investigator discretion may be administered. The infusion rate may also be decreased by 50 percent.

5.3.3 Serotonin Syndrome

In non-clinical assessments, prexasertib interacts with multiple 5-hydroxytryptamine (5-HT) receptors. Patients should be monitored for symptoms of serotonin syndrome, including autonomic changes (tachycardia, mydriasis, diaphoresis, or body temperature increases), mental status changes (restlessness or anxiety), and neurological hyperexcitability (tremor, hyperreflexia, or myoclonus).

If observed, appropriate treatment (e.g., cyproheptadine or octreotide) may be used as a treatment. As of 13 January 2017, serotonin syndrome had not been reported for any patient receiving prexasertib.

5.4 General Concomitant Medication and Supportive Care Guidelines

Investigators should use appropriate supportive medications to address toxicities that arise during the study, including but not limited to antiemetics, antidiarrheals, and blood product transfusion.

5.4.1 Antibiotic and Granulocyte Colony Stimulating Factor Administration

Prophylactic antibiotics should be considered for use in patients that have experienced febrile neutropenia or patients deemed at higher risk for febrile neutropenia by the treating investigator.

Prophylactic Granulocyte Colony Stimulating Factor (G-CSF) such as filgrastim (or equivalent), as well as pegfilgrastim (or equivalent), are permitted in accordance with the American Society of Clinical Oncology (ASCO) guidelines. Primary or secondary prophylactic G-CSF support is permitted.

5.4.2 Medications Known or Suspected to cause Prolonged QTc Intervals or Torsades de Pointes

On days when prexasertib (LY2606368) is administered, patients should avoid taking multiple concomitant medications that are known or suspected to cause prolonged QTc or Torsades de Pointes (see **Appendix B**). If possible, alternative agents should be considered.

5.4.3 Other Anticancer Therapy

No other investigational or commercial agents or therapies may be administered with the intent to treat the participant's malignancy, with the exception of palliative radiation therapy to non-target lesions which may be administered with the principal investigator’s agreement. Bisphosphonates are permitted. Maintenance hormonal therapy for breast or prostate cancer patients will be permitted as long as the therapy was started prior to the initiation of the study medication. Consideration of other medications for pre-existing conditions may be approved on a case-by-case basis by the overall principal investigator.

5.4.4 Other Concomitant Medication Guidelines

The potential risk of a clinical DDI with either CYP1A2 or CYP2D6 substrates is minimal. Therefore, there are no recommended restrictions on CYP1A2 or CYP2D6 substrates.

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression, and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue indefinitely or until one of the following criteria applies:

- Disease progression (unless the participant is exhibiting clinical benefit as agreed upon with the principal investigator)
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Pregnancy
- Participant demonstrates an inability or unwillingness to comply with the trial regimen
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

The research team will update the relevant Off Treatment information in OnCore.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Geoffrey Shapiro, MD, PhD at (617) 632-4942.

5.6 Duration of Follow Up

Participants will be followed for serious adverse events (SAEs) for 30 days after removal from protocol therapy or until death, whichever occurs first. Participants will be followed for survival status only until death after removal from protocol therapy. Survival follow up will be performed

by review of the medical record, contact with care providers, and/or telephone contact as needed every 3 to 4 months.

5.7 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the CRF.

The research team will update the relevant Off Study information in OnCore.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following sections. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 Expected Toxicities

The table below summarizes treatment-emergent AEs considered expected for prexasertib (LY2606368) serious adverse reaction reporting.

Table 4: Toxicities Considered Expected

System Organ Class	Event Preferred Term
Blood and lymphatic system disorders	Neutropenia *(including neutrophil count decreased)*
	Thrombocytopenia *(including platelet count decreased)*
	Anemia *(including hemoglobin and hematocrit decreased)*
	Leukopenia *(including white blood cell count decreased)*
	Febrile neutropenia
	Lymphopenia *(including lymphocyte count decreased)*
Infections and infestations	Sepsis *(including urosepsis)*

6.2 Specific Toxicity Management

6.2.1 Hematologic Toxicity Management

6.2.1.1 Neutropenia

Neutropenia has been the most frequent and severe toxicity following treatment with prexasertib (LY2606368) every 14 days, with grade 3 or greater decreases in neutrophil count deemed related to prexasertib treatment having been observed in approximately three-quarters of patients. In general, neutrophil counts nadir on day 8 and grade 4 decreases are often transient (e.g., less than 5 days). Febrile neutropenia has been reported in approximately 11% of patients, but no patients have died due to febrile neutropenia. Please refer to **Section 5.4.1** for G-CSF guidelines.

