

*FDA Oncologic Drugs Advisory Committee (ODAC)
Pediatric Subcommittee*

Sponsor Briefing Document

Meeting Date: 22 June 2017

Prexasertib (LY2606368)



Eli Lilly and Company

AVAILABLE FOR PUBLIC DISCLOSURE WITHOUT REDACTION

Approval Date: 25-May-2017 GMT

1. Table of Contents

Section	Page
1. Table of Contents.....	2
2. Executive Summary	6
3. Compound Overview and Mechanism of Action	8
4. Regulatory History.....	10
5. Nonclinical Data Supporting Pediatric Clinical Studies	11
5.1. Nonclinical Data in Pediatric Models	11
5.1.1. Neuroblastoma	13
5.1.2. Rhabdomyosarcoma.....	13
5.1.3. Other Pediatric Subtypes.....	15
5.2. Nonclinical Pharmacokinetics and Metabolism	16
5.3. Nonclinical Pharmacokinetic/Pharmacodynamic Model	17
5.4. Nonclinical Safety	17
6. Clinical Trial Experience in Adults	18
6.1. Overview	18
6.2. Study JTJA: First-in-Human Evaluation.....	20
6.2.1. Dose Escalation.....	20
6.2.2. Expansion Cohorts	20
6.2.3. Pharmacokinetic Summary.....	21
6.2.4. Biomarker Summary	21
6.3. NCI-Sponsored Phase 2 Study in Patients with Ovarian Cancer.....	21
7. Background and Rationale for Pediatric Indications	24
7.1. Overview	24
7.2. Neuroblastoma	24
7.2.1. Overview and Current Treatments.....	24
7.2.2. Selected Genetic Features Relevant to CHK1 Inhibition.....	24
7.3. RMS	25
7.3.1. Overview and Current Treatments.....	25
7.3.2. Selected Genetic Features Relevant to CHK1 Inhibition.....	25
8. Clinical Pediatric Development Plan	27
9. Clinical Trials in Pediatrics	28
9.1. Ongoing Pediatric Clinical Trial: Children’s Oncology Group-Sponsored Phase 1 Study.....	28
9.2. Proposed Pediatric Clinical Trial	28
9.2.1. Design and Objectives.....	28

9.2.2. Key Entry Criteria..... 29

9.2.3. Dose Selection 29

9.2.4. Safety Assessments 30

9.2.5. PK Assessments 30

9.2.6. Efficacy Assessments 30

9.2.7. Statistical Considerations 31

9.3. Combination Strategy for Future Trials 31

10. Overall Summary/Conclusions 32

11. References 33

12. Appendix 1: Innovative Medicines Initiative 38

13. Appendix 2: Current or Potential Challenges in Pediatric
Development..... 40

13.1. Epidemiology and Enrollment 40

13.2. Complexity of Pediatric Cancer Regimens 40

13.3. Translation of Nonclinical Data to Clinical Studies 41

13.4. Risk-Benefit in Pediatric Patients 41

13.5. Predictive Biomarkers 42

List of Tables

Table	Page
Table 1. EC ₅₀ (μM) Comparison of Prexasertib and Standard of Care in Pediatric Cancer Cell Lines	11
Table 2. Summary of In Vivo Responses to Prexasertib Monotherapy in Neuroblastoma and Sarcoma Models, As Observed by Lilly	12
Table 3. Lilly-Sponsored Clinical Trials with Prexasertib	19
Table 4. Exploratory Investigator-Sponsored Clinical Trials with Prexasertib	19
Table 5. Prexasertib Exposures across Various BSA Ranges.....	30

List of Figures

Figure	Page
Figure 1. Prexasertib monotherapy treatment results in neuroblastoma regression.	13
Figure 2. Efficacy of prexasertib as a monotherapy varies between subtypes of pediatric sarcoma.	14
Figure 3. Prexasertib in combination with doxorubicin prevents the development of acquired resistance in aRMS.	15
Figure 4. Prexasertib monotherapy treatment in a PDX model of DSRCT.	16
Figure 5. Efficacy of prexasertib as a monotherapy in pediatric bony tumors (osteosarcoma and Ewing’s sarcoma).....	16
Figure 6. Best response for target lesions by patient, among patients treated with prexasertib.	23
Figure 7. Illustration of prexasertib clinical pediatric development plan.	27

2. Executive Summary

Prexasertib, an inhibitor of checkpoint kinase 1 (CHK1), disrupts DNA replication, induces DNA damage, and subsequently prevents repair, leading to death by replication catastrophe (King et al. 2015). Compelling nonclinical efficacy data have been observed in in vivo models of neuroblastoma and rhabdomyosarcoma (RMS). As a result, it is hypothesized that prexasertib may improve therapeutic outcomes for pediatric patients with these tumors.

Although prexasertib is in the early stages of clinical development, it is positioned to be a first-in-class CHK1 inhibitor. The ongoing studies are in Phases 1 and 2; no Phase 3 studies have been initiated. The current development strategy includes assessments as a monotherapy, as a combination therapy with modalities or agents that cause genotoxic DNA damage (for example, cisplatin, radiation, and antimetabolites), and as a combination therapy with targeted agents (for example, cetuximab; ralimetinib, a p38 inhibitor; and LY3023414, a phosphatidylinositol-3-kinase [PI3K]/mammalian target of rapamycin [mTOR] inhibitor). Durable objective responses have been observed in adult patients with advanced cancer (Hong et al. 2016). Hematologic toxicity, including transient Grade 3 or 4 neutropenia (89%), thrombocytopenia (29%), and anemia (31%), are the most frequent toxicities when a dose of 105 mg/m² prexasertib is administered as a 1-hour infusion every 14 days (Hong et al. 2016). Febrile neutropenia rates are approximately 10% (Hong et al. 2016). Nonhematologic toxicities such as fatigue and gastrointestinal toxicities occur at much lower frequencies and are typically Grade 1 or 2 in severity. Overall, the current clinical data in adults support the clinical development of prexasertib in pediatric patients.

The nonclinical data in pediatric models of cancer and the clinical data in adults informed a Phase 1 study sponsored by the Children's Oncology Group (COG), which is assessing prexasertib as a monotherapy in pediatric patients with recurrent or refractory solid tumors. Based on the results of this COG study and supported by the nonclinical data in pediatric models, a subsequent study is proposed in pediatric patients with relapsed or refractory neuroblastoma or RMS. Data from the initial ongoing and completed studies will support potential evaluations in other solid or hematologic tumors, as well as in combination with cytotoxic chemotherapy and/or targeted agents.

In summary, as a CHK1 inhibitor, prexasertib represents a novel addition to cell-cycle and DNA damage inhibitors, classes of agents that have well-established benefits for patients with cancer. Notably, prexasertib is the first CHK1 inhibitor to demonstrate anticancer activity as a monotherapy in adults whose disease is refractory to currently available chemotherapies, while the safety profile has been acceptable and toxicities manageable in this setting. Prexasertib has demonstrated striking activity in multiple animal models of pediatric cancer, including neuroblastoma and RMS. Consequently, based on the observations of prexasertib activity in adult patients and pediatric nonclinical models, in addition to the lack of effective treatments for pediatric patients whose neuroblastoma or RMS is refractory to current therapies, it is the aim of the clinical trial proposed in this Briefing Document to explore whether prexasertib has antitumor activity in pediatric patients with relapsed or refractory neuroblastoma or RMS.

Moreover, Eli Lilly and Company (Lilly) is committed to the pursuit of further advancement in the treatment of pediatric cancers, one aspect of being an industry co-leader for a public-private partnership sponsored in the European Union (EU) through the Innovative Medicines Initiative (IMI Grant Agreement No 116064 “ITCC-P4”), which will deliver a comprehensive preclinical pediatric proof-of-concept platform for solid tumors in collaboration with 21 major pharma and EU pediatric research institutions. Refer to Appendix 1 (Section 12) for further details about the IMI.

3. Compound Overview and Mechanism of Action

Checkpoint kinase 1 is a multifunctional protein kinase that regulates DNA replication and the DNA damage response (DDR) (Dai and Grant 2010). In response to exogenous DNA damage, CHK1 mediates cell-cycle arrest to allow time for DNA repair or, if the damage is extensive, to trigger apoptosis. It is essential for homologous recombination-mediated repair of double-stranded DNA breaks. It also affects the initiation of DNA replication origin firing, stabilization of replication forks, resolution of replication stress, and coordination of mitosis, even in the absence of exogenous DNA damage (McNeely et al. 2014).

Traditionally, CHK1 inhibitors were developed solely as chemopotentiating agents to be given in combination with cytotoxic chemotherapy. However, recent advances in understanding the cell cycle and DNA damage repair pathways have led to the development of a new generation of inhibitors targeting these mechanisms that may be used either as a monotherapy or in combination with other agents. Inhibitors of CHK1 (Hong et al. 2016), ataxia telangiectasia-mutated (ATM) kinase (Pike 2016), ataxia telangiectasia and Rad3-related (ATR) kinase, and WEE1 (Dobbelstein and Sørensen 2015) are all in clinical development. These kinases are crucial for maintaining genomic integrity by reducing replication stress and for mediating the responses for the repair of DNA damage. Although early CHK1 inhibitors developed in combination with cytotoxic chemotherapy were not successful for a variety of reasons, newer CHK1 inhibitors such as prexasertib, LY2880070, and CCT245737 are being evaluated as monotherapies and combinations with other agents (Lin et al. 2017).

Prexasertib is an adenosine triphosphate-competitive inhibitor of CHK1. In an *in vitro* enzyme assay, prexasertib inhibits the activity of CHK1 with a half maximal inhibitory concentration (IC_{50}) $<1nM$ and an inhibition binding constant of $0.9nM$. The compound is a selective protein kinase inhibitor demonstrating an $IC_{50} <35nM$ against only 3 of 224 kinases in a kinase screening panel (King et al. 2015). Only CHK2 and the p90S6 kinases were inhibited by prexasertib at an $IC_{50} <10nM$.

Prexasertib has potent effects on cells *in vitro* as a single agent, 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, keeping late replication origins silent until late synthesis phase (S phase). Treatment with prexasertib induced phosphorylation of H2A histone family member X (γ H2AX), a marker of DNA damage, in S-phase cells, and induction depended on proteins that regulate replication origin firing. Dysregulation of replication origin activation increased the number of replication forks and resulted in replication catastrophe accompanied by appearance of deleterious double-stranded DNA breaks. Checkpoint abrogation by prexasertib allowed cells with damaged DNA to prematurely enter mitosis and die. *In vivo*, prexasertib monotherapy induced markers of replication stress and DNA damage in tumors from a Calu-6 xenograft mouse model and significantly inhibited tumor growth. (King et al. 2015)

In vitro results from King et al. (2015) were recapitulated in studies with cell lines derived from B-/T-cell progenitor acute lymphoblastic leukemia. Namely, single-agent prexasertib induced γ H2AX and increased the fraction of cells in S phase. Single-agent prexasertib also inhibited

cell growth and induced apoptosis. Interestingly, reduction in cell viability due to treatment with prexasertib was selective for primary leukemic cells; peripheral mononuclear cells from healthy donors were relatively unaffected. In combination experiments, prexasertib potentiated the activity of tyrosine kinase inhibitors imatinib and dasatinib in Philadelphia chromosome-positive cell lines and augmented the cytotoxicity of the antimetabolite clofarabine in Philadelphia chromosome-negative cell lines (Ghelli Luserna Di Rorà et al. 2016).

The use of prexasertib was also evaluated in head and neck squamous cell carcinoma (HNSCC). In HNSCC cell lines, single-agent prexasertib caused replication stress, as evidenced by accumulation of S-phase cells and induction of γ H2AX. Single-agent prexasertib inhibited proliferation, reduced survival, and induced apoptosis. In the context of combination treatment, prexasertib modestly augmented the antiproliferative and proapoptotic activity of cetuximab and/or irradiation (IR). In vivo, prexasertib delayed tumor growth in mouse HNSCC xenografts as a monotherapy and as a combination with cetuximab and/or IR, with the triple combination demonstrating the greatest antitumor efficacy. (Zeng et al. 2017)

Prexasertib was identified as having potent in vitro and in vivo antitumor activity in primary colorectal cancer cells that were enriched for cancer stem cells (CRC-SCs), which are a subpopulation of cells associated with therapeutic resistance and patient relapse. In vitro, prexasertib lowered the clonogenic potential of sensitive CRC-SC cell lines irrespective of *K-ras* mutation status and reduced the fraction of cells displaying cancer stem cell (CSC) markers. Similarly, prexasertib decreased the CSC fraction in sensitive in vivo xenograft models. Mechanistic in vitro studies in sensitive CRC-SCs demonstrated that prexasertib treatment induced DNA damage, accumulation of cells with S-phase DNA content, premature mitosis, and markers of apoptosis. Prexasertib sensitivity was associated with markers of activated DDR and elevated replication stress, namely basal levels of phosphorylated ATM, replication protein A (RPA)32, and γ H2AX, as well as *TP53* mutation (Manic et al. 2017).

