Abbreviated Title: CRLX101 in mCRPC

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Title: A Single Arm Phase II Study Combining CRLX101, a Nanoparticle Camptothecin, with Enzalutamide in Patients with Progressive Metastatic Castration Resistant Prostate Cancer Following Prior Enzalutamide Treatment

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Investigational Agents:

Drug Name:	CRLX101
IND Number:	138099
Sponsor:	Center for Cancer Research
Manufacturer:	Ellipses Pharma

Commercial Agents: Enzalutamide

PRÉCIS

Background:

- Enzalutamide is established as first-line hormonal therapy in participants with metastatic castration resistant prostate cancer (mCRPC). However, it is increasingly recognized that acquired resistance to therapy (e.g. AR overexpression, AR-V7) could limit the durability of response to therapy
- Upregulation of HIF-1α in hypoxic tumor cells provides a mechanism of acquired resistance to current hormonal therapies and chemotherapies. Acquired resistance increases angiogenesis and metastasis, leading to disease progression
- Targeting the hypoxia driven tumor microenvironment (e.g. down-regulation of HIF-1α) in addition to the androgen receptor (e.g. enzalutamide) has synergistic activity against prostate cancer cell line models (e.g. LNCaP, 22Rv1).
- CRLX101 is a nanoparticle drug conjugate composed of 20(S)-camptothecin (a potent and highly selective topoisomerase I inhibitor with anti-HIF-1α properties) conjugated to a linear, cyclodextrin-polyethylene glycol-based polymer
- CRLX101 has been to shown to be safe, tolerable, and efficacious in numerous Phase II clinical investigations in a variety of tumor subtypes.
- Preclinical and clinical studies have shown CRLX101 significantly down-regulates HIF-1α, impacting tumor-driven angiogenesis.
- The treatment combination of CRLX101 and enzalutamide provides a reasonable approach to re-sensitizing prostate cancer cells to hormonal therapy via synergistic antitumor activity and inhibition of acquired resistance

Objectives:

- *Primary Objective:* To evaluate the anti-tumor activity of CRLX101 at the recommended phase II dose (RP2D) in combination with enzalutamide with respect to treatment response, defined as ≥50% PSA decline or stable disease on imaging following 5 months of treatment.

Eligibility:

- Patients must have progressive mCRPC per Prostate Cancer Working Group 3 (PCWG3)
- Patients must be at least 18 years of age and able to give informed consent
- ECOG Performance Status ≤ 2
- Evaluable metastatic disease on bone scan or measurable disease on CT Scan per PCWG3 and/ or RECIST
- Patients must have had disease progression while receiving prior enzalutamide treatment

Design:

- The study will be conducted using an optimal two stage Phase II design (8 participants, expandable to 21 participants total) aimed to determine the percentage of participants with a PSA decline of greater than 50% or stable disease at 5 months.
- The first 3 to 6 participants enrolled on study will follow a lead-in dosing scheme to confirm the safety of the combination (CRLX101 12 mg/m^2 every 2 weeks for the first two cycles, followed by CRLX101 15 mg/m^2 every 2 weeks at the start of cycle 3, with enzalutamide 160 mg administered once daily starting on cycle 1 day 2) prior to initiation of the optimal two stage study design.
- For participants enrolled on study following the lead-in, the confirmed tolerable dose of CRLX101 will be administered via IV infusion every 2 weeks. Enzalutamide 160 mg will be administered orally once daily beginning on cycle 1 day 2.
- Blood and urine will be collected at multiple time points for PK and PD analyses.
- Tumor assessments will be made using ^{99}Tc bone scintography and/or CT scan (chest, abdomen, and pelvis) at baseline, prior to Cycle 3 and every 3 cycles thereafter.
- The accrual ceiling for the study is set at 30 participants.

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STATEMENT OF COMPLIANCE

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

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

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

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

1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

1.1.1.1 To evaluate the anti-tumor activity of CRLX101 at the recommended phase II dose (RP2D) combined with enzalutamide with respect to treatment response, defined as ≥50% PSA decline or stable disease on imaging following 5 months of treatment.

1.1.2 Secondary Objectives

1.1.2.1 To evaluate the duration of response as defined by a sustained >30% decline in PSA, overall survival, and changes in measurable disease as determined by Response

Evaluation Criteria in Solid Tumors (RECIST) and Prostate Cancer Working Group 3 (PCWG3) for the treatment combination.

1.1.2.2 To validate the recommended phase 2 dose (RP2D) of CRLX101 in combination with enzalutamide

1.1.3 Exploratory Objectives

1.1.3.1 To evaluate the pharmacokinetic profile of CRLX101 (both total drug and released camptothecin) and enzalutamide in plasma.

1.1.3.2 To evaluate the underlying mechanism of camptothecin-induced cystitis.

1.1.3.3 To evaluate the pharmacodynamic activity of CRLX101 and enzalutamide using surrogate biomarkers to measure acquired treatment resistance mediated by angiogenesis and androgen receptor signaling (e.g. VEGF, CTCs, AR-V7).

1.1.3.4 To evaluate the activity of CRLX101 and enzalutamide with respect to the prevalence of circulating tumor cells with high genomic instability.

1.1.3.5 To explore possible correlations between clinical response and biomarkers of DNA damage response (e.g. DNA damage response panel).

1.2 BACKGROUND AND RATIONALE

1.2.1 Overview

Prostate cancer is the most common cancer diagnosis among males in the United States, with 180,890 estimated new cases each year, 26,120 of these cases resulting in death(1). Advancement in disease detection as well as improvements in the treatment of castration resistant disease has greatly contributed to more favorable patient outcomes. Androgen deprivation therapy (ADT) has long been a mainstay of initial prostate cancer management, but the disease inevitably progresses and becomes resistant to androgen ablation. Uncontrolled tumor growth despite adequate castrate levels of androgens (serum testosterone <50 ng/mL) indicates the failure of ADT and the development of castrate resistant prostate cancer (CRPC)(2). Advanced CRPC carries significant morbidity and mortality, and necessitates a transition in treatment. Treatment options with the capability to manage CRPC were limited until the U.S. Food and Drug Administration (FDA) approval of docetaxel in 2004. Two pivotal phase III randomized trials, TAX 327 and SWOG 99-16, demonstrated an overall survival benefit (with prednisone or estramustine, respectively) compared to mitoxantrone and prednisone.(3, 4) As of result, docetaxel would remain the only approved treatment for CRPC for approximately one decade.

Following the approval of a second line chemotherapeutic, cabazitaxel, in 2010,(5) CRPC pharmacologic management has progressed rapidly. Continued drug development led to the first therapeutic vaccine for asymptomatic disease and a radioactive therapeutic agent for metastatic bone disease (sipuleucel-T and radium-223 respectively).(6, 7) Improvements in anti-androgen therapy provided a significant overall survival benefit in patients with metastatic CRPC (mCRPC). Two agents with improved pharmacologic properties to down-regulate androgen signaling, abiraterone acetate and enzalutamide, were initially approved in the post-docetaxel patient population via the COU-AA-301 and AFFIRM trials respectively.(8, 9) Additional

studies investigating first line therapy in patients with mCRPC (COU-AA-302 and PREVAIL) led to the approval of both abiraterone acetate and enzalutamide for primary disease management.(10, 11) Currently, abiraterone acetate, enzalutamide, docetaxel, cabazitaxel, sipuleucel-T, and radium-223 are all FDA approved therapies for patients with CRPC.

Current approaches to maximizing efficacy of currently approved therapies include optimal treatment sequencing and limiting acquired resistance. The latter approach can be accomplished via combining treatment modalities, often incorporating agents that target resistance pathways that lead to CRPC or pluripotent tumor cells of the microenvironment. This clinical study is focused on combining the currently accepted standard of care (androgen receptor blockade via enzalutamide) with an experimental agent shown to reduce the metabolic signaling of hypoxic tumor (HIF-1α down-regulation via CRLX101). Dual inhibition of the androgen receptor (AR) and hypoxia inducible factor-1α (HIF-1α) in heavily pre-treated patients with progressive mCRPC is proposed to counteract acquired resistance and re-sensitize prostate cancer tumor cells to treatment.

1.2.2 Enzalutamide

Enzalutamide is a current standard of care therapy for patients with metastatic castration resistant prostate cancer (mCRPC). Developed as a second-generation androgen receptor antagonist (ARA), enzalutamide has significantly improved target affinity over previous ARAs (e.g. nilutamide, bicalutamide, and flutamide).(12, 13) Enzalutamide exhibits stronger affinity to the androgen receptor than previous ARAs while also preventing nuclear translocation, DNA binding, and signaling to co-activators (12). Initially approved in docetaxel refractory patients, enzalutamide has since received U.S. FDA approval as first line therapy for patients with mCRPC. Further investigation of enzalutamide is ongoing, evaluating the use of the drug in potential combination regimens and at different stages of prostate cancer (e.g. enzalutamide in combination with PROSTVAC in patients with mCRPC, neoadjuvant enzalutamide and androgen deprivation in newly diagnosed prostate cancer).