Table 5: Management of Neutropenia

Toxicity	Prexasertib Dose Management
Uncomplicated Grade 3 Neutropenia (*ANC < 1 K/uL*)	Hold infusion until recovery to grade 2, resume at same dose level.
Grade 3 Neutropenia Associated with a Fever or Infection (*ANC < 1 K/uL*)	Hold infusion until recovery to grade 2, resume with one dose level reduction.
Uncomplicated Grade 4 Neutropenia < 7 Consecutive Days in Duration (*ANC < 0.5 K/uL*)	Hold infusion until recovery to grade 2, resume at same dose level.
Grade 4 Neutropenia ≥ 7 Consecutive Days in Duration (*ANC < 0.5 K/uL*)	Hold infusion until recovery to grade 2, resume with one dose level reduction.

6.2.1.2 Thrombocytopenia

Grade 3 or greater thrombocytopenia has been observed in approximately one fifth of patients.

Table 6: Management of Thrombocytopenia	
Toxicity	**Prexasertib Dose Management**
Grade 3 Platelet Count Decrease - Not Associated with Bleeding (*Platelet count 25 - 50 K/uL*)	Hold infusion until recovery to grade 2, resume at same dose level.
Grade 3 Platelet Count Decrease - Associated with Bleeding (*Platelet count 25 - 50 K/uL*) ***OR*** **Grade 4 Platelet Count Decrease** (*Platelet count < 25 K/uL*)	Hold infusion until recovery to grade 2, resume with one dose level reduction.

6.2.1.3 Anemia

Patients should receive blood transfusions as deemed necessary in the judgment of the treating investigator. Participants may be dose reduced for anemia ≥ grade 2 and considered at least possibly related to prexasertib if the treating investigator believes it to be in the best interest of the participant.

6.2.2 Non-hematologic Toxicity Management

6.2.2.1 Fatigue

Participants experiencing ≥ grade 3 fatigue should have their prexasertib (LY2606368) infusion held until recovery to ≤ grade 2. Upon resuming, the prexasertib dose should be reduced to the next lowest dose level unless the fatigue was attributed to anemia and has resolved following transfusion. In that case, the participant may be maintained at their current dose level if the treating investigator feels it is in the participant's best interest.

Participants with intolerable grade 2 fatigue may have their prexasertib held and/or reduced to the next lowest dose level if it is considered to be in their best interest by the treating investigator.

6.2.2.2 Nausea/vomiting

Patients experiencing nausea or vomiting should be offered appropriate supportive care, including the use of antiemetic therapy and IV hydration if needed. Participants experiencing ≥ grade 3 nausea or vomiting (or intolerable grade 2) despite optimal medical management should have prexasertib dose-reduced to the next lowest dose level.

6.2.2.3 Management of Other Non-hematological Toxicity

For any other ≥ grade 3 non-hematological toxicity which cannot be managed despite optimal supportive care, prexasertib should be held until recovery to ≤ grade 1 or baseline. Upon resuming, prexasertib should be dose-reduced to the next lowest dose level. Exceptions include:

- Photosensitivity, rash, or neuropathy, in which cases prexasertib may be resumed upon resolution to grade 2 if deemed appropriate by the treating investigator.
- Non-clinically significant laboratory abnormalities. Prexasertib may be continued without interruption at the current dose level if deemed appropriate by the treating investigator, or may be held and/or dose reduced at the treating investigator's discretion.
- Asymptomatic laboratory abnormalities that resolve to ≤ grade 1 or baseline within 48 hours of repletion. Prexasertib may be resumed at the current dose level if deemed appropriate by the treating investigator, or may be dose reduced at the treating investigator's discretion. Repletion and treatment may occur on the same day.

Patients experiencing intolerable grade 2 toxicities may have their infusion of prexasertib delayed at the treating investigator's discretion. Upon resolution, the dose of prexasertib may be reduced if the treating investigator considers a dose reduction appropriate.

6.3 Dose Modifications/Delays

Participants who are benefiting from prexasertib (LY2606368) in the opinion of the treating investigator may have their infusion held for up to 4 weeks to allow for recovery of toxicity. Participants requiring a longer hold should be removed from the trial. Exceptions to this requirement are possible should the principal investigator agree that the patient may continue despite the length of time off drug.

6.3.1 Dose Modifications

A maximum of two dose reductions are allowed. If a patient requires more than two dose reductions, they should be removed from the trial. Once a patient's dose has been reduced, it may be re-escalated to the previous dose level if the toxicity has resolved to baseline or ≤ grade 1 and with agreement from the principal investigator.

Table 7: Dose Modifications	
Dose Reduction Level	**Prexasertib Dose**
-1	80 mg/m^2
-2	60 mg/m^2

If the study medication is placed on hold for toxicity, the counting of cycle days and assessment schedule will continue without interruption. For example, a patient who does not receive their prexasertib (LY2606368) infusion on cycle 2 day 15 due to toxicity will proceed with their next scheduled visit as previously planned (cycle 3 day 1) with additional interim visits as needed for clinical management. The cycle will not restart for dosing delays due to toxicity.