Based on nonclinical data, it is postulated that tumors that have high levels of replication stress and/or defects in DNA damage repair pathways will be susceptible to the effects of a CHK1 inhibitor like prexasertib. Although the focus of the clinical program to date has been on adult tumors, given that some pediatric tumors have features consistent with high levels of replication stress and/or defects in DNA damage repair pathways, it is hypothesized that prexasertib also may improve therapeutic outcomes for pediatric patients with these tumors.

4. Regulatory History

The initial investigational new drug application (IND) for prexasertib was in effect in 2010 to support a Phase 1 clinical study (I4D-MC-JTJA [JTJA]) in patients with advanced cancer. Currently, there are 6 ongoing and 1 completed Lilly-sponsored clinical trials (Phase 1 or 2) in adults in the United States and Europe, plus 5 exploratory investigator-sponsored studies in adult and/or pediatric patients.

The US Food and Drug Administration (FDA) Office of Orphan Products Development granted prexasertib an orphan drug designation for the treatment of anal cancer in 2015.

As a result of the observed antitumor activity in adult cancer patients, the animal pediatric models of neuroblastoma or RMS for prexasertib, the lack of effective treatments for pediatric patients who have failed on or been refractory to current therapies, and the dosing information that will be obtained from the ongoing COG-sponsored study in children, Lilly plans to assess the safety and efficacy of prexasertib as monotherapy in pediatric patients with relapsed or refractory neuroblastoma or RMS. Lilly plans to submit a Proposed Pediatric Study Request (PPSR) to support a Written Request (WR) issued by the FDA that includes the type of studies to be conducted, study design and goals, and the age groups to be studied. Lilly will develop and submit to the FDA an initial Pediatric Study Plan (PSP) consistent with the Pediatric Research Equity Act (PREA) (21 USC §355c).

A Pediatric Investigation Plan (PIP) has not yet been submitted to the European Medicines Agency (EMA) but will be subject to the EMA procedure.

Prexasertib is not approved for marketing in the United States or any other country worldwide.

5. Nonclinical Data Supporting Pediatric Clinical Studies

The antitumor efficacy of prexasertib was evaluated in a panel of pediatric tumor cell lines and in more than 25 mouse xenograft models of pediatric or adolescent/young adult tumors.

As summarized in Section 5.1, the data demonstrate that prexasertib has compelling activity as a monotherapy in murine in vivo xenograft models of human pediatric neuroblastoma and several subtypes of pediatric sarcoma such as embryonal rhabdomyosarcoma (eRMS), alveolar rhabdomyosarcoma (aRMS), and desmoplastic small round cell tumor (DSRCT). In addition, prexasertib in combination with doxorubicin, cyclophosphamide, or irinotecan led to a durable complete regression in an in vivo xenograft model of RMS. Collectively, these nonclinical data provide a strong rationale for evaluating the therapeutic potential of prexasertib in pediatric patients.

5.1. Nonclinical Data in Pediatric Models

The first observed indication of the potential for prexasertib as a therapy for pediatric tumors was derived from an in vitro screen of prexasertib across a series of pediatric cancer cell lines.

Surprisingly, single-digit nanomolar sensitivity to prexasertib that was superior to standard-of-care agents was observed in the majority of pediatric cancer cell lines evaluated in vitro (Table 1). A subsequent analysis of prexasertib-treated pediatric sarcoma cell lines showed increased DNA damage, CHK1 phosphorylation, and MAPK pathway activation.

Table 1. EC₅₀ (μM) Comparison of Prexasertib and Standard of Care in Pediatric Cancer Cell Lines

Cancer Type	Cell Line	Prexasertib	Doxorubicin	Vincristine	Cisplatin
Ewing's sarcoma	RD-ES	0.003	0.267	<0.01	2.944
	SK-NM-C	<0.001	0.074	0.052	0.214
Neuroblastoma	KELLY	<0.001	0.030	0.026	1.660
	TGW	0.001	0.190	0.001	3.499
	IMR-32	<0.001	0.010	0.006	0.005
	SH-SY5Y	<0.001	0.038	0.017	0.420
Osteosarcoma	SJSA1	0.001	>0.200	0.015	13.260
	HOS	<0.001	0.040	0.015	6.774
	SAOS-2	0.001	0.043	<0.01	1.445
RMS	TE 381.T	0.001	0.026	0.013	2.040
	SJCRH30	<0.001	0.007	<0.01	1.384
	RD	<0.001	0.013	<0.01	0.781

Abbreviations: EC₅₀ = half maximal effective concentration; RMS = rhabdomyosarcoma.

Source: May et al. 2016.

To determine whether the in vitro results translated to in vivo efficacy, mice bearing cell-derived xenografts (CDXs) or patient-derived xenografts (PDXs) of pediatric or adolescent/young adult tumor types were treated with 4 weekly cycles of 10 mg/kg prexasertib administered subcutaneously twice daily (BID) for 3 consecutive days, followed by a 4-day dosing holiday. Pharmacokinetic/pharmacodynamic (PK/PD) modeling of nonclinical xenograft data predicts that this dose and schedule is clinically relevant (Hong et al. 2016). Using this dose and schedule, significant monotherapy activity was observed in multiple models of neuroblastoma and pediatric sarcoma (for example, eRMS, aRMS, and DSRCT) and in single models of osteosarcoma and Ewing's sarcoma, but not in single models of hepatoblastoma, retinoblastoma, or rhabdoid tumor. Table 2 presents a summary of in vivo responses to prexasertib monotherapy treatment in CDX and PDX sarcoma models, as observed by Lilly.

Table 2. Summary of In Vivo Responses to Prexasertib Monotherapy in Neuroblastoma and Sarcoma Models, As Observed by Lilly

Tumor Type	Age of Patients, y ^a	Response to Prexasertib
Neuroblastoma	1-4	2/3 complete response 1/3 stable disease
Alveolar RMS	17-24	2/2 complete response Acquired resistance observed
Embryonal RMS	2-7	1/3 complete response 1/3 stable disease
DSRCT	20	1/1 complete response
Ewing's sarcoma	8-17	1/4 stable disease
Osteosarcoma	11-19	1/4 stable disease

Abbreviations: DSRCT = desmoplastic small round cell tumor; RMS = rhabdomyosarcoma.

^a Age range of patients at time of original tumor collection.

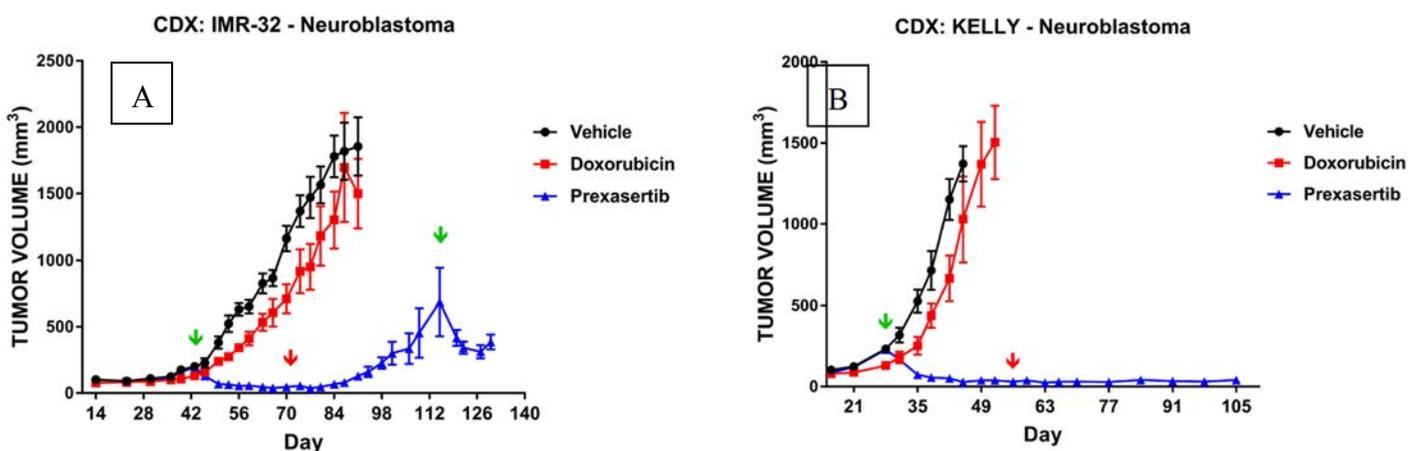
Source: May et al. 2015.

In addition, through a collaboration with the National Cancer Institute's (NCI's) pediatric preclinical testing program (PPTP) and pediatric preclinical testing consortium (PPTC), prexasertib was evaluated in additional childhood solid tumor and leukemia models. Consistent with Lilly-derived data, prexasertib demonstrated marked in vitro inhibition across a panel of 24 cell lines with a median IC₅₀ of 3.2nM. The most sensitive cell line was a *MYCN*-amplified neuroblastoma line. Similarly, when a dose comparable to that used in the Lilly experiments (10 mg/kg administered BID for 3 consecutive days) was used, 8 of the 12 models assessed had a complete response (CR) or partial response (PR) and prexasertib showed a similar level of tumor-regressing activity as irinotecan. Notably, a CR or PR was achieved in each of the 6 neuroblastoma models when prexasertib was administered as a monotherapy. Responses were also observed in a rhabdoid model and 1 of 2 RMS models. Insensitive models included

Ewing's sarcoma and osteosarcoma (n=2). Lilly continues to collaborate with the PPTC to extend these findings and understand what combination agents may further augment the observed monotherapy activity.

5.1.1. Neuroblastoma

As described in Section 5.1, neuroblastoma models were among the most sensitive to prexasertib. As a result, prexasertib was further investigated in several cell-line-derived xenograft mouse models of neuroblastoma. Within 24 hours, single-agent prexasertib promoted γ H2AX-positive double-stranded DNA breaks and phosphorylation of DNA damage sensors ATM kinase and DNA-dependent protein kinase catalytic subunit (DNA-PKcs), leading to neuroblastoma cell death in vitro. In vivo, neuroblastoma xenografts rapidly regressed following prexasertib administration, independent of the starting tumor volume (Figure 1). All told, when prexasertib is administered at a dose of 10 mg/kg BID, the combined data generated both at Lilly and at the PPTC indicate significant sensitivity to prexasertib in 9 of 9 models in neuroblastoma xenografts. Overall, these data demonstrate that prexasertib is a specific inhibitor of CHK1 in neuroblastoma and leads to DNA damage and cell death in preclinical models of this pediatric malignancy.



Abbreviation: CDX = mice bearing cell-derived xenograft.