Following a Phase I/II study of enzalutamide that demonstrated safety and suggested efficacy in both chemotherapy naïve and chemotherapy treated patients with mCRPC(14), two phase III studies were initiated: AFFIRM and PREVAIL. The AFFIRM trial enrolled 1199 patients with progressive mCRPC on docetaxel, who were then randomized 2:1 to enzalutamide 160 mg/day (n=800) or placebo (n=399). The overall survival favored patients randomized to enzalutamide versus placebo, 18.4 months to 13.6 months respectively. This 4.8 month improvement in survival represented a 37% risk reduction in death (hazard ratio (HR) 0.63; 95% CI: 0.53 to 0.75; P<0.001), the largest relative and absolute improvement in overall survival in an appropriately powered phase III study in prostate cancer. Median time to progression (TTP) based on radiographic findings was 8.3 vs. 2.9 months (HR: 0.40; 95% CI 0.35 to 0.47; P<0.001). Modest increases in fatigue, hot flashes, diarrhea, musculoskeletal pain, and headaches were reported in the enzalutamide group, but this was possibly related to the substantially longer monitoring time for patients on the treatment compared to the placebo group. Therefore, there were minimal concerns about the side effect profile of enzalutamide compared to placebo.(9) The U.S. FDA approved enzalutamide for patients with chemotherapy-refractory mCRPC in August of 2012.

The PREVAIL trial enrolled 1717 patients with chemotherapy naïve mCRPC. Patients were randomized 1:1 to enzalutamide 160 mg/day (n=872) or placebo (n=845), with co-primary

endpoints of radiographic progression free survival and overall survival. The overall survival favored patients in the enzalutamide arm, with a 29% decrease in the risk of death at time of follow-up (HR 0.71; 95% CI, 0.60 to 0.84; P<0.001)(11). Estimates of median overall survival were 32.4 months and 30.2 months for the enzalutamide and placebo arms respectively. At 12 months, the rate of radiographic progression free survival was 65% and 14% for patients on enzalutamide and placebo respectively (HR 0.19; 95% CI, 0.15 to 0.23; P<0.001). Fatigue and hypertension were the most common clinically relevant adverse events associated with enzalutamide(11). The U.S. FDA approved enzalutamide for chemotherapy-naïve mCRPC patients in August of 2014.

1.2.2.1 Acquired Resistance in mCRPC

Disease progression following monotherapy with enzalutamide is inevitable. Response to therapy diminishes over time, primarily due to acquired resistance of prostate cancer cells. While several mechanisms of resistance have been reported in the literature, splice variants of the androgen receptor have received the most attention. Currently, androgen receptor splice variant 7 (AR-V7) is a biomarker of enzalutamide resistance that is approaching clinical utility. Antonararkis et. al. demonstrated that the presence of AR-V7, a splice variant without a ligand binding domain, was up-regulated in patients following treatment with either abiraterone acetate or enzalutamide. Importantly, Antonararkis et al. also validated the use of reverse transcriptase polymerase chain reaction (RT-PCR) to accurately detect AR-V7 messenger RNA in circulating tumor cells (CTCs)(15). Other means of detecting AR splice variants (e.g. droplet digital PCR) have been developed and are currently in use in clinical trial settings. It is accepted that the enumeration of CTC plasma concentrations can provide information about disease progression (16).

A recent cohort analysis of patients with CRPC prior to abiraterone or enzalutamide treatment reported 107 of 150 patients (71.3%) harbored AR pathway alterations, a majority of which were direct alterations affecting the AR through amplification and mutation. Common AR mutations (e.g. T878A, W742C, L702H) and varying levels of AR splice variants were present in these patients. Alterations in AR pathway members (*NCOR1*, *NCOR2*, *FOXA1*) and the AR regulated gene, *ZBTB16*, were also present. Excluding the alterations of the AR pathway, 65% of patients had tumors with actionable aberrations, including alterations of pathways associated with HIF-1α signaling (PI3K/Akt/mTOR,(17), Wnt/β-catenin(18)) and mechanisms of DNA repair(19). In a separate study, increased AR copy number was found in a higher proportion of patients who did not respond to abiraterone than patients who responded(20).

1.2.3 AR, HIF-1α and Intratumoral Hypoxia

Intratumoral hypoxia is a common feature of prostate cancers that are associated with poor prognosis (21). Prostate cancer tumor cells acquire the ability to adapt to hypoxic environments caused by androgen deprivation, enhancing transcriptional activity of the androgen receptor.(22, 23) Hypoxia Inducible Factor (HIF)-1α mediates key hypoxia-associated genes involved in angiogenesis, metabolism, survival, immunity and invasion(24-26). Several studies suggested the cross-talk between the HIF-1α and AR pathways, with emphasis on a potential ternary complex (AR, HIF-1α, and β-catenin) responsible for AR target genes (27-31). Given HIF-1α and AR are key regulators of multiple cellular processes in prostate cancer, dual targeting of both axes represents an attractive therapeutic approach.

Several experiments were initiated in an androgen-dependent prostate cancer cell line, LNCaP, to evaluate the effect of combining enzalutamide with HIF-1α inhibition. LNCaP cells cultured in the presence of low concentration of DHT under hypoxic conditions (simulated using $CoCl_2$) had increased transcription of AR-dependent genes (KLK3, FKBP5, TMPRSS2) and hypoxia-dependent genes (VEGF, ENO1, LDHA). Under the same conditions, chetomin, a disrupter of an interaction of HIF-1α with its transcriptional co-activator, p300, in combination with enzalutamide resulted in a significant decrease of the mRNA transcripts of AR-dependent and hypoxia-dependent genes. The direct knockdown of HIF-1α expression (via HIF-1α siRNA) in combination with enzalutamide suppressed AR transcriptional activation and VEGF protein expression in LNCaP cells. Similar effects on AR transcriptional activity and VEGF expression were also shown in 22Rv1 cells, an androgen resistant prostate cancer cell line. Simultaneous treatment with HIF-1α siRNA synergistically enhances the inhibitory effect of enzalutamide on LNCaP cell viability through increased apoptosis. The treatment combination was also effective at reducing the growth rate of the enzalutamide-resistant 22Rv1 prostate cancer cell line, which suggests that HIF-1α inhibition can restore sensitivity to enzalutamide-resistant cells(32).