6.3.2 Overdose

There is no known antidote for over-dosage of prexasertib (LY2606368). In the case of suspected overdose, monitor hematologic parameters, serum chemistry, vital signs, cardiac function, and provide supportive care as necessary.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (**Section 7.1**) and the characteristics of an observed AE (**Sections 7.2 and 7.3**) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

A list of the expected adverse events associated with the investigational agent administered in this study can be found in **Section 6.1**.

7.1.1 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.03. A copy of the CTCAE version 4.03 can be downloaded from the CTEP web site

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent that is listed above should be reported only if the AE varies in nature, intensity or frequency from the expected toxicity information which is provided in Section 7.1.
  - Other AEs for the protocol that do not require expedited reporting are outlined in section 7.4 - Expedited Adverse Event Reporting, under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.2 Serious Adverse Events

A serious adverse event (SAE) is any adverse event that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment that results in one of the following outcomes:

- Death
- Hospitalization for greater than 24 hours
- Prolonging an existing inpatient hospitalization
- A life-threatening experience (that is, immediate risk of dying)
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Considered significant by the investigator for any other reason

Previously planned (prior to signing the informed consent form) surgeries, and non-disease related elective surgeries planned during the course of the study, should not be reported as SAEs unless the underlying medical condition has worsened or appeared during the course of the study. Events that occur prior to the first administration of study medication should not be reported as SAEs.

Preplanned hospitalizations or procedures for preexisting conditions that are already recorded in the patient's medical history at the time of study enrollment should not be considered SAEs. Hospitalization or prolongation of hospitalization without a precipitating clinical AE (e.g., for the administration of study therapy or other protocol-required procedure) should not be considered SAEs.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based on appropriate medical judgment, they

may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Death due to disease progression should not be reported as an SAE unless the investigator deems it to be related to the use of study drug.

Study site personnel must alert Eli Lilly of any SAE as soon as possible and no later than 24 hours of the investigator and/or institution receiving notification of the SAE experienced by a patient participating in the study. The SAE reports should be on a standard reporting form (e.g. CIOMS, MedWatch 3500a, or similar) and are to be sent to Eli Lilly via fax at 1-866-644-1697.

7.3 Expedited Adverse Event Reporting

7.3.1 Investigators **must** report to the Overall PI any SAE that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment.

7.3.2 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.3.3 Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting to the Overall PI or the DFCI IRB. However, they still must be reported through the routine reporting mechanism (i.e. case report form).

Table 8: Expedited AE Reporting Exclusions

CTCAE SOC	Adverse Event	Grade	Resulting in Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
Investigations	Neutrophil count decreased	4	No	Definite, probable, or possible	*Only* applies if the AE does not involve a hospitalization/prolongation of hospitalization (otherwise needs to be reported)
Investigations	White blood cell decreased	4	No	Definite, probable, or possible	*Only* applies if the AE does not involve a hospitalization/prolongation of hospitalization (otherwise needs to be reported)
Investigations	Platelet count decreased	4	No	Definite, probable, or possible	*Only* applies if the AE does not involve a hospitalization/prolongation

					of hospitalization (otherwise needs to be reported)
Investigations	Lymphocyte count decreased	4	No	Definite, probable, or possible	*Only* applies if the AE does not involve a hospitalization/prolongation of hospitalization (otherwise needs to be reported)

7.4 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any SAE that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.5 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.6 Routine Adverse Event Reporting

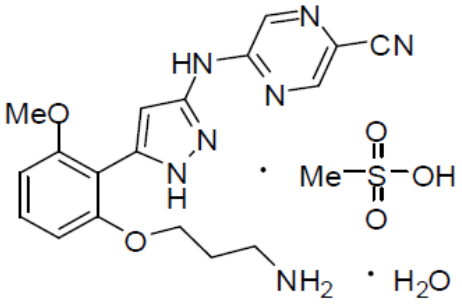
All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the AEs and potential risks associated with the investigational agent administered in this study can be found in **Section 6.1**.

8.1 LY2606368/Prexasertib

8.1.1 Description



Structure of LY2606368 mesylate monohydrate (LSN2940930).

Chemical name: 2-Pyrazinecarbonitrile, 5-[[5-[2-(3-aminopropoxy)-6-methoxyphenyl]-1H-pyrazol-3-yl]amino] monomesylate monohydrate

Molecular formula: $C_{19}H_{25}N_7O_6S$

Molecular weight: 479.51

pKa: 10.0 (basic) and 3.23 (basic)

pH: 5.51 (water slurry 10 mg/mL)

Specific Rotation, $[\alpha]^{20}_D$: Not applicable, achiral

pI: Not applicable

Solubility: Methanol 8.44 mg/mL (slightly soluble)

8.1.2 **Form**

Prexasertib (LY2606368) for injection is supplied for clinical trial use as a lyophilized, yellow to white solid, in glass vials, and is composed of prexasertib mesylate monohydrate and the inactive ingredient sulfobutylether-β-cyclodextrin (Captisol). The drug product is available in two vial strengths containing either 20 mg or 40 mg of the base compound prexasertib. The 40 mg strength vial contains a 3% excess whereas the 20 mg strength vial contains a 5% excess to facilitate the withdrawal of the label amount (40 mg/vial and 20 mg/vial, respectively) for application with an appropriate device, such as an infusion set.