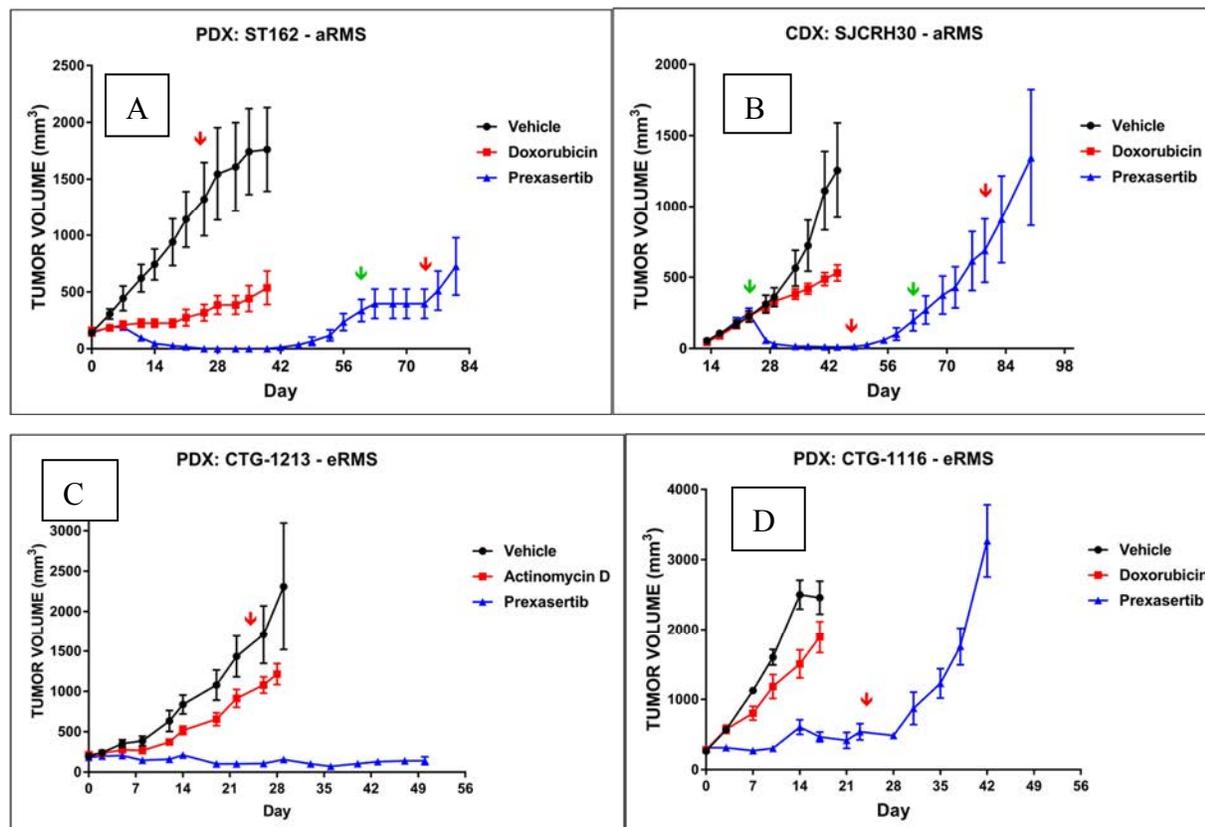
IMR-32 (A) and KELLY (B) xenograft regression was observed. Dosing interval for IMR-32 model was Days 43–62; dosing interval for KELLY model was Days 28–51. Unless otherwise indicated by a green arrow, the start of a treatment schedule was Day 0; a red arrow marks the end of a treatment period.

Figure 1. Prexasertib monotherapy treatment results in neuroblastoma regression.

5.1.2. Rhabdomyosarcoma

Prexasertib elicited strong monotherapy activity in 6 of 8 Lilly pediatric RMS in vivo models (alveolar and embryonal), ranging from stable disease (SD) (1/8) to complete regression (5/8), and in 1 of 2 PPTC in vivo models when prexasertib is administered at a dose of 10 mg/kg BID. Data from Lilly models are shown in Figure 2. In the SJCRH30 aRMS model, the complete

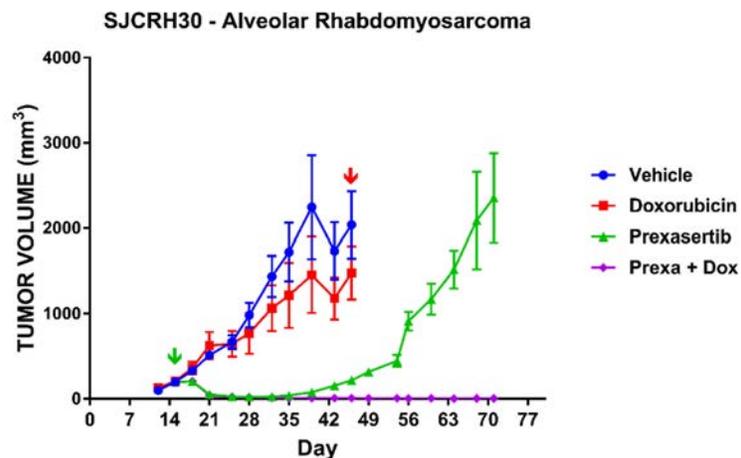
regression observed during treatment was reversed upon withdrawal of drug, and subsequent regrowth was resistant to further treatment with prexasertib at 10 mg/kg. The mechanism of this resistance is currently under investigation. In the SJCRH30 and the Rh41 models, prexasertib at a dose of 10 mg/kg plus doxorubicin, cyclophosphamide, or irinotecan led to a durable, complete regression with no development of acquired resistance. Representative prexasertib-plus-doxorubicin tumor regression data from the SJCRH30 model are shown in [Figure 3](#).



Abbreviations: aRMS = alveolar rhabdomyosarcoma; CDX = mice bearing cell-derived xenograft; eRMS = embryonal rhabdomyosarcoma; PDX = patient-derived xenograft.

Two aRMS xenograft models (A and B) are responsive to initial prexasertib monotherapy treatment. Upon tumor regrowth, both models were rechallenged with prexasertib. ST162 (A) responded with stable disease, while SJCRH30 (B) tumors were unresponsive. (C) A PDX model of eRMS responded with tumor regression to prexasertib treatment, while growth of another eRMS model (D) was delayed with prexasertib administration. Treatment commenced at Day 0 or as indicated by a green arrow; cessation of treatment is marked by a red arrow.

Figure 2. Efficacy of prexasertib as a monotherapy varies between subtypes of pediatric sarcoma.



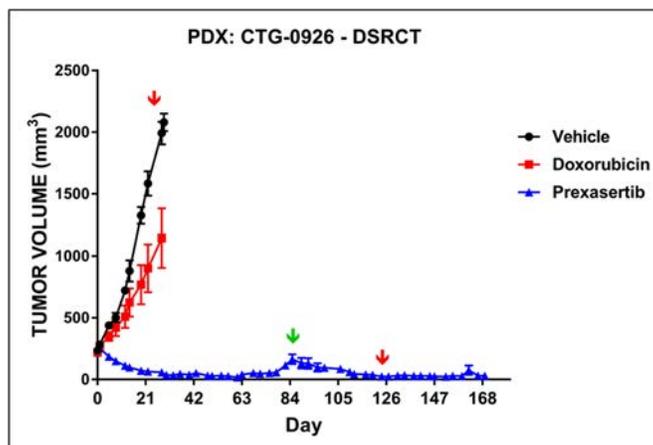
Abbreviations: aRMS = alveolar rhabdomyosarcoma; dox = doxorubicin; prexa = prexasertib.

The green arrow indicates dosing initiation and the red arrow marks the end of the treatment period.

Figure 3. Prexasertib in combination with doxorubicin prevents the development of acquired resistance in aRMS.

5.1.3. Other Pediatric Subtypes

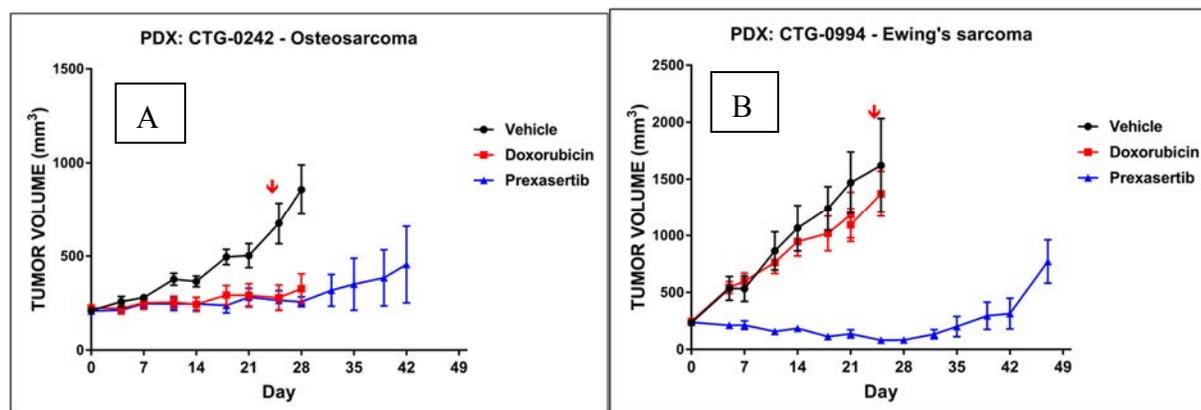
Although the strongest data, and therefore the focus of the proposed pediatric trial, are for neuroblastoma, eRMS, and aRMS, prexasertib also impedes the growth of other human PDX and CDX models of sarcoma. Prexasertib at 10 mg/kg BID elicited a CR as a monotherapy in 1 (of 1) pediatric DSRCT PDX model, tumor regression in 1 of 4 Ewing's sarcoma models, and SD in 1 of 4 osteosarcoma models (Figure 4 and Figure 5). Relatively insensitive tumor models in vivo include hepatoblastoma and retinoblastoma.



Abbreviations: DSRCT = desmoplastic small round cell tumor; PDX = patient-derived xenograft.

Residual DSRCT PDX tumors following treatment retained sensitivity to subsequent treatments. Treatment commenced at Day 0 or as indicated by a green arrow; cessation of treatment is marked by a red arrow.

Figure 4. Prexasertib monotherapy treatment in a PDX model of DSRCT.



Abbreviation: PDX = patient-derived xenograft. Growth of an osteosarcoma model (A) and a Ewing's sarcoma model (B) was delayed with prexasertib administration. The start of a treatment schedule was Day 0; a red arrow marks the end of a treatment period.

Figure 5. Efficacy of prexasertib as a monotherapy in pediatric bony tumors (osteosarcoma and Ewing's sarcoma).

5.2. Nonclinical Pharmacokinetics and Metabolism

Prexasertib exhibited dose-dependent exposure increase, rapid clearance, good tissue distribution, no accumulation following repeated dosing, and no sex-related differences in both rats and dogs. Multiple pathways (metabolism and excretion) are involved in the elimination of prexasertib.

5.3. Nonclinical Pharmacokinetic/Pharmacodynamic Model

An indirect response PK/PD model was developed to link prexasertib plasma concentrations with the inhibition of phosphorylated CHK1 (pCHK1) and tumor growth response in Calu-6 xenografts following prexasertib administration (Hong et al. 2016). The IC_{50} determined from the nonclinical monotherapy PK/PD model is 14.1 ng/mL. The predicted human systemic prexasertib exposure that correlates with the maximum tumor responses with prexasertib monotherapy is an area under the plasma concentration-versus-time curve from 0 to 72 hours ($AUC_{(0-72)}$) of 1896 ng·hr/mL.

5.4. Nonclinical Safety

The nonclinical safety assessment program for prexasertib is consistent with the International Conference on Harmonisation (ICH) S9 guidance for pharmaceuticals intended for treatment of advanced or life-threatening cancers (ICH 2009). The toxicity of prexasertib has been evaluated in repeat-dose toxicity studies (3 cycles of 3 daily doses, with 4 days between cycles, followed by a 2-week reversibility period) in adult rats and dogs to support early clinical development. Additional studies in adult animals are planned to support further clinical trials and global registration.

The toxicities of prexasertib in adult patients closely reflect those seen in nonclinical animal studies and are consistent with the expected pharmacology of prexasertib and the predicted effects of a cytotoxic therapeutic on proliferating cell populations. These effects are expected to be monitorable and clinically manageable in the context of treatment of advanced cancers in a pediatric patient population.

6. Clinical Trial Experience in Adults

6.1. Overview

Although prexasertib is in the early stages of clinical development, compelling clinical activity has been observed in subsets of patients, including those with high-grade serous ovarian cancer (HGSOC), and it is positioned to be a first-in-class CHK1 inhibitor. To date, the clinical data available from studies in adults support initiating studies in pediatric patients.

The current development strategy includes evaluating the molecule as a monotherapy, as a combination therapy with agents/modalities that cause genotoxic DNA damage (for example, cisplatin, radiation, and antimetabolites), and as a combination therapy with targeted agents (for example, cetuximab, ralimetinib [a p38 inhibitor], LY3023414 [a PI3K/mTOR inhibitor]). There are 11 ongoing or completed Phase 1 or 2 clinical trials (refer to [Table 3](#) and [Table 4](#)) in adults. (Refer to Section 9 for details regarding one ongoing Phase 1 trial in pediatric patients [NCT02808650].)