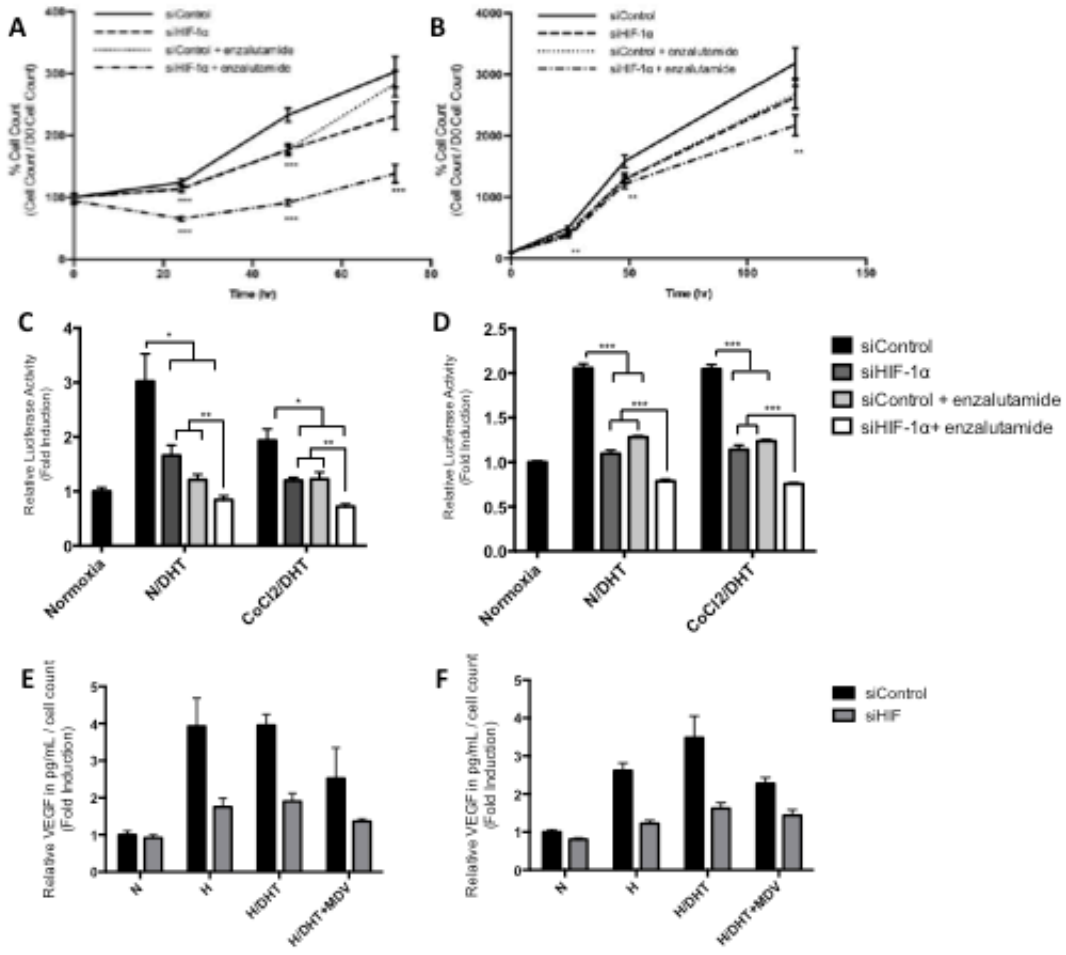

Figure 1**:** Synergy of dual AR inhibition and HIF-1α knockdown in both enzalutamide sensitive and enzalutamide resistant prostate cancer cell lines. Under hypoxic conditions, combination treatment with enzalutamide and HIF-1α siRNA led to decreased cell proliferation in both LNCaP (A) and 22Rv1 (B) cells. Under normoxic and hypoxic conditions, the combination treatment significantly reduced the luciferase activity of Androgen Receptor Response

Element (ARE) in both LNCaP (C) and 22Rv1 (D) cell lines, demonstrating a down-regulation of AR transcriptional activity. Similarly, the treatment combination demonstrated a reduction of downstream VEGF protein concentration in LNCaP (E) and 22Rv1 (F) cells.

Multiple drugs have been shown to down-regulate the activity of HIF-1α.(33, 34) Camptothecins were found to modulate HIF-1α expression through a Topoisomerase I (Top1) dependent mechanism(35). Topotecan was shown to inhibit HIF-1α protein accumulation independently of proteosomal degradation in a time and dose dependent fashion. The accumulation of HIF-1α mRNA was not affected rather a decrease of HIF-1α protein translation was observed following metronomic topotecan treatment of U251 cells. The presence of Top1 for the facilitation of RNA transcription is necessary for topotecan-induced inhibition of HIF-1α protein accumulation(36). Kummar et. al. evaluated topotecan's effects on HIF-1α in a pilot trial assessing patients with advanced solid tumors over-expressing HIF-1α(37). Biopsies of 4 patients showed undetectable levels of HIF-1α following treatment with topotecan in addition to decreased levels of VEGF and GLUT-1 mRNA(37). Decreased tumor blood flow and permeability were observed by DCE-MRI in 7 of 10 patients after 1 cycle. One patient had a partial response accompanied by inhibition of HIF-1α in tumor and reduction in tumor blood flow on DCE-MRI. This trial demonstrated that topotecan could decrease HIF-1α expression in advanced solid tumors.

1.2.4 Camptothecin Derivatives Investigated in Prostate Cancer

Camptothecin (CPT) is a potent Top1 inhibitor that failed clinical development due to poor solubility and high systemic toxicity. CPT derivatives such as irinotecan and topotecan have clinical utility for the treatment of a limited subset of advanced solid tumors. Both irinotecan and topotecan demonstrated improvements in plasma solubility but decreased target affinity compared to CPT. The primary cellular target of CPT is the Top1-DNA cleavage complex. CPT stabilizes the Top1-DNA cleavage complex during DNA replication and prevents Top1-mediated DNA re-ligation, leading to double strand breaks. The accumulation of double-strand breaks via CPT leads to apoptosis.

Camptothecin has shown activity in several prostate cancer cell lines (e.g. PC3, DU145, LNCaP).(38-40) The administration of CPT in LNCaP cells was shown to down-regulate expression of the AR and PSA.(41) Camptothecin was shown to suppress the expression of matrix-metalloproteinase-9 (MMP-9) and VEGF through inhibition of NF-κB activity in DU145 cells.(42)

Topotecan use in prostate cancer has been limited, in part, due to its low efficacy and high non-target toxicity. In a phase II study, 15 men with metastatic, PSA-progressive disease after primary androgen deprivation were given irinotecan at a dose of 125 mg/m^2 weekly for 4 weeks followed by two weeks of rest (6 week cycles). None of the 15 patients had a decline in PSA of greater than 50%, and eight patients had stable disease as a best response. Hematologic and gastrointestinal toxicities were common, with granulocytopenia and diarrhea occurring most frequently.(43) In another phase II study, 26 men with hormone resistant prostate cancer who failed one or two prior androgen ablative therapies were administered 21-day continuous intravenous infusion of topotecan at a dose of 0.5 mg/m^2 every 28 days. Of the 24 evaluable patients, four patients had PSA decline of 50% from baseline, 5 patients had stable disease as the best response, and the median (overall) survival was 9 months. Eight of 24 patients experienced grade 4 hematologic toxicity.(44) The discovery of topotecan and CPT as small molecule

inhibitors of HIF-1α has renewed interest in this class of drugs with development of novel derivatives or finding alternative dosing schedules. Indeed recent studies have shown that metronomic dosing increases the efficacy of topotecan in models of human prostate cancer.[45]

Treatment of hypoxic 22Rv1 cells with topotecan has been shown to knockdown HIF-1α protein concentration. Decreased nuclear translocation of the full length AR and AR-V7 splice variant was found to be associated with topotecan-mediated knockdown of HIF-1α. Furthermore, the decreased nuclear AR-V7 levels following topotecan treatment was associated with increased sensitivity to enzalutamide.[46] These findings support the hypothesis that camptothecin-mediated knockdown of HIF-1α could modulate enzalutamide resistance.

1.2.5 CRLX101

CRLX101, formerly named IT-101, is a nanoparticle-drug conjugate (NDC) comprised of a linear cyclodextrin-polyethylene glycol-base polymer conjugated to multiple 20(S)-camptothecin (CPT) molecules (Poly-CD-PEG-Camptothecin) (CRLX101 Investigator's Brochure). Upon reconstitution, CRLX101 self-assembles into nanoparticles 20 to 30 nm in diameter and releases CPT over prolonged periods of time in a pH dependent manner. The unique properties of this nanoparticle allow for prolonged circulation in the plasma compartment, decreased hepatic and renal clearance, decreased recognition via the mononuclear phagocyte system, and enhanced localization to the tumor.(47, 48)

Utilizing the Enhance Permeability and Retention (EPR)[49] effect, CRLX101 is able to selectively penetrate tumor tissue due to abnormally "leaky vasculature" and remain within the tumor for a prolonged period of time. CRLX101 provides sustained release of CPT from the polymer in the tumor for prolonged periods of time while maintaining a slow rate of clearance of unconjugated CPT into the plasma compartment. Animal xenograft models indicate that CRLX101 accumulates in solid tumors and releases CPT over several days to give sustained inhibition of its target. CRLX101 thus is proposed to optimize the potential antitumor activity by prolonging exposure in tumor tissue, providing more consistent tumor exposure compared to metronomic topotecan therapy, and improving tolerability with low systemic exposure.

Drug localization analyses have been conducted in tumor collected from subjects with HER-2 negative gastric cancer who enrolled in a clinical trial investigating CRLX101 monotherapy. Pre- and post- treatment biopsies involving tumor tissue and healthy adjacent tissue were collected from 10 subjects, 9 of whom had evaluable biopsies. Biopsies from all subjects were analyzed for differential drug accumulation between tumor and normal tissue using immunofluorescence techniques. Post-treatment biopsies were obtained between 24 and 48 hours after a single dose of CRLX101 and CPT were visualized by direct fluorescent excitation of tissues. Of the 9 patients with evaluable biopsies, 7 showed clear evidence of CPT within the post-treatment tumor while only one patient showed potential evidence of CPT in the post-treatment normal tissue. The images obtained of these tumor samples show bright and highly resolved CPT signal specific to tumor tissue following treatment with CRLX101, with rare events of CPT signal co-localized with anti-PEG signal (Figure 2). A decrease in Carbonic Anhydrase XI (CAXI, a downstream effector of HIF-1α) was shown in 6 patients with tumor biopsies obtained pre and post-treatment with CRLX101. A separate study collected pre- and post- treatment biopsies from 3 subjects with ovarian cancer receiving CRLX101 15 mg/m^2 monotherapy, 2 of whom had biopsies of sufficient quality for analysis. The post treatment tumor tissue showed evidence that CRLX101

and CPT are still present 6 days after the first dose of CRLX101. Additionally, staining with an antibody against γ-H2AX (used to visualize double strand DNA breaks) suggested that DNA damage persists for at least 6 days after the first dose of CRLX101 (CRLX101 Investigator's Brochure).