Reconstituting the 40 or 20 mg vial contents with 19 mL or 10 mL of water, respectively, yields a clear yellow solution with a concentration of 2 mg/mL of prexasertib, pH 4 to 6, and an osmotic pressure ratio of 344 mOsm.

The reconstituted solution may be diluted with dextrose 5% injection (D5W) prior to administration.

8.1.3 **Storage and Stability**

The drug product is stable when stored at room temperature at 10˚C - 30˚C (50˚F to 86˚F). Do not freeze or refrigerate prexasertib (LY2606368).

The reconstituted formulation is stable for at least 24 hours at room temperature; however, since the reconstituted drug product does not contain a preservative, the unused solution must be discarded after 6 hours. The infusion should be completed within 4 hours of priming the infusion set.

The investigator or pharmacist will ensure that all investigational products are stored in a secure area, under recommended storage conditions and in accordance with applicable regulatory requirements. The site should monitor the minimum and maximum temperature and maintain temperature logs to confirm the correct storage of all investigational products used in the trial.

8.1.4 **Compatibility**

Prexasertib (LY2606368) is incompatible with solutions containing saline or Lactated Ringer's and must not be mixed or administered simultaneously with other drugs through the same infusion line.

8.1.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.1.6 **Availability**

Prexasertib (LY2606368) is an investigational agent that will be supplied by Eli Lilly and Company.

8.1.7 **Preparation**

Prexasertib (LY2606368) diluted in D5W is compatible with standard IV solution bags constructed of polyolefin or polyvinyl chloride (PVC). Contact with PVC bags should be limited to not more than 6 hours. Administration sets constructed of PVC (with DEHP or TOTM), polyethyelene-lined (paclitaxel) may be used. Administration sets with integrated filters or filter extension sets MUST be used for all patients. Compatibility with filters fabricated from polyether sulfone has been demonstrated.

Table 9 illustrates the compatible materials for prexasertib administration.

Table 9: Compatible Materials for Prexasertib Administration

Vendor	Description	Item Tested	Primary Contact Surface	Comparable Administration Supplies
Baxter	IntraVia Empty IV Infusion Bag, 250 mL	2B8012	Polyolefin Minimal PVC (with DEHP) Contact	Polyolefin, Excel (BBraun)
Baxter	Paclitaxel IV Infusion Set with 0.2 µm Filter	2C7557	Polyolefin Lined PVC without DEHP Polyether sulfone filter	Alaris, 10011433 Alaris, 10015048 Baxter Extension Set 0.2micron Filter (1C8363) Kawasumi, IV Add On Extension, IV-F02Y
Baxter	Interlink Infusion Set	2C6419S	PVC and DEHP	Alaris 72013E
BBraun	IV Administration Set	V1415-15	PVC and TOTM	None Identified
Baxter	5% Dextrose, USP, 250 mL	2B0062	PVC and DEHP	None Identified

8.1.7.1 Prexasertib 20 mg:

Prexasertib for injection, 20 mg: Reconstituted by adding 10 mL of water for injection and shaking. The resulting solution contains 2 mg/mL of prexasertib for injection.

Multiple vials of prexasertib will be required to prepare most dosage strengths. Obtain the number of vials needed to prepare the desired dose and reconstitute each vial with 10 mL of water for injection.

Transfer the total volume of solution required into an IV bag for subsequent dilution. Reconstituted drug product from the vials should be diluted with dextrose 5% injection (D5W) to a final volume of no more than 250 mL. If less than 250 mL is used, the concentration of prexasertib must be maintained within a range of 0.08 mg/mL - 1.6 mg/mL of prexasertib.

Reconstituted or diluted prexasertib should NOT be used if it appears turbid or contains foreign particles.

8.1.7.2 Prexasertib 40 mg:

Prexasertib for injection, 40 mg: Reconstituted by adding 19 mL of water for injection and shaking. The resulting solution contains 2 mg/mL of prexasertib for injection.

Multiple vials of prexasertib will be required to prepare most dosage strengths. Obtain the number of vials needed to prepare the desired dose and reconstitute each vial with 19 mL of water for injection.

Transfer the total volume of solution required into an IV bag for subsequent dilution. Reconstituted drug product from the vials should be diluted with D5W to a final volume of no more than 250 mL. If less than 250 mL is used, the concentration of prexasertib must be maintained within a range of 0.08 mg/mL - 1.6 mg/mL of prexasertib.

Reconstituted or diluted prexasertib should NOT be used if it appears turbid or contains foreign particles.