Results are available from 2 of the studies in adults and are summarized in Sections 6.2 and 6.3. In a Phase 1 assessment, hematologic toxicity was common, with neutropenia (93% [Grade 3/4, 90%]), leukopenia (82% [Grade 3/4, 71%]), anemia (69% [Grade 3/4, 31%]), thrombocytopenia (53% [Grade 3/4, 29%]) being reported. Notably, Grade 4 neutropenia was observed at all doses and schedules of treatment. Febrile neutropenia rates are approximately 10%. The neutrophil nadir consistently occurs approximately 1 week after each dose, and the duration of Grade 4 neutropenia is transient (typically <5 days). The transient nature of the neutropenia may account for why febrile neutropenia rates are not higher. Although prexasertib is administered every 14 days, the vast majority of patients did not require dose reductions or delays as a result of the neutropenia, but the neutropenia did not attenuate with repeat cycles of dosing. Interestingly, the extent and nature of prior therapy did not correlate with the extent of neutropenia. Current protocols allow for the use of granulocyte colony-stimulating factor (G-CSF), including prophylactic G-CSF, and in some patients this may reduce the extent and duration of neutropenia. It is not yet known whether the etiology of the neutropenia should be attributed to nonspecific cell cycles effects or is specific to inhibition of CHK1. Nonhematologic toxicities such as fatigue and gastrointestinal toxicities occur at a much lower frequency and are typically Grade 1 or 2 in severity. Durable objective responses have been observed in heavily pretreated patients with HNSCC, SCC of the anus, and HGSOC. (Hong et al. 2016; Lee et al. 2016)

[Table 3](#) and [Table 4](#) list the ongoing or completed studies in the prexasertib clinical trial program, including studies in patients with solid tumors, hematologic malignancy, and a Phase 1 study in pediatric patients.

Table 3. Lilly-Sponsored Clinical Trials with Prexasertib

Protocol Number	Study	
Phase	Status	Study Description
NCT Number		
I4D-MC-JTJA (JTJA) NCT 01115790 Phase 1	Completed	A dose-escalation study of prexasertib in patients with advanced cancer followed by cohort expansions in patients with squamous cell carcinomas
I4D-JE-JTJK (JTJK) NCT02514603 Phase 1	Ongoing	A dose-escalation study of prexasertib in Japanese patients
I4D-MC-JTJF (JTJF) NCT02124148 Phase 1	Ongoing	Dose escalation followed by cohort expansions of prexasertib in combination with chemotherapy or targeted agents in patients with advanced cancer
I4D-MC-JTJI (JTJI) NCT02555644 Phase 1	Ongoing	Dose escalation followed by cohort expansion of prexasertib in combination with chemotherapy and radiation in patients with locally advanced head and neck cancer
I4D-EW-JTJG (JTJG) NCT02778126 Phase 1	Ongoing	A [¹⁴ C] radiotracer study with prexasertib in patients with advanced cancer
I4D-MC-JTJL (JTJL) NCT02860780 Phase 1	Ongoing	A dose-escalation study of prexasertib and ralimetinib in patients with advanced cancer followed by cohort expansions of the combination in patients with <i>K-ras</i> - or <i>BRAF</i> -mutated colorectal cancer or non-small cell lung cancer
I4D-MC-JTJH (JTJH) NCT02735980 Phase 2	Ongoing	A Phase 2 study of prexasertib in patients with extensive stage small cell lung cancer who have either platinum-sensitive or platinum-resistant/refractory disease

Table 4. Exploratory Investigator-Sponsored Clinical Trials with Prexasertib

NCT Number	Study	
Phase	Status	Study Description
NCT02649764 Phase 1	Ongoing	A dose-escalation study of prexasertib in combination with cytarabine and fludarabine in patients with relapsed/refractory acute myelogenous leukemia or high-risk myelodysplastic syndrome
NCT03057145 Phase 1	Ongoing	A dose-escalation study of the combination of prexasertib and olaparib in patients with advanced solid tumors
NCT02808650 Phase 1	Ongoing	A dose-escalation study of prexasertib in pediatric patients with recurrent or refractory solid tumors
NCT02203513 Phase 2	Ongoing	A single-arm pilot study of prexasertib in patients with <i>BRCA1</i> -/ <i>BRCA2</i> -mutation-associated breast or ovarian cancer, non-high-risk triple-negative breast cancer, high-grade serous ovarian cancer at low genetic risk, and metastatic castrate-resistant prostate cancer
NCT02873975 Phase 2	Ongoing	A study of prexasertib in patients with advanced solid tumors with replicative stress or homologous repair deficiency

6.2. Study JTJA: First-in-Human Evaluation

The first-in-human Study JTJA consisted of a dose-escalation phase in patients with advanced or metastatic cancer followed by Phase 1b dose-expansion cohorts in patients with squamous cell carcinomas. A total of 146 patients were enrolled in this study, which is now completed.

The primary objective was to determine the recommended Phase 2 dose (RP2D) and schedule of prexasertib.

6.2.1. Dose Escalation

Prexasertib was administered as a 1-hour infusion starting at 10 mg/m² on Days 1 to 3 (schedule 1; n=27) or starting at 40 mg/m² on Day 1 (schedule 2; n=18) every 14 days. Dose escalation of prexasertib for schedules 1 and 2 established maximum tolerated doses (MTDs) of 40 mg/m² and 105 mg/m², respectively. All of the dose-limiting toxicities (DLTs) were hematologic in nature (Grade 4 neutropenia, thrombocytopenia, and/or leukopenia lasting >5 days, Grade 3 thrombocytopenia with bleeding [epistaxis], or febrile neutropenia). The most frequently observed adverse event (AE) was neutropenia, which was predominantly Grade 4 (73%) (Hong et al. 2016). At doses at or below the MTDs, the duration of Grade 4 neutropenia was transient (typically <5 days). Three patients (7%) experienced febrile neutropenia. There were no deaths or discontinuations as a result of febrile neutropenia. Two of the 43 evaluable patients had a PR (4.4%) and 15 patients (33.3%) had SD; both patients with an objective response had SCC (HNSCC and SCC of the anus) (Hong et al. 2016).

Based on comparisons of the safety, efficacy, predicted target inhibition, and PK/PD simulations, the Day 1 dosing schedule every 14 days at a dose of 105 mg/m² was selected for further evaluation in the expansion cohorts consisting of patients with SCC.

6.2.2. Expansion Cohorts

The expansion cohorts included 57 patients with recurrent/metastatic HNSCC and demonstrated that prexasertib had an acceptable safety profile to support further investigation with modest preliminary activity in heavily pretreated patients. Consistent with what was observed in dose escalation, the most frequent AE was a transient decrease in neutrophil/leukocyte count, which occurred in 91% of patients (Grade 4 in 63% of patients). Ten patients (18%) experienced febrile neutropenia. No patients discontinued treatment or died due to febrile neutropenia. Other study drug-related AEs occurring in >10% of patients included thrombocytopenia (44%), anemia (25%), fatigue (23%), and headache (14%). The majority of nonhematologic AEs were Grade 1 or 2 in severity. Three patients (5%) had a PR and 25 patients (44%) had SD. The duration of response for the 3 patients with an objective response was 4.8, 7.0, and 12.4 months. (Bendell et al. 2015b)

The expansion cohorts also contained 26 patients with metastatic or recurrent SCC of the anus. Again, the most frequently reported AE was neutropenia, which occurred in 92% of patients (Grade 4: 77%); 1 patient (4%) experienced febrile neutropenia. Other drug-related AEs included thrombocytopenia (58%), anemia (38%), and fatigue (31%). Nonhematologic AEs included nausea (19%), diarrhea (15%), and anorexia and headache (12% each), which were

mostly Grade 1 or 2. One patient (4%) had a CR, 3 patients (12%) had a PR, and 11 patients (42%) had SD, for an overall response rate (ORR) of 15% and a disease-control rate (DCR) of 58%. The median duration of disease control was 4.2 months. The median PFS was 2.8 months. One patient had a prolonged duration of response (10.1 months) before discontinuing therapy. (Bendell et al. 2015a)

6.2.3. Pharmacokinetic Summary

Analysis of prexasertib PK data from adult cancer patients in Study JTJA across the dose range of 10 to 130 mg/m² demonstrated a multicompartment PK profile with dose- and time-independent PK behavior, as well as consistent PK behavior within (intra) and between (inter) patients after single- and multiple-dose administration using 2 different schedules of administration (Hong et al. 2016). There was no intercycle accumulation of prexasertib between Cycle 1 and Cycle 2 following the adult RP2D. A body surface area (BSA)-based dose regimen was shown to be appropriate and justified for prexasertib administration.

The systemic exposure following administration of 105 mg/m² is greater than the median systemic exposure (AUC₍₀₋₇₂₎) predicted before clinical investigation to achieve the maximal monotherapy tumor response with prexasertib (Section 5.3; Hong et al. 2016). Moreover, the average plasma concentration over the first 72 hours following 105 mg/m² is greater than the IC₅₀ determined from the nonclinical Calu-6 xenograft PK/PD model indicating that the prexasertib exposure achieved after 105 mg/m² is in a predicted therapeutic range (Section 5.3; Hong et al. 2016). Therefore, the prexasertib PK profile following 105 mg/m² every 14 days (RP2D) was determined to be suitable for achieving the median exposure predicted for maximal monotherapy tumor response in nonclinical xenograft models, while minimizing the accumulation of prexasertib after repeat administration.

6.2.4. Biomarker Summary

To identify genomic biomarkers associated with single-agent drug response, pretreatment tissues (archived or biopsy) from patients with HNSCC or SCC of the anus in Study JTJA were analyzed by next-generation sequencing. The clinical biomarker findings support the hypothesis that oncogene-induced replication stress (that is, arising from HPV E6/E7 and/or PARK2/FBXW7 loss-dependent cyclin E1 dysregulation) in the context of attenuated DDR (that is, *BRCA1/BRCA2*, *MRE11A*, or *ATR* mutations) may sensitize patients to prexasertib monotherapy. (Martinez et al. 2017)

6.3. NCI-Sponsored Phase 2 Study in Patients with Ovarian Cancer

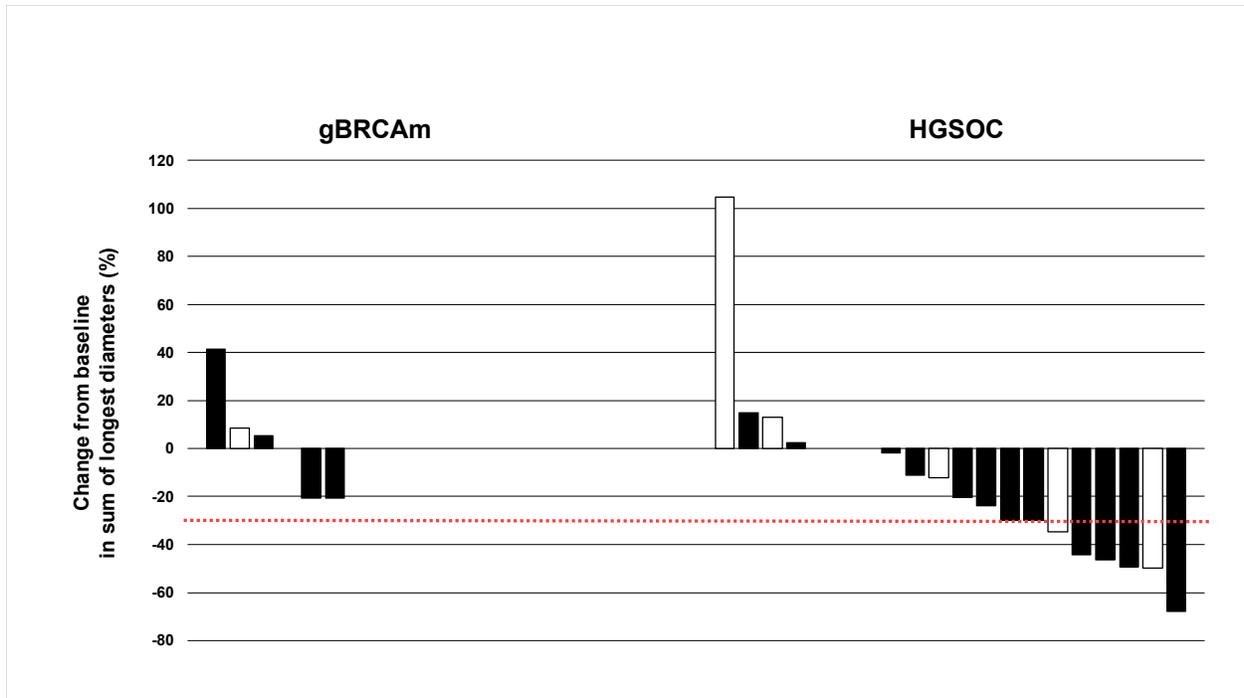
The NCI (principal investigator: Jung-min Lee, MD) is sponsoring a Phase 2 pilot study with a primary objective of determining the ORR of prexasertib monotherapy in patients with breast cancer, ovarian cancer, or prostate cancer (NCT02203513). The estimated enrollment for the study is up to 144 patients. Patients receive prexasertib on Days 1 and 15 of a 28-day cycle at the RP2D of 105 mg/m² (NCI 2017b).