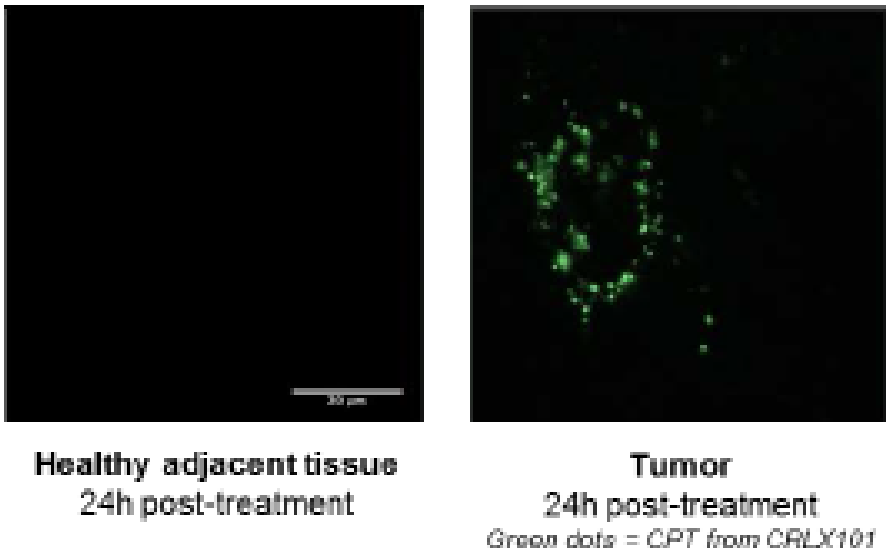

Figure 2: Immunofluorescence visualization in gastric cancer tumor tissue and adjacent normal tissue after the first dose with CRLX101 in subjects on the Gastric Cancer Investigator-Sponsored Trial. Left: Healthy adjacent tissue – 24 hour post-treatment; Right: Tumor - 24 hour post-treatment;

The payload of CRLX101 is CPT, a very potent topoisomerase 1 (topo-1) inhibitor, is largely active during cell replication, it is expected to produce effects on rapidly dividing normal tissues such as bone marrow and gastrointestinal mucosa are anticipated. Possible toxicities include: neutropenia, thrombocytopenia, anemia, nausea, vomiting, diarrhea, mucositis, anorexia, and cystitis. However, nanoparticle drug conjugate (NDC) technology mitigated significantly these adverse events leading to a better safety profile due to accumulation of CPT in tumor cells. Commonly reported toxicities in clinical trials with CRLX101 are anemia, fatigue, cystitis, nausea, hematuria, neutropenia, diarrhea, vomiting, dysuria, constipation, thrombocytopenia, decreased appetite, and infusion-related reaction.

1.2.5.1 CRLX101 and HIF-1α Inhibition

The HIF-1α inhibitory properties of camptothecin have been assessed in several breast cancer models. A comparison of camptothecin (CPT) and topotecan (TPT) administration in SUM159 cells, a breast cancer stem cell line, showed the cells were more resistant to the cytotoxic effects of TPT than CPT (IC_{50} values of 35.3 nM vs. 8.6 nM, respectively). CPT showed a more potent

effect of decreasing HIF-1α accumulation in response to hypoxia, with TPT requiring greater than a 1000-fold higher dose to decrease HIF-1α compared to CPT. The combination of CRLX101 with bevacizumab, a VEGF inhibitor, was assessed in NOD/SCID mice injected with SUM159 cells. Combination therapy resulted in decreased tumor diameter, with higher doses of CRLX101 in combination with bevacizumab exhibiting the greatest effect. In regions of hypoxic tumor, the addition of CRLX101 to bevacizumab resulted in significant down-regulation of HIF-1α (Figure 3). CRLX101 alone had the most significant effect on tumor volume compared to the combination with bevacizumab.[50] In addition, CRLX101 has shown activity in PC3 cells *in vitro*.[40]

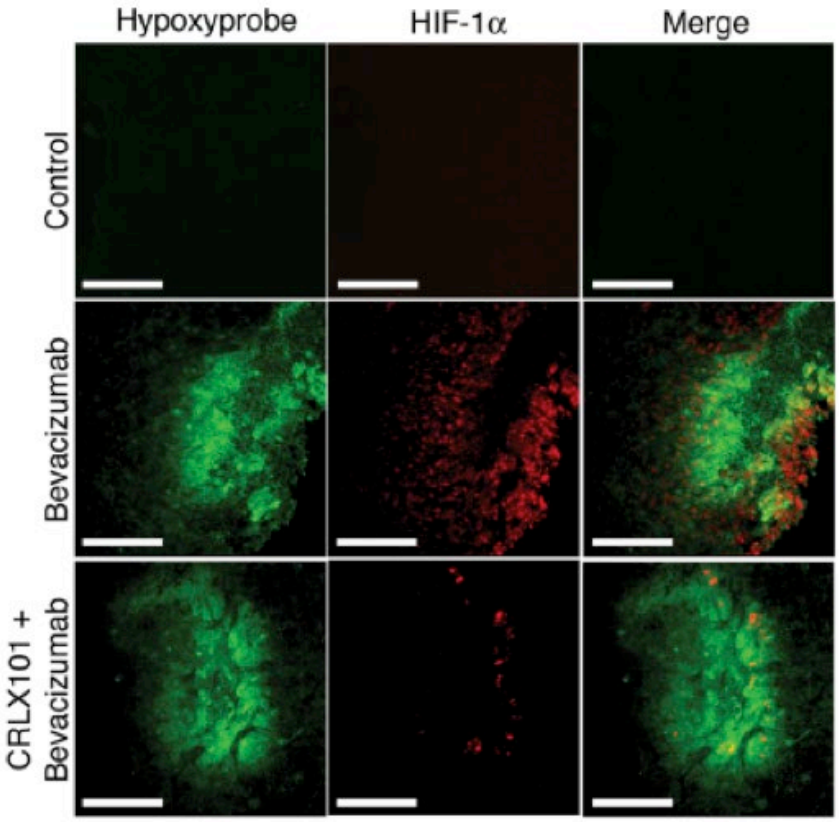

Figure 3: Hypoxyprobe staining of low oxygen zones (green) and HIF-1α staining (red) of tumors from a bevacizumab-treated mouse and a CRLX101 + bevacizumab-treated mouse. Scale bars 200 μM.

CRLX101 maintains or improves tumor perfusion and reduces tumor density. CEUS and PA imaging, used to measure blood flow volume and oxygen saturation respectively, of LM2-4 tumor xenografts characterized the ability of CRLX101 to restore functional vasculature to dense tumor tissue, enabling enhanced delivery of a secondary agent in combination (Figure 4). Paradoxically, high doses of CRLX101 did not promote the formation of micrometastases. CRLX101 with and without bevacizumab were evaluated in a post-surgical metastatic breast

cancer model of advanced breast cancer (YFP-SCID mice orthotopically implanted with LM2-4 cells, followed by primary tumor resection when 400-500 mm^3). CRLX101 prevented the emergence of new metastases in mice with no apparent metastases at the start of treatment and caused the regression of metastases in mice with apparent metastases. High doses of CRLX101 significantly extended the survival of mice with metastatic disease, showing no significant benefit with the addition of bevacizumab.[51]

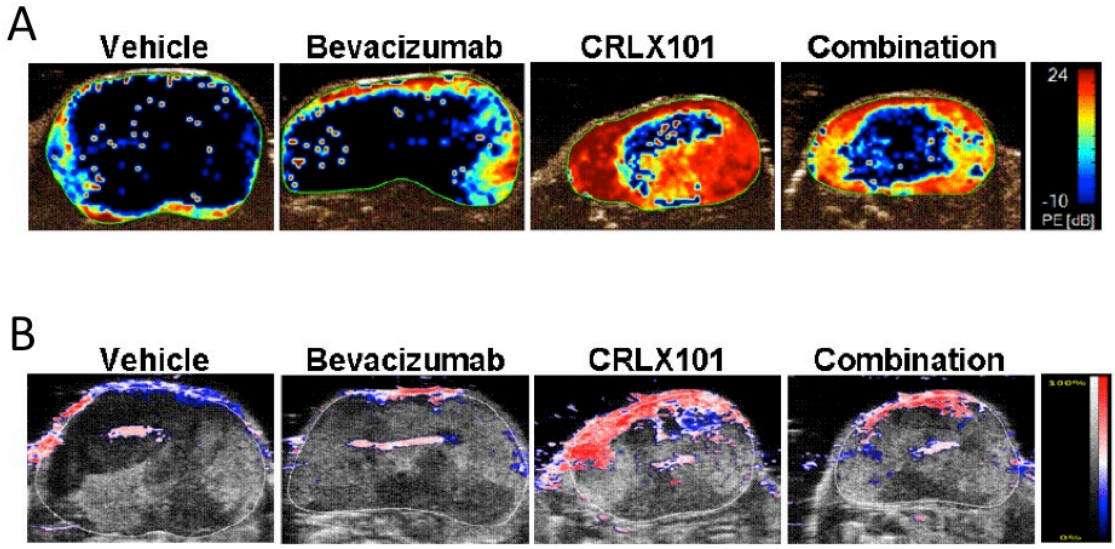

Figure 4: CEUS and PA imaging of primary LM2-4 tumors. (A) Representative CEUS images of one tumor from each therapy group overlaid with parametric color mapping to show areas of high (red), low (blue) or no perfusion (black). (B) Representative PA images of the same tumors overlaid with color mapping to indicate areas of high (red) and low tissue oxygenation (blue).