8.1.8 **Administration**

Prexasertib (LY2606368) should be administered IV over 1 hour (+/- 5 minute infusion window) using a central or free flowing peripheral IV line with an in-line, sterile, non-pyrogenic, 0.22-µm filter. Reconstituted or diluted prexasertib should NOT be used if it appears turbid or contains foreign particles.

8.1.9 **Ordering**

Drug supply will be ordered from Eli Lilly by site pharmacy personnel.

8.1.10 **Accountability**

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.11 **Destruction and Return**

Expired supplies of prexasertib (LY2606368) should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form. At the end of the study, unused supplies of prexasertib should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

9.1.1 Exploratory Tumor Biopsy – Replicative Stress and HR Cohorts

Mandatory fresh tumor biopsies will be obtained from the patients enrolled to the replicative stress or HR cohorts during Stage 1 of the 2-stage trial. The biopsies will be obtained at baseline (any time prior to the first infusion).

Core biopsy samples should be obtained for analysis. Three-to-four biopsy passes utilizing a 16-18 gauge needle are preferable, but a 20 gauge core needle biopsy is also acceptable. Less than the goal amount of tissue is acceptable for the biopsy procedure, and should be based upon the clinical judgment of the treating investigator and the clinician performing the procedure.

Biopsy samples should be fresh frozen. A plastic cryomold should be labeled with the protocol number, study subject number, and the exact date and time of collection. Several drops of OCT compound will be placed into the cryomold and at time of biopsy the sample will be placed into OCT, covered with additional drops of OCT and then placed upon dry ice immediately. The specimen will then be stored at -80ºC until used.

The frozen samples should be shipped to the Broad Institute Center for Cancer Precision Medicine. The baseline tumor tissue sample should be shipped at the same time as the germline blood sample as described below in **Section 9.1.3**.

Biopsy samples should be sent on dry ice to:

Broad Institute Center for Cancer Precision Medicine
320 Charles Street
ATTN: Samples Lab
Cambridge, MA 02141

9.1.2 Blood sample collection – All Cohorts

For **all** patients enrolling on trial, blood samples will be collected to be analyzed for cell-free DNA (cfDNA). A sample will be collected at baseline (prior to the first infusion), at visits immediately following restaging scans (i.e., cycle 3 day 1, cycle 5 day 1, cycle 7 day 1, etc.) and at the off study visit. Samples collected on days where a prexasertib infusion will be given can be collected anytime pre-infusion.

For the patients enrolling during Stage 1 of the trial in the replicative stress or HR cohorts **only**, a germline blood sample will be collected at baseline (any time prior to the first infusion) to be paired with the pre-treatment fresh tumor biopsy obtained for WES.

9.1.2.1 Germline Blood Sample for WES Collection Procedure (HR and Replicative Stress Cohorts):

Blood sample should be labeled with the protocol number, study subject number, and the date of

collection.

- One 10 mL sample will be drawn into an EDTA containing test tube.
- Freeze immediately upright at -80ºC until shipping.
- **Both the germline blood sample and pre-treatment fresh tumor biopsy samples should be shipped to the Broad Institute at the same time for processing.**
- The samples should be sent to the following address to be processed:
  Broad Institute Center for Cancer Precision Medicine
  320 Charles Street
  ATTN: Samples Lab
  Cambridge, MA 02141

9.1.2.2 cfDNA Sample Collection Procedure (All Cohorts):

1. Draw two 9 mL blood samples into Streck Tubes.
2. Blood samples should be labeled with: cfDNA, protocol number, study subject number, and the date of the draw.
3. Within 2 hours of the draw time, cfDNA samples should be processed by the DF/HCC Clinical Trials Core laboratory as follows:
   1. Immediately centrifuge for 10 minutes at 1500 (+\- 150) x g. NOTE: Brake switch must be off so the cell/plasma interface is not disturbed.
   2. Pipette the plasma layer into a 15 mL tube labeled "CFDNA/with patient #". Do not ship. NOTE: Do not dip the tip of the pipette into the plasma/cell interface. Leave a thin plasma layer intact over the interface.
   3. Centrifuge the 15 mL tube containing the plasma only for 10 minutes at 3000 (+/- 150) x g.
   4. Transfer using a fresh pipette, the supernatant into a second 15 mL tube labeled "CFDNA super.do not ship". NOTE: Leave about 0.3 mL of supernatant in the centrifuged 15 mL tube. This leftover 0.3 mL contains cellular debris.
   5. Using a fresh pipette, transfer 1 mL of plasma from the "super.do not ship" tube into max four (4) 2 mL cryovials labeled CFDNA.ship.patient#/
   6. Freeze immediately upright at -70ºC or colder until shipping.

4. Samples should then be sent to the Broad Institute at:
   Broad Institute of MIT and Harvard
   75 Ames Street
   Lab 75A-4045
   Cambridge, MA 02142

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 14 days prior to start of protocol therapy, with the exception of the informed consent and baseline tumor imaging which may be obtained up to 28 days prior to the start of protocol therapy. Assessments must be performed prior to

administration of any study agent.