Preliminary results from 32 heavily pretreated female patients with HGSOC were presented at the meeting of the European Society for Medical Oncology (ESMO) in October 2016 (Lee et al.

2016). The interim data included 7 patients in Group 1 (patients with a documented deleterious germline *BRCA1/BRCA2* mutation [gBRCA1/2m]) and 25 patients in Group 2 (patients with a negative family history of hereditary breast ovarian cancer syndrome, or negative gBRCA1/2m test) (Lee et al. 2016).

Transient Grade 4 neutropenia was observed in 69% of patients and resolved to Grade ≤ 2 within 8 days after onset. Two patients (6%) had febrile neutropenia. A total of 56% patients received G-CSF. Other toxicities included decreased white blood cell count (78%), anemia (66%), and decreased platelet count (34%). Nonhematologic AEs were generally mild and included fatigue, nausea, vomiting, and diarrhea (9% each). One patient experienced Grade 3 diarrhea and vomiting during infusion. Only 1 patient (3%) required a dose reduction (80 mg/m²). (Lee et al. 2016)

Figure 6 presents a plot of best responses for target lesions by patient, among patients treated with prexasertib. Of the 25 patients treated in Group 2, 20 were evaluable for tumor response. A total of 7 (35%) evaluable patients achieved PR (including 2 whose disease was platinum-sensitive and 5 whose disease was platinum-resistant or -refractory). The median duration of response was 6 months [range: 2-13 months], and 5 (25%) patients achieved SD lasting at least 4 months, for a DCR of 60%. Notably, an expected response rate for this patient heavily pretreated population with existing agents is approximately 15%. Of the 7 patients treated in Group 1, 6 were evaluated for tumor response. None of the evaluable patients in Group 1 achieved CR or PR, but 4 (67%) achieved SD lasting at least 4 months, for a DCR of 67%. These preliminary results suggest that prexasertib could be a novel approach to improve the outcomes of patients with HGSOc. (Lee et al. 2016)



Abbreviations: *gBRCAm* = germline *BRCA* mutated; HGSOC = high-grade serous ovarian cancer.
White bars signify platinum-sensitive disease; black bars signify platinum-resistant or -refractory disease.

Figure 6. Best response for target lesions by patient, among patients treated with prexasertib.

7. Background and Rationale for Pediatric Indications

7.1. Overview

Although initial treatments may result in benefit, options for pediatric patients with neuroblastoma or RMS whose disease relapses or is refractory to treatment are limited. As described in Section 5.1, the most compelling nonclinical data with prexasertib are in models of neuroblastoma and both eRMS and aRMS. In addition, it is hypothesized that tumors having high levels of replication stress and/or defects in DNA damage repair pathways will be susceptible to the effects of a CHK1 inhibitor such as prexasertib. As described in Sections 7.2 and 7.3, alterations found in neuroblastoma or RMS such as *MYCN* amplification/overexpression or deletion of 11q may increase replication stress and/or result in impaired DDR, providing further rationale for testing prexasertib in these tumors.

7.2. Neuroblastoma

7.2.1. Overview and Current Treatments

Current therapy for high-risk neuroblastoma (>18 months of age and having metastatic or locoregional disease with evidence of *MYCN* amplification) is multimodal, including chemotherapy, surgery, external beam radiotherapy, and myeloablative chemotherapy with autologous stem cell transplant. Therapy with 13-cis-retinoic acid, dinutuximab, or ¹³¹I-meta-iodobenzylguanidine (MIBG) may also be used. Approximately 70% of those receiving initial therapy for high-risk disease subsequently relapse or never achieve remission; for these patients, there is no standard of care and new therapies are needed. Cytotoxic chemotherapy regimens for relapsed and refractory neuroblastoma typically revolve around the use of topotecan or irinotecan, in combination with agents such as cyclophosphamide and temozolomide. Although these regimens affect objective tumor responses, long-term survival is poor. (Morgenstern et al. 2013)

7.2.2. Selected Genetic Features Relevant to CHK1 Inhibition

The most consistent genetic alteration in neuroblastoma is amplification of the *MYCN* oncogene on chromosome 2p24, which occurs in 22% of tumors (Newman and Nuchtern 2016). Overexpression of *MYC* is associated with elevated replication stress possibly due to excessive use of replication origins, depletion of cofactors necessary for sustained replication, and transcription-replication collision (Rohban and Campaner 2015). The ATR-CHK1 pathway is critical for suppression of oncogene-induced replication stress (Lecona and Fernández-Capetillo 2014; Dobbstein and Sørensen 2015), and reliance on this pathway for tolerance of replication stress in neuroblastoma is supported by elevated CHK1 expression in *MYCN*-amplified primary tumors (Cole et al. 2011).

As a result, it is hypothesized that tumors with *MYCN* amplification will have increased sensitivity to a CHK1 inhibitor such as prexasertib. This hypothesis is supported by published data in which an RNA interference (RNAi) screen of neuroblastoma cell lines identified CHK1 loss as potentially inhibiting proliferation, and sensitivity to CHK1 inhibition positively correlated

with total *MYC* (*MYC* and *MYCN*) expression (Cole et al. 2011). Furthermore, expression of *MYCN* in non-neuroblastoma retinal epithelial cells induced CHK1 autophosphorylation and increased sensitivity to CHK1 inhibitor (Cole et al. 2011). In a *MYCN*-driven transgenic mouse model of neuroblastoma, CHK1 inhibitor CCT244747 demonstrated monotherapy antitumor efficacy (Walton et al. 2012).

MYCN amplification is inversely correlated with deletion of 11q, which is a recurrent segmental chromosomal aberration occurring in 40% of neuroblastoma tumors (Newman and Nuchtern 2016). The DDR gene *ATM* is located in the most frequently deleted region in neuroblastoma, namely 11q22-q23, and 14/50 tumor samples were found to have complete hemizygous *ATM* deletion (Mandriota et al. 2015). *ATM* is critical for the cellular response to double-stranded DNA breaks, and cells lacking *ATM* are particularly vulnerable to inhibition of ATR (Kwok et al. 2016), a kinase that functions upstream of CHK1 in the response to replication stress and single-stranded DNA breaks. Treatment with prexasertib induces DNA breaks (King et al. 2015) and activates *ATM* (Section 5.1.1), but further research is needed to understand whether *ATM* loss confers increased sensitivity to CHK1 inhibition. Other genes integral to the DDR such as *H2AFX* and *MRE11A* are also located on the distal arm of 11q. Loss of function mutations in *MRE11A* have been observed in patients deriving clinical benefit from prexasertib.

Collectively, these data suggest that subsets of neuroblastoma tumors will have increased replication stress and/or defects in DNA damage repair pathways and provide additional rationale for the investigation of prexasertib monotherapy in neuroblastoma.

7.3. RMS

7.3.1. Overview and Current Treatments

In general, a multimodal treatment approach is used in RMS, which often includes systemic chemotherapy in conjunction with either surgery or radiation therapy, or both, to maximize local tumor control (NCI 2017c). In COG studies, the standard chemotherapy consists of vincristine, actinomycin d, and cyclophosphamide. Chemotherapeutic agents used in recurrent RMS include carboplatin, doxorubicin, etoposide, ifosfamide, cyclophosphamide, topotecan, irinotecan, and vincristine. Although advances have been made in improving the outcomes of patients with low- and intermediate-risk disease, little progress has been made in children with high-risk metastatic RMS.

7.3.2. Selected Genetic Features Relevant to CHK1 Inhibition

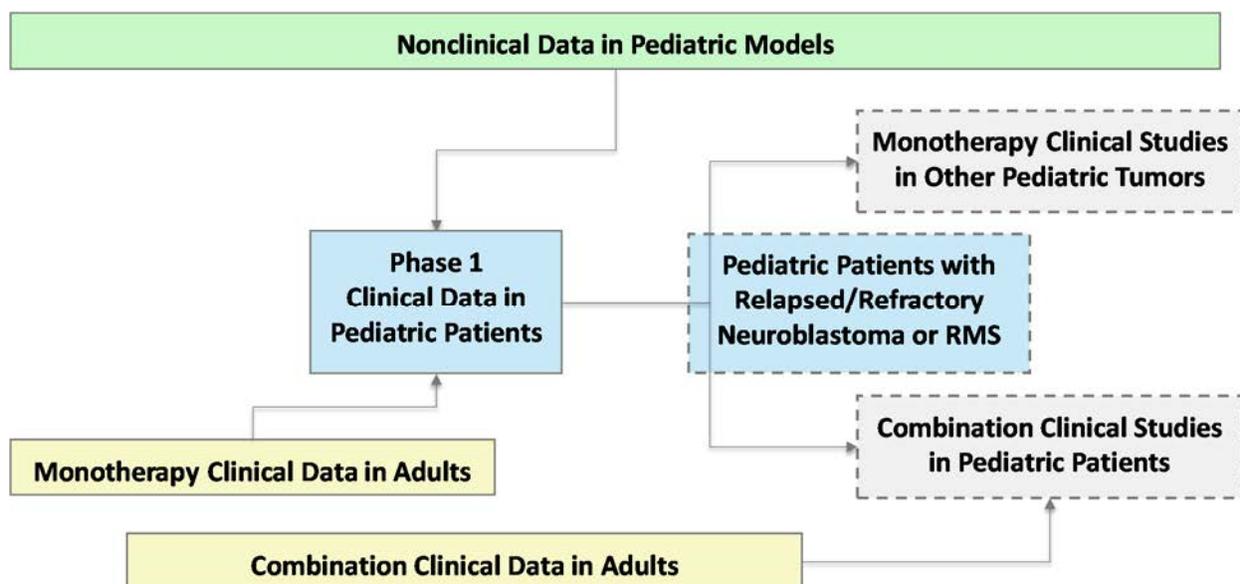
As described in Section 7.2.2, it is hypothesized tumors with *MYCN* amplification will have increased sensitivity to a CHK1 inhibitor such as prexasertib. Similar to neuroblastoma, amplification of *MYCN* has been observed in both aRMS and eRMS (Williamson et al. 2005; Malempati and Hawkins 2012). Additionally, translocations t(2;13) and t(1;13) that result in *PAX3/FOXO1* and *PAX7/FOXO1* fusion genes are often observed in aRMS (80%) (Ognjanovic et al. 2009; Malempati and Hawkins 2012). *PAX3-FOXO1* or *PAX7-FOXO1* fusion genes correlate with *MYCN* amplification (25%) or *MYCN* overexpression (55%) and poor prognosis in aRMS (Tonelli et al. 2012). The potential of *MYCN* amplifications and

PAX3-FOXO1 or *PAX7-FOXO1* fusions to increase replication stress provides additional rationale for the investigation of prexasertib in eRMS and aRMS.

8. Clinical Pediatric Development Plan

Prexasertib has demonstrated activity both as a monotherapy and in combination with other agents in multiple nonclinical models of pediatric cancers. In clinical studies in adult patients, monotherapy efficacy has been observed and the safety profile is acceptable for development in pediatric patients. Additionally, the capacity to combine with radiation, cytotoxic chemotherapy, and targeted agents is being assessed. These data support clinical evaluation in pediatric patients and a Phase 1 study to determine the RP2D in pediatric patients with recurrent or refractory solid tumors has been initiated by the COG (Section 9.1).