CRLX101's ability to down-regulate HIF-1α was also demonstrated in a preclinical mouse model of advanced ovarian cancer. Preliminary Phase II clinical data assessing CRLX101 monotherapy in patients with advanced platinum-resistant ovarian cancer demonstrated benefit in patients with RECIST-evaluable disease. Three of 19 patients had tumor reductions greater than or equal to 30%.[52]

1.2.5.2 CRLX101 Preclinical Toxicology

Dose-limiting toxicities in rat and dog were largely body weight losses. Reductions in neutrophil and platelet counts were observed in the acute dog toxicity study and reversible reductions in neutrophil and platelet counts were observed at the mid-dose in the sub-acute multi-dose dog toxicity study (CRLX101 Investigator's Brochure). Reversible reductions in neutrophil and increases in platelet counts were observed at the high dose in the sub-acute multi-dose rat toxicity study.

1.2.5.3 CRLX101 Pharmacokinetics

The plasma exposure (as assessed by AUC_{all} [period of 24 hours post end of infusion]) of the polymer-conjugated and unconjugated camptothecin increased in a dose-dependent manner (6 to 15 mg/m^2). Comparison of the polymer-conjugated to unconjugated CPT exposure showed a

~11-fold increase in exposure for conjugated CPT relative to unconjugated CPT. The mean clearance and volume of distribution values for the conjugated CPT over the dose range evaluated were dose independent and ranged from 0.0914 to 0.132 L/hr and 2.33 to 4.63 L respectively. The $T_{1/2}$ values appeared to be dose dependent with individual subject $T_{1/2}$ values ranging from 19.9 to 42.0 hours and 27.5 to 111 hours for the conjugated and unconjugated species respectively.

The polymer-conjugated and unconjugated camptothecin is primarily cleared via renal excretion. *In vitro* pharmacologic evaluation of CRLX101 with S9 liver microsomal fractions indicated no metabolites nor induction of cytochrome P450 enzymes following incubation up to 120 minutes. Additional studies showed CRLX101 bound moderately to rat RBCs and did not significantly inhibit CYP450 enzymes. In clinical evaluation of urinary excretion during the first 48 hours, the majority of CPT excreted was in the polymer-conjugated (16.2% of the total CRLX101 dose) compared to the unconjugated form (4.4% of the total CRLX101 dose). The majority of polymer-conjugated CPT was excreted in the first 24 hours, with a noteworthy decline in the 24- to 48-hour collection. Urinary clearance of unconjugated CPT was notably higher than that for polymer-conjugated CPT over all observation periods. Urinary clearance of unconjugated CPT in the first 48 hours following administration was similar over all collection periods and was not correlated with dose or creatinine clearance.

The majority of subjects receiving CRLX101 15 mg/m^2 or 18 mg/m^2 every 2 weeks (Q2W) schedule had measurable levels of unconjugated CPT in plasma at 14 days post CRLX101 administration. The values represented less than 3.1% of the respective mean C_{max} values for each dose level. The lower dose group (12 mg/m^2) on a Q2W schedule did not have any measurable levels of unconjugated CPT in their plasma by 14 days post CRLX101 administration. A dosing interval greater than one week is required to avoid a significant carry-over of unconjugated plasma CPT from one dose to the next. No polymer-conjugated CPT was detectable in plasma at 14 days post administration of CRLX101 for all Q2W administration groups. The data suggests CRLX101 dosed at 15 mg/m^2 maintains a consistent low concentration of CPT in tumor tissue for 14 days post administration.

1.2.5.4 CRLX101 Clinical Development Overview

The safety and efficacy information for CRLX101 contained herein is based on the data available as of June 29, 2020.

As of March 12, 2020, there have been 6 clinical trials sponsored by Cerulean (prior to April 17, 2017) and NewLink Genetics (after April 17, 2017): CRLX-001, CRLX-002, CRLX101-208, CRLX101-209, CRLX101-102, and CRLX101-OVAR0801. Overall, 286 subjects received at least 1 dose of CRLX101 at doses ranging from 6 to 18 mg/m^2 in these 6 NewLink Genetics-sponsored studies

There have been 8 investigator-sponsored trials (ISTs) with CRLX101, and as of March 12, 2020, 193 subjects received at least 1 dose of CRLX101 at starting doses ranging from 12 to 15 mg/m^2 in these ISTs.

When tested in the every two week (Q2W) schedule, the MTD/RP2D of CRLX101 monotherapy was determined to be 15 mg/m^2 IV. CRLX101 has generally well-tolerated, and has shown

preliminary anti-tumor activity in subjects with solid tumors such as ovarian cancer and renal cell carcinoma.

1.2.5.5 Dosing Considerations for CRLX101

1.2.5.5.1 CRLX101 Monotherapy

Patients with advanced solid malignancies were enrolled to an open-label, single-arm dose escalation study (Study CRLX-001). CRLX101 was administered intravenously over 60 minutes initially weekly at 6, 12, and 18 mg/m^2, and later every other week at 12, 15, and 18 mg/m^2. The MTD was determined to be 15 mg/m^2 Q2W, and an expansion Phase 2a study was completed. See Section 1.2.5.7.1 for Adverse Event Information.

More intensive once weekly dosing was assessed in patients with advanced solid tumors (Arm 1 of Study CRLX101-102). CRLX101 was given once weekly at either 12 mg/m^2 (N=7) or 15 mg/m^2 (N=8) intravenously over 1-2 hours for 7 weeks. Plasma pharmacokinetic analysis was performed at weeks 1, 3, and 7, measuring total conjugated CPT $AUC_{0\text{-}168h}$ and unconjugated CPT $AUC_{0\text{-}168h}$. In plasma, >90% of the total CPT remains as the conjugated form, and the plasma exposure for both unconjugated and conjugated CPT from week 1 to 7 with no evidence of significant drug accumulation. One Grade 3 adverse event was reported for a subject receiving the 12 mg/m^2 dose, which was considered a dose limiting toxicity (febrile neutropenia). The MTD for Arm 1 (CRLX101 QW alone) in this study was determined to be 15 mg/m^2 once weekly.(53)

1.2.5.5.2 CRLX101 Combination Therapy

A Phase II study with a lead-in Phase I dose escalation combined CRLX101 and bevacizumab in patients with metastatic renal cell carcinoma (mRCC). The dose escalation assessed a 12 mg/m^2 dose of CRLX101 (given every 2 weeks) in combination with the FDA approved dose of bevacizumab (10 mg/m^2 given every 2 weeks) in dose level 1 (N=3) and the 15 mg/m^2 dose of CRLX101 in combination with bevacizumab in dose level 2 (N=3). The 15 mg/m^2 dose of CRLX101 was determined to be the recommended Phase II dose and 27 total patients were evaluated for safety on the study. Bevacizumab, a drug without significant overlapping toxicities, did not exhibit additive toxicity when used in combination with CRLX101. An observed increase in Grade 3/4 cystitis with CRLX101 and bevacizumab is likely attributed to the impaired healing of the bladder tissue following the accumulation of camptothecin (CRLX101 Investigator's Brochure).(54) See Section 1.2.5.7.2 for more information on adverse events and Section 1.2.5.9 for more information on camptothecin-induced cystitis.