	Table 10: Study Calendar								
	Screening[a]	**Cycle 1 Day 1**	**Cycle 1 Day 8[h]**	**Cycle 1 Day 15[i]**	**Cycle 1 Day 22[h]**	**Cycle 2+ Day 1[j]**	**Cycle 2+ Day 15[i]**	**Off Treatment[k]**	**Every 3 Months After Discontinuing[l]**
Prexasertib (LY2606368)[b]		X		X		X	X		
Informed consent	X								
Demographics	X								
Medical history	X								
Physical exam	X	X	X	X	X	X	X	X	
Vital signs[c]	X	X	X	X	X	X	X	X	
Height	X								
Weight	X	X		X		X	X	X	
ECOG performance status	X	X		X		X	X	X	
CBC w/diff & Plt	X	X	X	X	X	X	X	X	
Serum chemistry[d]	X	X	X	X	X	X	X	X	
β-HCG[e]	X							X	
EKG[f]	X	X				X			
Adverse Events		X	X	X	X	X	X	X	
Radiologic evaluation	X	CT or MRI imaging of any disease-involved site. Radiologic measurements should be performed at the end of cycle 2 (cycle 2 day 28) and at the end of every 2 cycles of treatment thereafter (i.e. cycle 4 day 28, cycle 6 day 28, and so on). There is a ± 7 day window on imaging evaluations.						X	
Fresh Tumor Biopsy[g]	X								
Research Germline Blood Sample for	X								

	Table 10: Study Calendar								
	Screening[a]	**Cycle 1 Day 1**	**Cycle 1 Day 8[h]**	**Cycle 1 Day 15[i]**	**Cycle 1 Day 22[h]**	**Cycle 2+ Day 1[j]**	**Cycle 2+ Day 15[i]**	**Off Treatment[k]**	**Every 3 Months After Discontinuing[l]**
WES[g]									
Research Blood Samples for cfDNA		X	cfDNA samples to be collected at the visit immediately following a restaging scan (i.e., cycle 3 day 1, cycle 5 day 1, cycle 7 day 1, and so on) as described in **Section 9**.					X	
Telephone or Care Provider Contact									X

a) Screening evaluations are to be performed within 14 days of cycle 1 day 1, with the exception of the informed consent and baseline tumor imaging which may be obtained up to 28 days prior to cycle 1 day 1.
b) Prexasertib (LY2606368) dose as assigned; see administration guidelines in **Section 5**.
c) Heart rate, respiratory rate, blood pressure, temperature, and oxygen saturation (O_2 sat). On infusion days, vital signs are to be collected anytime pre-infusion (exact time of vital signs should be recorded). May be repeated as clinically indicated.
d) Sodium, potassium, chloride, CO_2, blood urea nitrogen (BUN), creatinine, glucose, albumin, total protein, alkaline phosphatase, total bilirubin, SGOT [AST], SGPT [ALT], globulin, and magnesium. Other tests may be ordered as clinically indicated.
e) Serum pregnancy test only required for women of childbearing potential. Childbearing potential defined as any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation or bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea >12 consecutive months; or women with a documented plasma follicle-stimulating hormone level >35µIU/mL).
f) To be performed at screening, at any time pre-dose on cycle 1 day 1, upon completion of the first infusion (+15 minute window from the end of the infusion), and 1 hour after the end of the infusion (+30 minute window). EKG to be completed pre-dose (any time prior to the infusion) on the first day of each subsequent cycle. Additional EKGs may be performed as clinically indicated at the treating investigator's discretion. If QTcF increases > 30 msec from screening EKG at any time point, EKG should be repeated at 10 minutes after the end of the infusion (± 5 minute window), 30 minutes after the end of the infusion (± 10 minute window), and 1 hour after the end of the infusion (+30 minute window). The exact time of all EKGs should be recorded.
g) Baseline fresh tumor biopsy and germline blood sample for WES required for the patients enrolled during Stage 1 of the HR and replicative stress cohorts. Please see **Section 9** for further detail.
h) Cycle 1 day 8 and cycle 1 day 22 may be shifted by ± 3 days for scheduling issues (vacations, holidays, adverse weather, etc.).
i) Day 15 assessments and infusion may shifted ± 1 day for scheduling issues (vacations, holidays, adverse weather, etc.).
j) The start of a subsequent cycle may be delayed by up to 7 days for scheduling issues (vacations, holidays, adverse weather, etc.).
k) Off-treatment evaluation. Note: follow up visits or other contact is required in order to identify SAEs during the 30 days following the end of study treatment.
l) Participants will be followed until death or withdrawal of consent after removal from protocol therapy for survival status only. This follow up will be performed by review of the medical record, contact with care providers, and/or telephone contact as needed every 3 to 4 months.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect

For the purposes of this study, participants should be re-evaluated for response at the end of every 2 cycles of treatment (every 8 weeks) as described in the **Study Calendar**. In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants 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.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area are not considered measurable unless there has been demonstrated progression in the lesion.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 28 days before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based

evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be

prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

MIBG (meta-iodobenzylguanidine).The following is recommended, to assure high quality images are obtained.