To date, the most consistent nonclinical data have been observed in models of neuroblastoma and RMS. As a result, once a pediatric dose has been established in the ongoing Phase 1, a Phase 2 study assessing prexasertib as a monotherapy in pediatric patients with relapsed/refractory neuroblastoma or eRMS/aRMS is proposed. The pediatric monotherapy clinical data, ongoing nonclinical experiments, and tolerability of combinations in adult patients will be used to inform future potential additional solid tumors, and/or combination studies in pediatric patients. Due to the safety profile of prexasertib, hematologic malignancies may be an attractive setting to assess prexasertib. An ongoing adult clinical study is assessing prexasertib in combination with cytotoxic chemotherapy in patients with acute myelogenous leukemia and these data will be used to inform potential future studies in pediatric hematologic malignancies. The clinical pediatric development plan for prexasertib is illustrated in Figure 7.



Abbreviation: RMS = rhabdomyosarcoma.

Yellow boxes represent adult clinical studies; blue boxes represent monotherapy in pediatric patients.

Figure 7. Illustration of prexasertib clinical pediatric development plan.

For details regarding current or potential challenges that have been identified regarding clinical trials in children, refer to Appendix 2 (Section 13).

9. Clinical Trials in Pediatrics

9.1. Ongoing Pediatric Clinical Trial: Children's Oncology Group-Sponsored Phase 1 Study

A Phase 1 study sponsored by the COG and entitled “A Phase 1 Study of LY2606368, a CHK1/2 Inhibitor, in Pediatric Patients With Recurrent or Refractory Solid Tumors, Including CNS Tumors” (study chair: Cynthia Wetmore) (NCT02808650) started enrollment in the United States in March 2017 (NCI 2017a). No data have been publically disclosed and no other studies in pediatric patients with prexasertib are currently ongoing.

The primary objectives are to estimate the MTD and/or RP2D, define and describe the toxicities of prexasertib, and characterize PK. Secondary objectives include preliminarily defining the antitumor activity of prexasertib and biomarker assessments.

Prexasertib is administered as a 1-hour intravenous infusion on Days 1 and 15 of a 28-day cycle. The rolling six Phase 1 trial design is used for dose escalation (Skolnik et al. 2008). Patients in the initial cohort will be treated at a dose of 80 mg/m² (predicted exposure less than the exposure at the adult RP2D; see Section 9.2.3). Up to 13 cycles may be administered, provided there is no disease progression or unacceptable toxicity.

Selected entry criteria are outlined below and a more comprehensive list is available at www.clinicaltrials.gov. Patients must have recurrent or refractory solid tumors, including central nervous system tumors for which there is no known curative therapy or therapy proven to prolong survival with an acceptable quality of life. Patients must be ≥ 12 months and ≤ 21 years of age, have an adequate performance status by Karnofsky or Lansky, be fully recovered from the acute toxic effects of all prior anticancer therapy, and have adequate hematologic, kidney, hepatic, and cardiac function. Tissue blocks or slides will be collected.

Patients will be excluded if they have an uncontrolled infection, received a prior solid organ transplantation, are pregnant or breastfeeding women, or are receiving prohibited concomitant treatments (for example, another investigational drug, other anticancer agents).

9.2. Proposed Pediatric Clinical Trial

9.2.1. Design and Objectives

The proposed study is a multicenter, nonrandomized, parallel-cohort Phase 2 study of prexasertib in pediatric patients with either neuroblastoma or eRMS/aRMS with relapsed or refractory disease and for whom no standard therapy is available. Patients will receive prexasertib every 14 days, at the recommended Phase 2 pediatric dose. The primary objective will be to estimate the investigator-assessed ORR. Other objectives will include assessment of safety, toxicity, PK, biomarker associations, and patient-reported outcomes; an independent central assessment of ORR; and estimation of secondary efficacy measures, such as DCR, duration of response, event-free survival (EFS), and overall survival (OS).

Cohort 1 will include children and adolescents with relapsed or refractory neuroblastoma while Cohort 2 will include patients with relapsed or refractory RMS.

9.2.2. Key Entry Criteria

Eligible patients will have a diagnosis of refractory or relapsed neuroblastoma (Cohort 1) or eRMS/aRMS (Cohort 2) and not be an appropriate candidate for surgery, radiotherapy, or other conventional systemic therapy. Patients will be ≥ 12 months and ≤ 21 years of age. They should have measurable or evaluable disease, discontinued all previous treatments for cancer, and recovered from the acute effects of therapy and meet protocol-defined intervals from prior therapy. The patients will have adequate performance status as defined by Lansky or Karnofsky and adequate hematologic, kidney, hepatic, and cardiac organ function as defined in the protocol.

Patients will be excluded if they have received more than 2 prior therapies for relapsed or refractory disease or have a known serious concomitant systemic disorder (for example, active infection).

9.2.3. Dose Selection

Pediatric dose selection will leverage the pediatric PK data from the ongoing Phase 1 study sponsored by the COG with the adult prexasertib population PK (pop PK) model and allometric scaling from adults to children. It is assumed that systemic exposures achieved in adult patients at 105 mg/m^2 every 14 days (adult RP2D) will be achieved in pediatric patients and will provide similar safety and efficacy profiles to adults. If systemic exposures greater than the adult RP2D are safely achieved at the pediatric MTD, this pediatric dose level may be chosen in order to maximize the systemic exposure and the potential for efficacy in pediatric patients.

In the adult monotherapy patient trial (Study JTJA) that was used to build a pop PK model, the lower end of the BSA value range was approximately 1.2 m^2 (31 kg). Therefore, a BSA of 1.2 m^2 was used as the upper limit for pediatric dose projections based on the allometric projection of systemic clearance (CL). A dose of 105 mg/m^2 is recommended for patients with BSA values $\geq 1.2 \text{ m}^2$, since PK simulations using the adult pop PK model demonstrate similar and overlapping exposure of prexasertib in patients with BSA values $\geq 1.2 \text{ m}^2$. As shown in [Table 5](#), prexasertib pediatric doses across various BSA ranges were identified to provide the equivalent systemic exposure to the adult RP2D. Note that the projected doses based on allometry in patients with BSA values $< 1.2 \text{ m}^2$ are considered to be a more conservative estimate, compared with extrapolating dose projections using the adult pop PK model linear regression analysis between the post hoc predicted CL values and BSA, which predicts higher starting doses in patients with BSA values $< 1.2 \text{ m}^2$.

Table 5. Prexasertib Exposures across Various BSA Ranges

Dose, mg/m ²	BSA Range, m ²	Approximate Age, y
90	0.50 to <0.60	1-2
95	0.60 to <1.2	3-11
105	≥1.2	>11

Abbreviation: BSA = body surface area.

Because the adult human clearance pathways have not been determined, prexasertib dosing is not planned in patients less than 1 year of age due to immature development (ontogeny) in cytochrome P450 (CYP) enzymes (Hines and McCarver 2002), which could impact the metabolic clearance of prexasertib.

Once human clearance pathways from the human ¹⁴C study (NCT02778126) have been determined, this information will be combined with available pediatric PK data to create a physiologically based PK model. This model may further refine the pediatric dose selection across age ranges and account for any CYP ontogeny (that is, ontogeny simulated using an age-based maturation function to account for developmental changes in hepatic CYP metabolism of prexasertib). It may also help inform which physiological factor(s) alter the prexasertib PK and how these might differ across pediatric age groups. Pediatric population simulations could then be performed in order to estimate the mean and range of systemic exposure across the projected doses for each age and BSA range.

9.2.4. Safety Assessments

Safety parameters, including AEs, physical examinations, vital signs, clinical laboratory tests, and ECGs, will be assessed throughout the study. Safety assessments will also be included in the long-term follow-up to monitor for nonacute toxicities. It is anticipated the weekly assessment of hematologic parameters will be required during Cycles 1 and 2. The aggregate safety data will be periodically reviewed.

9.2.5. PK Assessments

Pharmacokinetic samples will be collected for prexasertib following multiple cycles of treatment at designated time points to characterize the PK profile in pediatrics. Collectively, these data will be used to determine the population PK parameters for prexasertib in plasma (clearance, volume of distribution) and interindividual PK variability using nonlinear mixed-effect modeling implemented in NONMEM in order to describe the dose-concentration relationship in the target population. Covariate effects (such as age, weight, sex, etc) on the PK parameters of prexasertib will also be investigated.

9.2.6. Efficacy Assessments

Efficacy assessments will occur at baseline (within 28 days of first dose) and 8-week intervals thereafter. Patients will remain on treatment until they fulfill a criterion for discontinuation (for example, evidence of progressive disease, unacceptable toxicity). The modified International Neuroblastoma Response Criteria (INRC) (Cohort 1) or Response Evaluation Criteria in Solid

Tumors, Version 1.1 (RECIST 1.1) (Cohort 2) will be used for efficacy assessments (Brodeur et al. 1993; Eisenhauer et al. 2009). In addition, for Cohort 1, responses on MIBG scan will use a modified Curie score (Messina et al. 2006). Following treatment discontinuation, patients will continue to be followed for survival status.

9.2.7. Statistical Considerations

The study will enroll approximately 110 patients (55 in each cohort). The primary objective of the study is to estimate the ORR.

For each cohort, if the lower bound of a 95% confidence interval on the observed ORR excludes (is greater than) 15%, then prexasertib is considered to have superior ORR compared to the historical controls. A sample size of 55 patients for each cohort will provide at least 80% power to detect an increase in ORR from 15% to 30%.

A futility interim analysis is planned after the 20th patient has been enrolled and received the first postbaseline tumor assessment for each cohort. If 2 or fewer responders are observed in the first 20 patients, the cohort will be terminated for futility.

9.3. Combination Strategy for Future Trials

New treatments for pediatric tumors should provide a pathway to a cure. Although the initial assessment with prexasertib is as a monotherapy, it is recognized that combination therapy forms the basis for the most effective treatment approaches in pediatric tumors. As a result, identifying agents that can be safely and effectively combined with prexasertib is an important aim of the pediatric strategy. In nonclinical pediatric models, prexasertib has demonstrated increased efficacy when administered in combination with cytotoxic chemotherapeutic agents such as doxorubicin, cyclophosphamide, and irinotecan (Lowery et al. 2017). Clinical studies are ongoing in adult patients to understand the tolerability of prexasertib in combination with radiation and/or cytotoxic chemotherapy. In addition, the clinical monotherapy safety profile, and in particular the extent of hematologic toxicity in pediatric patients will be defined in the ongoing study sponsored by the COG. These assessments will provide an understanding of the types of cytotoxic chemotherapy agents that can be considered in future pediatric studies.

However, the emerging biology of CHK1 also provides a strong rationale to combine with targeted agents and ongoing clinical assessments in adults are assessing the tolerability of prexasertib with targeted agents (for example, PI3K/mTOR inhibitor). Given the low level of hematologic toxicity of many targeted agents, there may be attractive combination partners for prexasertib in future pediatric trials. Once the clinical monotherapy safety profile is defined in pediatric patients and there is an understanding of the monotherapy toxicity profile in pediatric patients, this information will be used to determine the combination strategy with targeted agents in pediatric tumors.

10. Overall Summary/Conclusions

In summary, prexasertib is positioned to be a first-in-class CHK1 inhibitor. The novel mechanism of action may provide a new approach to improving therapeutic options for pediatric cancer patients. The compelling nonclinical data in pediatric models and the safety and efficacy profile from adult clinical studies provide strong rationale to assess prexasertib in pediatric tumors. The results from an ongoing Phase 1 evaluation to determine the RP2D and safety profile of prexasertib will inform the proposed study in pediatric patients with neuroblastoma and RMS. Emerging nonclinical data and information from adult combination trials will inform additional pediatric tumors and/or combinations where prexasertib may be assessed.

As prexasertib's pediatric development plan is developed, both internal Lilly nonclinical research capabilities as well as external collaborations with PPTC, the IMI (Appendix 1, Section 12), and COG will be leveraged to inform the optimal development of this promising new molecule that hopefully will improve therapeutic options for pediatric patients with cancer.