More intensive once weekly dosing of CRLX101 in combination with bevacizumab was assessed in patients with advanced solid tumors (Arm 2 of Study CRLX101-102). 7 subjects were treated with CRLX101 at the 12 mg/m^2 dose intravenously once weekly, with one reported DLT (Grade 3 cystitis). Eight subjects were treated with CRLX101 at the 15 mg/m^2 dose, with 2 reported DLTs (both Grade 3/4 neutropenia). The first subject required a dosing delay of >2 weeks following the second week of dosing (meeting definition of DLT), whereas the second patient experienced febrile neutropenia 4 days following the Week 4 dose of CRLX101. The Safety Review Committee (SRC) deemed it reasonable to administer CRLX101 15 mg/m^2 once weekly for three weeks with one week off in combination with bevacizumab Q2W, thus declaring this dosing schema the MTD for this combination. Once weekly intravenous CRLX101 at 12 mg/m^2

in combination with bevacizumab Q2W was also considered an acceptable dose (CRLX101 Investigator's Brochure).[53]

1.2.5.6 Efficacy of CRLX101 Therapy

The following efficacy information includes data as of March 12, 2019 per CRLX101 Investigator's Brochure version 15.

CRLX-001 – Phase 1/2a Study: Of 44 subjects with advanced solid tumors treated with CRLX101 at the MTD (15 mg/m^2 IV Q2W), 28 (64%) subjects had a best overall response of stable disease (SD) per RECIST criteria. For a subset of subjects treated with NSCLC treated at the MTD, 16 patients (73%) subjects had SD. Stable disease was documented for 6 or more cycles of treatment with CRLX101 in 7 (16%) subjects treated at the MTD and in 4 (18%) subjects with NSCLC treated at the MTD. Median PFS for subjects treated with CRLX101 at the MTD and for those treated at the MTD in the NSCLC population (3.7 months and 4.4 months, respectively) was similar to the median PFS for these subjects on prior cancer therapy (4.0 months and 4.5 months, respectively).

CRLX-002 – Phase 2 NSCLC Study: The median OS (mOS) of patients treated with CRLX101 (15 mg/m^2 IV Q2W) + BSC was 6.3 months (95% CI: 4.70, 8.68). The mOS for the BSC alone arm, by comparison, was 11.9 months (95% CI: 6.74, not evaluable). More subjects in the CRLX101 + BSC arm were treated with surgery, XRT, and ≥2 lines of antineoplastic regimens compared with subjects in the BSC alone arm. Discontinuation due to symptomatic deterioration and withdrawal by subjects was reported at a higher frequency in the BSC alone arm. Thus, the survival estimate may be biased due to a much higher number of censored subjects (~50%) in the BSC arm.

CRLX101-208 – Phase 2 mRCC Study: As of March 12, 2020, this study completed accrual, but the clinical study report has yet to be finalized. The primary endpoint was median progression-free survival (mPFS) for the clear cell renal carcinoma population (n=102), with secondary endpoints including overall response rate, duration of response, and overall survival. mPFS was 3.7 months for the CRLX101 (15 mg/m^2 IV Q2W) + bevacizumab (10 mg/m^2 IV Q2W) treatment group vs. 3.9 months for the standard of care arm (HR: 1.25; p=0.822). The 95% confidence interval for PFS for the combination treatment group was 2.0 months to 4.3 months vs. 2.2 months to 5.4 months for the standard of care arm. Objective response rate by independent radiological review for the combination treatment group was 5% (2/42) compared with 14% (6/43) for the standard of care group (p=0.836).

CRLX101-209 – Phase 1b/2 Ovarian Cancer Study: A total of 30 patients received CRLX101 (12 mg/m^2 IV Q2W or 15 mg/m^2 IV Q2W) + paclitaxel (80 mg/kg on Day 1, 8, and 15 of a 28-day cycle). One of 30 subjects (3.3%) achieved a complete response, 7 of 30 subjects (23.3%) achieved a partial response and 16 of 30 subjects (53.3%) had stable disease. The median duration of response was 6.0 months. Of 19 subjects who had previously failed bevacizumab, 5 subjects (26.3%) achieved a partial response. Note: Efficacy data reporting cut-off was March 12, 2019.

CRLX101-102 – Phase 1 Advanced Solid Tumor Study: A total of 14 patients with advanced solid tumors receiving CRLX101 (12 mg/m^2 IV weekly or 15 mg/m^2 IV weekly) ± bevacizumab (10 mg/m^2 IV Q2W) were evaluable. Five subjects had PRs: one subject with SCLC receiving

CRLX101 15 mg/m^2 monotherapy, one subject with rectal cancer receiving CRLX101 12 mg/m^2 + bevacizumab, and one subject with RCC receiving CRLX101 12 mg/m^2 + bevacizumab. Eight subjects had stable disease.

1.2.5.7 Adverse Events with CRLX101 Therapy

The safety information for CRLX101 is based on the data available as of March 12, 2020. (CRLX101 Investigator's Brochure) Provided below is the summary of the overall safety assessment:

- Across all studies, 479 subjects experienced a total of 309 serious adverse events (SAEs) during the reporting period.
- During the reporting period, serious adverse reactions (SARs) have been reported in 13 subjects in the CRLX101-209 study and 7 subjects in Study CRLX101-102. None of the subjects in Study CRLX101-208 or in the Investigator-sponsored trials experienced an SAR during the reporting period
- A total of 20 deaths were reported during the reporting period, including 15 in Study CRLX101-208, 4 in Study CRLX101-209 and 1 in an Investigator-sponsored trial (NCI 16-C-0107).

1.2.5.7.1 CRLX101 Monotherapy/Dose Escalation Studies

Study CRLX-001 (Phase 1/2a Solid Tumors): Thrombocytopenia, neutropenia, anemia, and leukopenia were dose limiting following weekly administration of CRLX101 18 mg/m^2 via IV infusion. Thrombocytopenia and neutropenia were dose-limiting following treatment with CRLX101 18 mg/m^2 every other week (Q2W), leading to the MTD of CRLX101 15 mg/m^2 administered IV Q2W. The majority of TEAEs were Grade 1 or 2. The commonly reported treatment-related TEAEs (>10% of subjects) were: fatigue (23 subjects, 37%), cystitis (17 subjects, 27%); nausea (12 subjects, 19%); anemia (12 subjects, 19%); dysuria (11 subjects, 18%); hematuria (11 subjects, 18%); neutropenia (9 subjects, 15%);diarrhea (7 subjects, 11%); and vomiting (6 subjects, 10%). Among the 44 subjects treated at the MTD, of whom 22 had NSCLC, the commonly reported treatment-related TEAEs were fatigue (15 subjects, 34%); cystitis (11 subjects, 25%); nausea (10 subjects, 23%); anemia (6 subjects, 14%); dysuria (6 subjects, 14%); and neutropenia (5 subjects, 11%). Three subjects had serious infusion-related hypersensitivity, which were resolved without sequelae.

Study CRLX101-102 (Phase 1 Solid Tumors): In patients receiving CRLX101 12 mg/m^2 IV weekly, the following serious adverse events were observed: febrile neutropenia (1 subject, 14.3%), thrombocytopenia (1 subject, 14.3%), and small intestinal obstruction (1 subject, 14.3%). In patients receiving CRLX101 15 mg/m^2 IV weekly, the following serious adverse events were observed: pericarditis (1 subject, 12.5%), pain (1 subject, 12.5%), cognitive disorder (1 subject, 12.5%), dyspnea (1 subject, 12.5%), and pulmonary edema (1 subject, 12.5%).

Study CRLX-002 (Phase 2 NSCLC): A total of 97 subjects were treated with CRLX101 15 mg/m^2 IV Q2W + best supportive care (BSC).) At least 1 TEAE related to the study drug was reported for 53 subjects (55%) and at least one Grade ≥3 TEAE was reported for 18 subjects (19%). The commonly reported TEAEs regardless of grade (>88%) were: anemia (19 subjects, 20%), nausea (10 subjects, 10%), and cystitis (8 subjects, 8%). The following Grade ≥3 TEAEs

were reported in more than one patient: neutropenia (2 subjects, 2%). One TEAE with a fatal outcome was reported for one subject (atrial flutter, suspected to be related to study drug).

1.2.5.7.2 CRLX101 and Bevacizumab

A total of 49 subjects were treated with CRLX101 and bevacizumab on 3 different schedules (CRLX101 12 mg/m^2 QW and bevacizumab Q2W, N=7; 15 mg/m^2 CRLX101 QW and bevacizumab Q2W, N=4; 15 mg/m^2 CRLX101 Q2W and bevacizumab Q2W, N=55).