Patient preparation: Iodides, usually SSKI (saturated solution of potassium iodide), are administered to reduce thyroidal accumulation of free radioiodine, preferably beginning the day prior to injection and continuing for 3 additional days (4 days total). For infants and children, one drop t.i.d. is sufficient, for adolescents 2 drops t.i.d., and for adults 3 drops t.i.d. Participants and/or parents are asked about exposure to potential interfering agents. If none is noted, an indwelling intravenous line is established. The dose of MIBG is administered by slow intravenous injection over 90 seconds.

Images from the head to the distal lower extremities should be obtained.

I-123MIBG scintigraphy is performed to obtain both planar and tomographic images.

Planar: Anterior and posterior views from the top of the head to the proximal lower extremities are obtained for 10 minutes at 24 hours and occasionally at 48 hours following injection of 10 mCi/1.7 square meters of body surface area (~150 μCi/kg, maximum 10 mCi). Anterior views of the distal lower extremities are adequate. A large field of view dual head gamma camera with low energy collimators is preferred.

SPECT: Most participants receiving I-123 MIBG also undergo SPECT at 24 hours, using a single or multi-headed camera with a low energy collimator. The camera is rotated through 360 degrees, 120 projections at 25 seconds per stop. Data are reconstructed using filtered back projections with a Butterworth filter and a cut off frequency of 0.2-0.5. SPECT/CT may be performed at institutions with this capacity.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later data and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.1.3.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.3.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.3.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.3.4 Evaluation of 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.

For Participants with Measurable Disease (*i.e.*, Target Disease)

Table 11: RECIST Criteria for Evaluation of Response				
Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Overall Response when Confirmation is Required***
CR	CR	No	CR	≥4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Table 12: RECIST Criteria for Patients with Non-Measurable Disease		
Non-Target Lesions	**New Lesions**	**Overall Response**
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.4 Duration of Response

Duration of overall response: The duration of overall response 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, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.5 Progression-Free Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.1.6 Response Review

Evaluation of scans will be done centrally at the DFCI using the Tumor Metrics Core.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in **Section 7.0** (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or

data forms to the ODQ according to the schedule set by the ODQ.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

13. STATISTICAL CONSIDERATIONS

This is a three-arm phase II study exploring the anti-tumor activity of prexasertib (LY2606368) in patients with advanced cancer exhibiting either replicative stress, HR deficiency, or *CCNE1* amplification. The primary goal of the study will be to examine the progression-free rate at 4 months in all arms separately. Secondary efficacy measures will include assessment of objective response rate by RECIST 1.1 as well as toxicity using CTCAE version 4.0 criteria, overall survival (OS), and progression free survival (PFS).

Each arm will be conducted as a Simon's 2-stage design and will initially enroll 9 participants. If there are less than 2 subjects that are alive and progression-free at 4 months after initiation of prexasertib, that arm will be terminated. If 2 or more subjects are progression free by 4 months, then enrollment for that arm will be expanded to 19 subjects. A minimum of 6 patients must be alive and progression-free at 4 months for the cohort to be considered meaningful for further evaluation. If at any point a participant does not complete Cycle 1 dosing of the study agent, that participant may be replaced at the principal investigator's discretion.

Progression status by 4 months will be determined for all patients. We expect all patients to have this status documented. PFS will be defined as the time from study registration to documented disease progression per RECIST 1.1 criteria or death from any cause, whichever occurs first. OS will be defined as the time from study registration until death. Patients who have not experienced an event of interest by the time of analysis will be censored at the date they are last known to be progression-free for PFS and alive for OS.

This design provides 80% power to detect a progression-free rate at 4 months of 40% tested against a historical control rate of $\leq 15\%$[57] using a one-sided significance of 0.05.

13.1 Study Design/Endpoints

Primary Efficacy Measures:

1) Investigate the progression-free rate at 4 months in three cohorts of patients with tumors exhibiting:
    a. Replication stress, as defined by tumors with *MYC* amplification, C*CNE1* amplification, *Rb* loss, or *FBXW7* mutation

    b. HR deficiency, as defined by patients with either genomic or somatic alteration in a known HR gene, including *BRCA1*, *BRCA2*, *PALB2*, *RAD51C*, *RAD51D*, *ATR, ATM, CHK2,* or Fanconi anemia pathway genes

    c. *CCNE1* amplification, defined as tumors with 6-fold amplification or greater.

Secondary Efficacy Measures:

1) Investigate the toxicity and objective response rates to prexasertib in these cohorts.