11. References

- American Childhood Cancer Organization. Beltsville, MD. Neuroblastoma. Available at: <http://www.acco.org/neuroblastoma>. Accessed April 20, 2017.
- Bendell JC, Eng C, Moore KN, Burris HA, Meric-Bernstam F, Jones SF, Piha-Paul SA, Infante JR, Subbiah V, Bauer TM, Golden L, Lin J, Martinez R, Wijayawardana S, Lin AK, Hong DS. Checkpoint kinase (CHK) 1/2 inhibitor LY2606368 in a phase I, dose-expansion study in patients (pts) with metastatic squamous cell carcinoma (mSCC) of the anus. *J Clin Oncol*. 2015a;33(suppl): Abstract 3520.
- Bendell J, Grant S, Janku F, Infante J, William WN, Bauer TM, Piha-Paul S, Martinez R, Wijayawardana S, Lin J, Golden L, Lin AB, Hong D. Checkpoint kinase (CHK) 1/2 inhibitor LY2606368 in a phase I dose-expansion study in patients with squamous cell carcinoma of the head and neck. AACR American Association for Cancer Research 2015 Annual Meeting; April 18-22, 2015b; Philadelphia, PA. Abstract CT240.
- Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V, Castelberry RP, De Bernardi B, Evans AE, Favrot M, Hedborg F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. *J Clin Oncol*. 1993;11(8):1466-1477.
- Cole KA, Huggins J, Laquaglia M, Hulderman CE, Russell MR, Bosse K, Diskin SJ, Attiyeh EF, Sennett R, Norris G, Laudenslager M, Wood AC, Mayes PA, Jagannathan J, Winter C, Mosse YP, Maris JM. RNAi screen of the protein kinome identifies checkpoint kinase 1 (CHK1) as a therapeutic target in neuroblastoma. *Proc Natl Acad Sci U S A*. 2011;108(8):3336-3341.
- Dai Y, Grant S. New insights into checkpoint kinase 1 in the DNA damage response signaling network. *Clin Cancer Res*. 2010;16(2):376-383.
- Dobbelstein M, Sørensen CS. Exploiting replicative stress to treat cancer. *Nat Rev Drug Discov*. 2015;14(6):405-423.
- Domchek SM, Aghajanian C, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, Balmaña J, Mitchell G, Fried G, Stemmer SM, Hubert A, Rosengarten O, Loman N, Robertson JD, Mann H, Kaufman B. Efficacy and safety of olaparib monotherapy in germline BRCA1/2 mutation carriers with advanced ovarian cancer and three or more lines of prior therapy. *Gynecol Oncol*. 2016;140(2):199-203.
- Eisenhauer EA, Therasse P, Bogaert J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2) 228-247.
- Ghelli Luserna Di Rorà A, Iacobucci I, Imbrogno E, Papayannidis C, Derenzini E, Ferrari A, Guadagnuolo V, Robustelli V, Parisi S, Sartor C, Abbenante MC, Paolini S, Martinelli G. Prexasertib, a Chk1/Chk2 inhibitor, increases the effectiveness of conventional therapy in B-/T-cell progenitor acute lymphoblastic leukemia. *Oncotarget*. 2016;7(33):53377-53391.
- Hines RN, McCarver DG. The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. *J Pharmacol Exp Ther*. 2002;300(2):355-360.

- Hong D, Infante J, Janku F, Jones S, Nguyen LM, Burris H, Naing A, Bauer TM, Piha-Paul S, Johnson FM, Kurzrock R, Golden L, Hynes S, Lin J, Lin AB, Bendell J. Phase I study of LY2606368, a checkpoint kinase 1 inhibitor, in patients with advanced cancer. *J Clin Oncol*. 2016;34(15):1764-1771.
- [ICH] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline: Nonclinical Evaluation for Anticancer Pharmaceuticals S9, October 29, 2009. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S9/Step4/S9_Step4_Guideline.pdf. Accessed April 18, 2017.
- King C, Diaz HB, McNeely S, Barnard D, Dempsey J, Blosser W, Beckmann R, Barda D, Marshall MS. LY2606368 causes replication catastrophe and antitumor effects through CHK1-dependent mechanisms. *Mol Cancer Ther*. 2015;14(9):2004-2013.
- Kwok M, Davies N, Agathangelou A, Smith E, Oldreive C, Petermann E, Stewart G, Brown J, Lau A, Pratt G, Parry H, Taylor M, Moss P, Hillmen P, Stankovic T. ATR inhibition induces synthetic lethality and overcomes chemoresistance in TP53- or ATM-defective chronic lymphocytic leukemia cells. *Blood*. 2016;127(5):582-595.
- Lecona E and Fernández-Capetillo O. Replication stress and cancer: it takes two to tango. *Exp Cell Res*. 2014;329(1):26-34.
- Lee J-M, Karzai FH, Zimmer A, Annunziata CM, Lipkowitz S, Parker B, Houston N, Ekwede I, Kohn EC. A phase II study of the cell cycle checkpoint kinases 1 and 2 inhibitor (LY2606368; prexasertib monomesylate monohydrate) in sporadic high-grade serous ovarian cancer (HGSOC) and germline *BRCA* mutation-associated ovarian cancer (gBRCAm+ OvCa). Paper presented at: European Society for Medical Oncology (ESMO) 2016 Congress; October 7-11, 2016; Copenhagen, Denmark. *Ann Oncol*. 2016;27 (suppl 6): 855O.
- Lin AB, McNeely SC, Beckmann RP. Achieving precision death with cell cycle inhibitors that target DNA replication and repair. *Clin Cancer Res*. 2017 Mar 22. pii: clincanres.0083.2016. doi: 10.1158/1078-0432.CCR-16-0083. [Epub ahead of print]
- Lowery CD, VanWye AB, Dowless M, Blosser W, Falcon BL, Stewart J, Stephens J, Beckmann RP, Bence Lin A, Stancato LF. The Checkpoint Kinase 1 Inhibitor Prexasertib Induces Regression of Preclinical Models of Human Neuroblastoma. *Clin Cancer Res*. 2017 Mar 7. pii: clincanres.2876.2016. doi: 10.1158/1078-0432.CCR-16-2876. [Epub ahead of print]
- Malempati S, Hawkins DS, Rhabdomyosarcoma: review of the Children's Oncology Group (COG) Soft-Tissue Sarcoma Committee experience and rationale for current COG studies. *Pediatr Blood Cancer*. 2012;59(1):5-10.
- Mandriota SJ, Valentijn LJ, Lesne L, Betts DR, Marino D, Boudal-Khoshbeen M, London WB, Rougemont AL, Attiyeh EF, Maris JM, Hogarty MD, Koster J, Molenaar JJ, Versteeg R, Ansari M, Gumy-Pause F. Ataxia-telangiectasia mutated (ATM) silencing promotes neuroblastoma progression through a MYCN independent mechanism. *Oncotarget*. 2015;6(21):18558-18576.
- Manic G, Signore M, Sistigu A, Russo G, Corradi F, Siteni S, Musella M, Vitale S, De Angelis ML, Pallocca M, Amoreo CA, Sperati F, Di Franco S, Barresi S, Policicchio E, De Luca G, De Nicola F, Mottolese M, Zeuner A, Fanciulli M, Stassi G, Maugeri-Saccà M, Baiocchi M,

- Tartaglia M, Vitale I, De Maria R. CHK1-targeted therapy to deplete DNA replication-stressed, p53-deficient, hyperdiploid colorectal cancer stem cells. *Gut*. 2017 Apr 7. pii: gutjnl-2016-312623. doi: 10.1136/gutjnl-2016-312623. [Epub ahead of print]
- Martinez R, Wijayawardana SR, Hong D, Bendell J, Russell AM, Beckmann RP, Lin AB. A clinical genomic biomarker study of the CHK1 inhibitor prexasertib in advanced head and neck squamous cancer and squamous cell carcinoma of the anus. Poster presented at: AACR American Association for Cancer Research 2017 Annual Meeting; April 1-5, 2017; Washington, DC. Poster 1778/8.
- Matthay KK, Maris JM, Schleiermacher G, Nakagawara A, Mackall CL, Diller L, Weiss WA. Neuroblastoma. *Nat Rev Dis Primers*. 2016;2:16078.
- May CD, Beckmann R, Blosser W, Dowless M, VanWye A, Burke T, Oakley G, Stephens J, Stewart J, Falcon B, Stancato L. Targeting checkpoint kinase 1 (CHK1) with the small molecule inhibitor LY2606368 mesylate monohydrate in models of high-risk pediatric cancer yields significant antitumor effects. [abstract]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research; 2016 Apr 16-20; New Orleans, LA. Philadelphia (PA): AACR; *Cancer Res*. 2016;76(14 Suppl). Abstract nr 2458.
- May C, Beckman R, Blosser W, Dowless M, Burke T, Oakley G 3rd, Stephens J, Stancato L. The CHK1/CHK2 small molecule inhibitor LY2606368 mesylate monohydrate demonstrates anti-tumor activity across a wide range of preclinical models for pediatric and adult soft tissue sarcoma. Poster presented at: The Connective Tissue Oncology Society 20th Annual Meeting; November 4-7, 2015; Salt Lake City, UT. Poster 203. 2330517.
- Messina JA, Cheng SC, Franc BL, Charron M, Shulkin B, To B, Maris JM, Yanik G, Hawkins RA, Matthay KK. Evaluation of semi-quantitative scoring system for metaiodobenzylguanidine (mIBG) scans in patients with relapsed neuroblastoma. *Pediatr Blood Cancer*. 2006;47(7):865-874.
- McNeely S, Beckmann R, Bence Lin AK. CHEK again: revisiting the development of CHK1 inhibitors for cancer therapy. *Pharmacol Ther*. 2014;142(1):1-10.
- Morgenstern DA, Baruchel S, Irwin MS. Current and future strategies for relapsed neuroblastoma: challenges on the road to precision therapy. *J Pediatr Hematol Oncol*. 2013;35(5):337-347.
- [NCI] National Cancer Institute. A Phase I Study of LY2606368, a CHK1/2 Inhibitor, in Pediatric Patients With Recurrent or Refractory Solid Tumors, Including CNS Tumors. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000- [cited April 20, 2017a]. Available at: <https://clinicaltrials.gov/ct2/show/study/NCT02808650> NLM Identifier: NCT02808650.
- [NCI] National Cancer Institute. A Phase II Single Arm Pilot Study of the Chk1/2 Inhibitor (LY2606368) in BRCA1/2 Mutation Associated Breast or Ovarian Cancer, Triple Negative Breast Cancer, High Grade Serous Ovarian Cancer, and Metastatic Castrate-Resistant Prostate Cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000- [cited April 19, 2017b]. Available at: <https://clinicaltrials.gov/ct2/show/study/NCT02203513> NLM Identifier: NCT02203513.