For patients treated with CRLX101-208, at least 1 TEAE related to study treatment (CRLX101 15 mg/m^2 IV Q2W + bevacizumab 10 mg/m^2 IV Q2W) was reported for 54 subjects (98.2%) and at least 1 Grade ≥3 TEAE was reported for 17 subjects (34.7%). The commonly reported TEAEs regardless of grade (>10%) were: fatigue (21 subjects, 38.2%), nausea (16 subjects, 29.1%), constipation (10 subjects, 18.2%), anemia (8 subjects, 14.5%), decreased appetite (7 subjects, 12.7%), non-infective cystitis (7 subjects, 12.7%), diarrhea (6 subjects, 10.9%), headache (6 subjects, 10.9%), epistaxis (6 subjects, 10.9%) and proteinuria (6 subjects, 10.9%). The following Grade ≥3 TEAEs were reported in more than one subject: fatigue (4 subjects, 7.3%), anemia (3 subjects, 5.5%), hypertension (3 subjects, 5.5%), vomiting (1 subject, 1.8%), and headache (1 subject, 1.8%). One TEAE with a fatal outcome was reported for one subject receiving CRLX101 15 mg/m^2 IV Q2W and bevacizumab 10 mg/m^2 IV Q2W (sudden death, suspected to be related to study drug)

For patients treated on CRLX101-102, the following serious adverse events were reported in the CRLX101 12 mg/m^2 IV weekly + bevacizumab 10 mg/m^2 IV Q2W: vomiting (2 subjects, 28.6%), nausea (1 subject, 14.3%), constipation (1 subject, 14.3%), chest pain (1 subject, 14.3%), urinary tract infection (1 subject, 14.3%), tremor (1 subject, 14.3%), noninfective cystitis (1 subject, 14.3%), hematuria (1 subject, 14.3%), and renal failure (1 subject, 14.3%). No serious adverse events reported in the CRLX101 15 mg/m^2 IV weekly + bevacizumab 10 mg/m^2 IV weekly treatment group.

1.2.5.7.3 CRLX101 and Paclitaxel

A total of 30 subjects were treated with CRLX101 and paclitaxel on 2 different dose cohorts: CRLX101 12 mg/m^2 IV Q2W and paclitaxel 80 mg/kg IV QW (3 out of 4 weeks), N=3; CRLX101 15 mg/m^2 IV Q2W and paclitaxel 80 mg/kg IV QW (3 out of 4 weeks), N=27). In the CRLX101 12 mg/m^2 Q2W + paclitaxel group, there was a report of an attempted suicide (1 subject, 33.3%). The following serious adverse events were reported in the CRLX101 15 mg/m^2 Q2W and paclitaxel group: small intestinal obstruction (2 subjects, 7.4%), infusion-related reaction (2 subjects, 7.4%), skin infections (2 subjects, 7.4%), uncoded respiratory disorders (2 subjects, 7.4%), abdominal pain (1 subject, 3.7%), ascites (1 subject, 3.7%), infectious enterocolitis (1 subject, 3.7%), hyperglycemia (1 subject, 3.7%), and non-infective cystitis (1 subject, 3.7%). There have been four deaths reported on this study: three subjects died of disease progression and the cause of death for one subject was not related to treatment or disease.

1.2.5.8 Hypersensitivity Reactions

Infusion related reactions (IR) have been observed in a small number of subjects treated with CRLX101. IRs are observed in other nanotechnologies and may occur in a higher incidence when CRLX101 is combined with weekly paclitaxel. Most IRs associated with CRLX101 are mild and moderate, reversible upon study drug interruption. In general, subjects restarted

CRLX101 infusion at a slower rate without recurrence. Mandatory premedication has been implemented in all CRLX101 protocols. In addition, it is recommended that the duration of CRLX101 infusion be prolonged from 1 hour to 2 hours for at least the first 4 infusions. In a few severe cases, a simple desensitization protocol allowed patients to continue therapy.

1.2.5.9 Camptothecin-Induced Cystitis

Cystitis is a common adverse event following CRLX101 administration. The proposed mechanism of toxicity involves the reclosing of the camptothecin lactone ring once the drug is cleared into the more acidic pH of the bladder. The closed lactone ring leads to reactivation of camptothecin, thus increasing the likelihood for off-target effect and subsequent toxicity in the bladder. The current management of the risk for cystitis involves dilution of the urine via increased hydration. Alkalinization of the urine is another proposed method of prophylaxis, which could be achieved via sodium bicarbonate administration. Although higher urine pH will limit the concentration of active camptothecin, it could lead to higher release of CPT from intact CRLX101 nanoparticles that are cleared renally. The use of sodium bicarbonate to circumvent high grade camptothecin-induced cystitis requires further investigation.

In vitro experiments were conducted at varying pH levels in urine following a 72-hour incubation at 37°C to measure the release of CPT from the CRLX101 nanoparticle and the ratio of lactone to carboxylate of the unconjugated CPT. While increasing pH lead to an increased rate of CPT release, the ratio of CPT species favored the carboxylate form, especially at pH values of 8 or greater (See Figure 5). Despite a higher concentration of total CPT, urine alkalinization led to a decrease in the active lactone form compared to lower urine pH values.

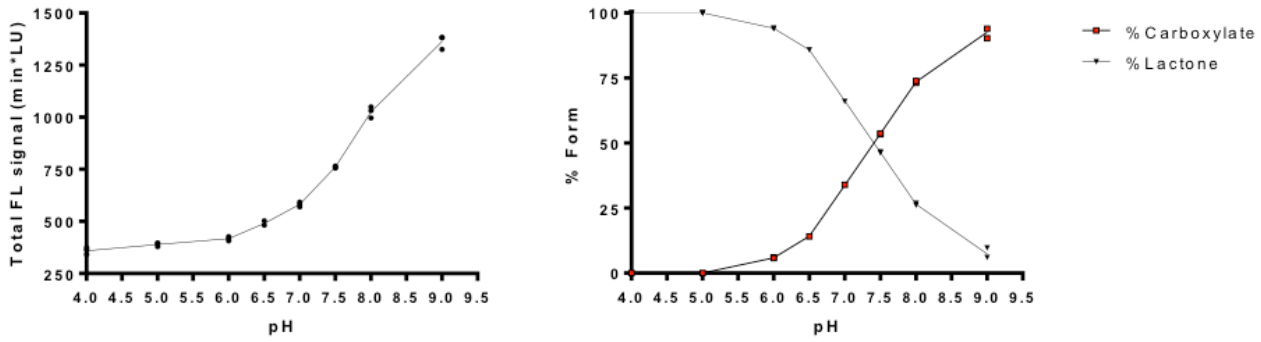

Figure 5: Total concentration of free CPT released from CRLX101 at various pH values (left) and the percentage of carboxylate form graphed against percentage of lactone form at various pH values (right).

Sodium Bicarbonate for Rapid Onset Urine Alkalinization

In a prospective open-label trial, 9 healthy volunteers were given 4 grams of sodium bicarbonate orally 3 times daily for 24 hours. All participants had a urine pH of at least 7 after 10 hours and a urine pH of at least 8 at 20 hours. During the 24 hour follow-up, no adverse effects or abnormal blood results were documented.[55]

1.2.6 Targeting DNA Damage in Prostate Cancer

Alterations of DNA repair pathways have been shown to be a significant mechanism of resistance following the failure of androgen deprivation therapy.[19] A phase II trial in patients

with mCRPC previously treated with chemotherapy and abiraterone/enzalutamide examined the efficacy of administering olaparib and evaluated the correlation of clinical response with the genomic signature. Following assessment of DNA repair genes, 16 patients were identified to have homozygous deletions, deleterious mutations, or both (*BRCA1/2*, *ATM*, Fanconi's anemia genes, *CHEK2*), with 14 of 16 patients responding to olaparib.[56] The characterization of DNA repair alterations in patients with progressive mCRPC before and after treatment with CRLX101, a topoisomerase I inhibitor, may provide useful information for future hypothesis generation and/or clinical investigation.

Identification of CTCs with high genomic instability from their phenotypic features alone has been used to classify patients with resistance to taxanes and AR targeted therapies. An algorithm with a cut point for high genomic instability (an indication of homologous recombination deficiency [HRD+]) was found to maximize prognostication for standard of care drugs. The overall prevalence of CTCs with high phenotypic genomic instability in patients following AR targeted therapy was 27% (36 of 134 patients), and was associated with inferior OS times (HR=7.69, p<0.0001).[59] In a follow-up study using this alogorithm, the CTC HRD+ phenotype cell counts were shown to increase on average from baseline (9 to 24 HRD+ CTCs/patient) in patients receiving abiraterone alone. By comparison, patients receiving abiraterone and veliparib (a PARP inhibitor [PARPi]) were shown to have a decrease in CTC HRD+ phenotype cell counts on average (17 to 3 HRD+ CTCs/patient).[60] The serial assessment of CTCs using this algorithm could aid in characterizing the effect of CRLX101 and enzalutamide on acquired resistance attributable to genomic instability.