Exploratory Endpoints:

1) WES will be performed on the pre-treatment biopsies collected from participants enrolled to the HR and replicative stress cohorts during Stage 1 to examine possible genomic determinants of sensitivity or resistance to CHK1 inhibition.

2) Clinical outcome will also be correlated to the tumor DNA levels obtained on the serial plasma samples submitted for cfDNA.

13.2 Sample Size, Accrual Rate and Study Duration

The planned sample size is a maximum of 19 patients in each arm. The replicative stress cohort is now closed and will not expand, having enrolled 10 participants. Allowing for replacement of participants who do not make it through one cycle of dosing, the estimate for the maximum sample size is 50 participants (with 35 yet to enroll as of 5 December 2017).

As of 5 December 2017, a total of 15 participants have been accrued. Adjusting for the current accrual level and slower estimated accrual for the *CCNE1* cohort, we anticipate accruing approximately 3-4 patients per quarter. An additional year of follow-up will be required on the last participant accrued to observe the patient's response following study therapy, for a total study length of about 4.5 years. Participants will be identified via targeted NextGen sequencing using the DFCI/BWH OncoPanel or another CLIA-certified method for genotyping.

Table 13: Accrual Targets

Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	3	+	0	=	3

Not Hispanic or Latino	25	+	22	=	47
Ethnic Category: Total of all subjects	28	+	22	=	50
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	0	+	0	=	0
Black or African American	2	+	0	=	2
Native Hawaiian or other Pacific Islander	0	+	0	=	0
White	26	+	22	=	48
Racial Category: Total of all subjects	28	+	22	=	50

13.3 Analysis of Primary and Secondary Endpoints

The primary and secondary analyses will include all eligible patients who completed at least one cycle of dosing. The exception to this includes the planned analysis of toxicity data, which will include all patients who received study drug regardless of eligibility and regardless of whether they completed a full cycle. Progression-free rate will be estimated as binomial proportion and overall survival and progression-free survival estimates and curves will be generated using the Kaplan-Meier method.

13.4 Analysis of Exploratory Endpoints

WES will be retrospectively performed on the pre-treatment biopsies obtained during the first stage of the HR and replicative stress study arms. The WES will profile the entire HR proficiency of the tumor, and will involve up to 100 genes of interest in DNA repair in order to obtain the entire HR state for each participant. The results of the tissue analyses will be compared to the clinical outcomes of the patients, and will hopefully help to further identify the genetic characteristics of patients who will respond to treatment with prexasertib (LY2606368) versus patients who will not. This data may help with future selection of patient populations appropriate for treatment with CHK1 therapy.

Clinical outcome will also be correlated to the tumor DNA levels obtained on the serial plasma samples submitted for cfDNA.

Data from correlative studies will be summarized using descriptive statistics and may be explored graphically.

13.5 Reporting and Exclusions

13.5.1 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first treatment.

13.5.2 Evaluation of the Primary Efficacy Endpoint

All eligible participants included in the study who complete at least one cycle of dosing will be assessed for response, even if there are major protocol therapy deviations. Each participant should be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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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 LIST OF DRUGS KNOWN TO PREDISPOSE TO TORSADE DE POINTES

Generic Name	Brand Name(s)
Amiodarone	Cordarone®, Pacerone®
Arsenic trioxide	Trisenox®
Astemizole	Hismanal®
Azithromycin	Zithromax®
Bepridil	Vascor®
Chloroquine	Aralen®
Chlorpromazine	Thorazine®
Cisapride	Propulsid®
Citalopram	Celexa®
Clarithromycin	Biaxin®
Disopyramide	Norpace®
Dofetilide	Tikosyn®
Domperidone	Motilium®
Droperidol	Inapsine®
Erythromycin	Erythrocin®, E.E.S. ®
Flecainide	Tambocor®
Halofantrine	Halfan®
Haloperidol	Haldol®
Ibutilide	Corvert®
Levomethadyl	Orlaam®
Mesoridazine	Serentil®
Methadone	Dolophine®, Methadose®
Moxifloxacin	Avelox®
Ondansetron*	Zofran®
Pentamidine	Pentam®, NebuPent®
Pimozide	Orap®
Probucol	Lorelco®
Procainamide	Pronestyl®, Procan®
Quinidine	Cardioquin®, Quinaglute®
Sotalol	Betapace®
Sparfloxacin	Zagam®
Terfenadine	Seldane®
Thioridazine	Mellaril®
Vandetanib	Caprelsa®

*when administered intravenously at high dose (32 mg).

Adapted from the University of Arizona Cancer Center for Education and Research on Therapeutics: "Torsades List: Drugs with a Risk of Torsades de Pointes," drugs that are generally accepted by the QTdrugs.org Advisory Board to carry a risk of Torsades de Pointes on the University of Arizona CERT website: http://www.crediblemeds.org/. This list is not meant to be considered all inclusive. See website for current list.