- [NCI] National Cancer Institute. Childhood Rhabdomyosarcoma Treatment (PDQ[®]) - Health Professional Version. Available at: https://www.cancer.gov/types/soft-tissue-sarcoma/hp/rhabdomyosarcoma-treatment-pdq#section/_130. Accessed April 19, 2017c.
- Newman EA, Nuchtern JG. Recent biologic and genetic advances in neuroblastoma: Implications for diagnostic, risk stratification, and treatment strategies. *Semin Pediatr Surg*. 2016;25(5):257-264.
- Ognjanovic S, Linabery AM, Charbonneau B, Ross JA. Trends in childhood rhabdomyosarcoma incidence and survival in the United States, 1975–2005. *Cancer*. 2009;115(18):4218-4226.
- Pastore G, Peris-Bonet R, Carli M, Martínez-García C, Sánchez de Toledo J, Steliarova-Foucher E. Childhood soft tissue sarcomas incidence and survival in European children (1978-1997): report from the Automated Childhood Cancer Information System project. *Eur J Cancer*. 2006;42(13):2136-2149.
- Pike KG. Discovery of AZD0156: The first potent and selective inhibitor of ATM kinase for clinical evaluation. Reference Module in Chemistry, Molecular Sciences and Chemical Engineering 2016.
- Ries LAG, Harkins D, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2003, National Cancer Institute. Bethesda, MD. Available at: http://seer.cancer.gov/csr/1975_2003. Accessed June 10, 2011.
- Rohban S, Campaner S. Myc induced replicative stress response: how to cope with it and exploit it. *Biochim Biophys Acta*. 2015;1849(5):517-524.
- Rose S. Rucaparib approved for ovarian cancer. *Cancer Discov*. 2017;7(2):120-121.
- [SEER] Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER Cancer Statistics Review 1975-2014. Table 28.1, Childhood Cancer, Age-Adjusted SEER Cancer Incidence and U.S. Death Rates, 2010-2014, By Primary Cancer Site, All Races, Males and Females. Available at: https://seer.cancer.gov/csr/1975_2014/browse_csr.php?sectionSEL=28&pageSEL=sect_28_table.01.html. Accessed April 20, 2017a.
- [SEER] Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER Cancer Statistics Review 1975-2014. Table 1.5, Age-Adjusted SEER Incidence and U.S. Death Rates and 5-Year Relative Survival (Percent), By Primary Cancer Site, Sex and Time Period, All Races. Available at : https://seer.cancer.gov/csr/1975_2014/browse_csr.php?sectionSEL=1&pageSEL=sect_01_table.05.html. Accessed April 20, 2017b.
- Skolnik JM, Barrett JS, Jayaraman B, Patel D, Adamson PC. Shortening the timeline of pediatric phase I trials: the rolling six design. *J Clin Oncol*. 2008;26(2):190-195.
- Stiller CA, McKinney PA, Bunch KJ, Bailey CC, Lewis IJ. Childhood cancer and ethnic group in Britain: a United Kingdom children's Cancer Study Group (UKCCSG) study. *Br J Cancer*. 1991;64(3):543-548.
- Tonelli R, McIntyre A, Camerin C, Walters ZS, Di Leo K, Selfe J, Purgato S, Missiaglia E, Tortori A, Renshaw J, Astolfi A, Taylor KR, Serravalle S, Bishop R, Nanni C, Valentijn LJ, Faccini A, Leuschner I, Formica S, Reis-Filho JS, Ambrosini V, Thway K, Franzoni M,

- Summersgill B, Marchelli R, Hrelia P, Cantelli-Forti G, Fanti S, Corradini R, Pession A, Shipley J. Antitumor activity of sustained N-myc reduction in rhabdomyosarcomas and transcriptional block by antigene therapy. *Clin Cancer Res.* 2012;18(3):796-807.
- Walton MI, Eve PD, Hayes A, Valenti MR, De Haven Brandon AK, Box G, Hallsworth A, Smith EL, Boxall KJ, Lainchbury M, Matthews TP, Jamin Y, Robinson SP, Aherne GW, Reader JC, Chesler L, Raynaud FI, Eccles SA, Collins I, Garrett MD. CCT244747 is a novel potent and selective CHK1 inhibitor with oral efficacy alone and in combination with genotoxic anticancer drugs. *Clin Cancer Res.* 2012;18(20):5650-5661.
- Williamson D, Lu YJ, Gordon T, Sciot R, Kelsey A, Fisher C, Poremba C, Anderson J, Pritchard-Jones K, Shipley J. Relationship between MYCN copy number and expression in rhabdomyosarcomas and correlation with adverse prognosis in the alveolar subtype. *J Clin Oncol.* 2005;23(4):880-888.
- Zeng L, Beggs RR, Cooper TS, Weaver AN, Yang ES. Combining Chk1/2 inhibition with cetuximab and radiation enhances in vitro and in vivo cytotoxicity in head and neck squamous cell carcinoma. *Mol Cancer Ther.* 2017 Jan 30. pii: molcanther.0352.2016. [Epub ahead of print]

12. Appendix 1: Innovative Medicines Initiative

The IMI is a public-private partnership sponsored in the EU. Lilly is a pharmaceutical co-leader of this initiative and applies the below charter principles to the oncology portfolio molecules that have potential benefit in pediatric patients.

Cancer remains the leading cause of disease-related death in children. For the approximately 25% of children who experience relapses of their malignant solid tumors, usually after very intensive first-line therapy, curative treatment options are scarce. Preclinical drug testing to identify promising treatment options that match the molecular make-up of the tumor is hampered by the fact that:

- i. molecular genetic data on pediatric solid tumors from relapsed patients and thus our understanding of tumor evolution and therapy resistance are very limited to date, and
- ii. for many of the high-risk entities, no appropriate and molecularly well-characterized patient-derived models and/or genetic mouse models are currently available.

To increase therapeutic successes of novel molecularly targeted compounds in children with solid malignancies, upfront preclinical testing of novel molecularly targeted compounds in a (saturated) repertoire of well-characterized models needs to be established. Since these tumors are overall genetically much less complex than their adult counterparts, it is anticipated that it will be easier to identify powerful predictive biomarkers to allow for accurate matching of targets and drugs.

To address this great-but-as-yet-unmet clinical need, the main objectives for the public-private partnership sponsored in the EU, ITCC-P4 Preclinical Pediatric Proof-of-Concept Platform, this preclinical platform projects are the following:

- Establish a representative collection of patient-derived in vitro and in vivo models (target: n=400) as well as genetic mouse models (target: maximum of 15 across multiple tumor types) of the most common pediatric solid high-risk entities including a significant proportion of models from relapses.
- Molecularly characterize and quality assess the models as well as the matching primary tumor samples and germline controls with state-of-the-art molecular diagnostic tools.
- Enable regulatory filings in the EU through the development of comprehensive preclinical data packages necessary to move drugs into clinical trials for children with solid tumors.
- Prioritize pediatric drug development using existing collections of molecular data for systematic target reports, followed by in vivo drug testing in faithful disease models including PK and pharmacodynamics where necessary (for example, brain penetration).
- Identify suitable biomarkers for future clinical stratification of patients across entities.
- Ultimately, the establishment of the ITCC-P4 platform over the long term will overcome a long-standing gap by enabling thorough molecular characterization of high-risk pediatric malignancies coupled with standardized preclinical testing procedures, and will thus greatly expedite the development of more precise and efficacious drugs for this

patient group. Developing this platform as a public-private partnership will create a model for collaboration that can hopefully be extended to other types of cancer entities and patient groups.

Lilly is pleased to be a leader in this initiative that is committed to the pursuit of further advancement in pediatric cancer treatment.

13. Appendix 2: Current or Potential Challenges in Pediatric Development

13.1. Epidemiology and Enrollment

The overall incidence rate of childhood cancer was 17.8 per 100,000 in the United States for the period 2010-2014, compared with 443 per 100,000 in adults (SEER 2017a; SEER 2017b).

Neuroblastoma is the most common extracranial solid tumor in childhood and the third most common childhood cancer. Nonetheless, they are rare, representing only 5% to 7% of pediatric cancers overall. The incidence of neuroblastoma in the United States is 1 case per 100,000 children, affecting approximately 500 to 1000 children per year (Newman and Nuchtern 2016; American Childhood Cancer Organization 2017). Worldwide, the incidence is approximately 6.8 cases per million for black populations and 9.7 cases per million for white populations (Matthay et al. 2016).

Rhabdomyosarcoma is the most common soft tissue tumor of childhood, responsible for approximately one half of all pediatric soft tissue sarcomas. Nonetheless, they are rare, representing only 3% to 4% of pediatric cancers overall. Approximately 350 new cases are diagnosed in the United States each year, and the annual incidence in children, adolescents, and young adults under the age of 20 is 4.3 cases per 1 million (Pastore et al. 2006; Ries et al. 2011). Two thirds of cases are diagnosed in children younger than 6 years of age, and there is a small male predominance (male-to-female ratio between 1.3 and 1.5). The incidence in African American patients is higher than in whites, most notably in those 15 to 19 years of age. The incidence appears to be lower in Asian populations, compared with predominantly white populations (Stiller et al. 1991). Rhabdomyosarcomas are classified based on histologic and biologic features of the tumor with the largest subgroups being embryonal (eRMS, 57%) and alveolar (aRMS, 23%) (Ognjanovic et al. 2009).

A significant challenge for the clinical development of prexasertib in neuroblastoma and RMS is the low incidence of these diseases, which is further exacerbated when focusing on relapsed/refractory patients. This presents recruitment challenges and makes conducting randomized assessments difficult. However, single-arm assessments are limited by the heterogeneity and variability of data with that of historical comparators. In order to address some of the recruitment challenges, it is anticipated that the proposed trial would be a global trial that would leverage international coalitions.

13.2. Complexity of Pediatric Cancer Regimens

As described in Section 7, pediatric patients with neuroblastoma or RMS are treated with a multimodal approach that may include systemic chemotherapy, surgery, radiation therapy, and/or stem-cell transplant. Such complexity can pose challenges for how to best incorporate newer anticancer treatments, such as prexasertib, into existing treatment paradigms in order to establish safety and efficacy. The ongoing Phase 1 COG study was designed to determine the dose and safety of prexasertib as monotherapy and will help inform future potential combinations with standard-of-care approaches.

13.3. Translation of Nonclinical Data to Clinical Studies

Nonclinical testing has the potential to be a powerful tool to better identify and validate therapeutic targets for individual pediatric cancers and standardize this level of evidence. However, there are limitations to nonclinical models, as they do not always fully translate to clinical outcomes.

At the current time, the rationale for assessing prexasertib in pediatric patients is based on nonclinical data. No data from patients with either neuroblastoma or RMS are available. In nonclinical studies, efficacy is observed when prexasertib is administered at a dose of 10 mg/kg BID for 3 days/week. Pharmacokinetic/pharmacodynamic modeling of the nonclinical xenograft data predicts that this dose and schedule will achieve a systemic exposure that results in a maximum tumor response with prexasertib monotherapy in nonclinical xenograft models and the systemic exposure predicted for maximal tumor response is achieved in adults following the prexasertib RP2D (Hong et al. 2016). However, it should be noted that the predictive capability of this approach (that is, translating efficacy results from murine xenograft models to humans) has inherent limitations, given the differences between animal models and humans (for example, PK profile, protein binding, tumor microenvironment, tumor size/location, target expression, immune system, and prior therapies in humans).

Additionally, the MTD in pediatric patients has not yet been defined in the ongoing study. Since pediatric PK data are not yet available and the adult pop PK model is empirical in nature (that is, not physiologically based), it is not known whether the prexasertib disposition kinetics will be altered in pediatric patients due to the impact of pediatric-specific covariates and/or due to differences in the distribution (for example, into fat, brain, etc.), metabolism (CYP functionality/maturation), and/or elimination (relative contribution of renal and nonrenal pathways) of prexasertib in the pediatric patient population. As a result, it is not yet certain whether the compelling preclinical data will translate to clinical benefit in pediatric patients.

13.4. Risk-Benefit in Pediatric Patients

Prexasertib is still at an early stage of development and the majority of the clinical experience has been in heavily pretreated patients with advanced or metastatic cancer. To date, the most frequent treatment-related acute toxicity observed in prexasertib monotherapy studies is transient Grade 4 neutropenia with an incidence of 73%, resulting in febrile neutropenia rates of approximately 10% (Hong et al. 2016). Given the resiliency of the hematopoietic system of pediatric patients, it is not yet known whether the same level of hematologic toxicity will occur in pediatric patients. The RP2D and schedule in adults is 105 mg/m² administered once every 2 weeks, and it is possible that the RP2D and/or schedule may be different in children and adolescents.

Prexasertib causes DNA damage as well as disrupts DNA damage-repair pathways. Other agents that inhibit DNA damage-repair pathways have been associated with secondary malignancies (Domchek et al. 2016; Rose 2017). None of the patients treated in adult trials with prexasertib have reported developing secondary malignancies. However, it is unknown whether this is a risk

in pediatric patients receiving prexasertib. Data will be collected during the pediatric program to monitor potential long-term toxicities.

13.5. Predictive Biomarkers

Clinically actionable prospective biomarkers for identifying patients that may be sensitive or resistant to the effects of prexasertib have not yet been identified. Emerging data suggest that tumors that have increased replication stress and/or defects in DNA damage-repair pathways may be more sensitive to the effects of CHK1 inhibitors like prexasertib (Lin et al. 2017). As described in Section 7, neuroblastoma and RMS have features that provide a strong biologic rationale for evaluating prexasertib in patients with these tumors. However, ongoing work is trying to define attributes of individual patients with these diseases that will predict sensitivity and/or resistance to prexasertib. Identification of such markers would further enhance the potential therapeutic benefit of prexasertib and expand the types of patients where prexasertib could be assessed.