1.3 Rationale

Acquired resistance to enzalutamide monotherapy limits the durability of response in both the first line and second line treatment setting.(61) Dual targeting of the AR and HIF-1α axis represents a novel therapeutic approach for CRPC treatment, with the inhibition of HIF-1α as a possible mechanism for overcoming enzalutamide resistance and potentiating anti-androgen/AR therapy.(32, 46) The treatment combination of enzalutamide and CRLX101, a HIF-1α inhibitor, is aimed to reduce cross-talk of both androgen receptor and HIF-1α signaling pathways, reverse enzalutamide resistance and increase time to progression. To test this concept, this pilot study will examine the efficacy of combination therapy to determine if further investigation is warranted.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 Eligibility Criteria

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histologically or cytologically confirmed prostate cancer confirmed by either the Laboratory of Pathology at the NIH Clinical Center or Walter Reed National Military Medical Center at Bethesda prior to starting this study. If no pathological specimen is available, patients may enroll with a pathologist's report

showing a histological diagnosis of prostate cancer and clinical course consistent with the disease.

2.1.1.2 Patients must have progressive mCRPC. There must be radiographic evidence of disease progression or biochemically (rising PSA levels on successive measurements) recurring disease despite adequate testosterone suppression.

2.1.1.3 Progression must be evidenced and documented by any of the following parameters:

- PSA progression defined by a minimum of two rising PSA levels with an interval of ≥1 week between each determination
- Appearance of one or more new lesions consistent with prostate cancer on bone scan
- New or growing lesions on CT scan

2.1.1.4 Patients must have metastatic disease, per RECIST 1.1(62). See Section **6.3** for the evaluation of measurable disease.

2.1.1.5 Patients must have received treatment with prior enzalutamide for two or more cycles and must have had evidence of disease progression while on enzalutamide.

2.1.1.6 Patients who have received antiandrogens such as flutamide, bicalutamide, or nilutamide for >6 months immediately before enrollment on this study must be off treatment for 4 weeks (6 weeks for bicalutamide) and demonstrate a continued rise in PSA. Patients on antiandrogens for <6 months must be off medication for 2 weeks.

2.1.1.7 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of enzalutamide and CRLX101 in patients <18 years of age and prostate cancer is not common in children <18 years of age, children are excluded from this study.

2.1.1.8 Patients must have adequate organ and marrow function as defined below:

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥100,000/mcL
– total bilirubin	within normal institutional limits; for patients with Gilbert's syndrome, total bilirubin ≤3.0 mg/dL
– hemoglobin	≥9 g/dL
– serum albumin	≥ 2.8 g/dL
– AST(SGOT)/ALT(SGPT)	≤2.5 X institutional upper limit of normal (<5 X institutional ULN for liver metastases)
– creatinine	within 1.5X normal institutional limits OR
– creatinine clearance	≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

2.1.1.9 Patients must have castrate levels of testosterone (<50 ng/dL [1.74 nmol/L]).

2.1.1.10 Patients must have undergone bilateral surgical castration or must continue on GnRH agonists/antagonists for the duration of the study.

2.1.1.11 Patients on 5-alpha reductase inhibitors such as finasteride or dutasteride must stop medication at least 28 days prior to study entry.

2.1.1.12 The effects of enzalutamide and CRLX101 on the developing human fetus are unknown. For this reason and because androgen receptor antagonists and topoisomerase I inhibitors as well as other therapeutic agents used in this trial are known to be teratogenic, all study subjects must agree to use a condom during the study treatment period and for 120 days following the last dose of study drug. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.13 ECOG performance status ≤2 (Karnofsky ≥60%, see **APPENDIX A**).

2.1.1.14 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who are receiving any other investigational agents. A minimum washout period of 28 days is required prior to the initiation of on study treatment, unless the patient is receiving immunotherapy, for which the minimum washout period will be 14 days. This is because Immune-related toxicities are distinct and unlikely to synergize with this protocol therapy, a shorter washout period is reasonable and customary in clinical trials.

2.1.2.2 Patients who have been treated with prior secondary hormonal manipulations with proposed investigational rationale for having efficacy against AR-V7 splice variants. This includes but is not limited to EPI-002 and AZD5312. (Note: patients previously treated with abiraterone, orteronel (TAK-700), apalutamide (ARN-509), galeterone, or VT-464 will be eligible for this study. Patients who have received prior chemotherapy will also be eligible for this study).

2.1.2.3 Patients with known 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.

2.1.2.4 Patients with a recent (within 1 year) history of seizure or any condition that, in the opinion of the investigator, significantly increases seizure risk. Also current or prior treatment with anti-epileptic medications for the treatment of seizures. Transient ischemic attack within 12 months prior to study enrollment will not be permitted.

2.1.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to enzalutamide, CRLX101, or other agents used in study.

2.1.2.6 Patients with a history within the last 3 years of another invasive malignancy (localized non-melanoma skin and bladder cancers are allowed).

2.1.2.7 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac

arrhythmia, uncontrolled hypertension (SBP>170/DBP>105), or psychiatric illness/social situations within 6 months that would limit compliance with study requirements.

2.1.2.8 Patients who have received palliative radiotherapy within 2 weeks of study entry and have not recovered to Grade 1 or baseline from associated toxicities. Note: Patients may receive palliative radiation once enrolled on study, as per guidance outline in Section **4.2.2**.The subject has not recovered to baseline or CTCAE ≤ Grade 1 from toxicity due to all prior therapies, including surgery, except alopecia and other non-clinically significant AEs.

2.1.2.9 Patients who are unable to swallow tablets or have a gastrointestinal disease that could hinder the absorption of enzalutamide

2.1.2.10 The use of any herbal products that may lower PSA levels (e.g. saw palmetto).

2.1.2.11 Patients with microscopic hematuria (defined as >100 RBCs on urinalysis) or worsening urinary symptoms within 7 days prior to the initiation of study treatment.

2.1.2.12 Known HIV-positive patients on antiretroviral therapy are ineligible because of potential pharmacokinetic interactions with study drugs. However, patients with long-standing (>5 years) HIV on antiretroviral therapy >1 month (undetectable HIV viral load and CD4 count > 150 cells/μL) may be eligible if the Principal Investigator or designee determines no anticipated clinically significant drug-drug interactions.

2.1.3 Inclusion of Minorities and Women

Men of all races and ethnic groups are eligible for this trial. Women are excluded as prostate cancer does not exist in this population.

2.1.4 Recruitment Strategies

This study will be listed on available websites (e.g. www.clinicaltrials.gov) and on NIH social media forums. Additionally, participants will be recruited from the current patient population at NIH.

2.2 SCREENING EVALUATION

Note: screening evaluation testing/procedures are conducted under the separate screening protocol, 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols).

The following tests may be obtained any time prior to enrollment:

- Pathological confirmation of diagnosis and PSA expression in either the Laboratory of Pathology at NIH Clinical Center, Walter Reed National Military Medical Center at Bethesda. However, if no pathologic specimen is available, participants may enroll with a pathologist's report showing a histologic diagnosis of prostate cancer and a clinical course consistent with the disease.

The following parameters will be obtained within 30 days prior to enrollment (unless otherwise noted):

- Tc-99 whole body scintigraphy
- CT of chest/abdomen /pelvis (MRI may be substituted at investigator's discretion).
- History and physical examination with ECOG and vital signs
- Serum PSA
- Complete blood count plus differential and platelet count
- Hepatic and Acute Care panels
- Testosterone level
- Urinalysis
- EKG

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

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

2.3.1 Treatment Assignment Procedures

2.3.1.1 Cohorts

Number	Name	Description
1	*Lead-in Cohort*	*First 3-6 participants with metastatic castration resistant prostate cancer receiving escalating doses of CRLX101*
2	*Combination Treatment Cohort*	*Up to 21 participants with metastatic castration resistant prostate cancer receiving tolerable dose of CRLX101*

2.3.1.2 Arms

Number	Name	Description
1	*Lead-in safety*	*Combination treatment of increasing dose of CRLX101 with enzalutamide*
2	*Efficacy*	*Tolerable dose of CRLX101 in combination with enzalutamide (8 participants, expandable to 21 total participants)*

2.3.1.3 Arm Assignment

Participants in cohort 1 will be directly assigned to arm 1 based on enrollment time in the study.

Participants in cohort 2 enrolled after the lead-in study will be directly assigned to arm 2.

2.4 BASELINE EVALUATION

The following parameters will be obtained within 14 days prior to start of treatment (unless otherwise noted):

2.4.1 Clinical Evaluation

- History and physical examination

- ECOG performance status
- Height, Weight
- Baseline EKG

2.4.2 Laboratory studies

- Complete blood count plus differential and platelet count
- Acute care panel
- Hepatic panel
- Mineral panel
- LDH, CK, Uric Acid, Total Protein
- Urinalysis within 7 days prior to treatment
- Serum PSA
- Activated partial thromboplastin time (APTT) and International normalized ratio (INR)

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is an open label single arm phase 2 study that will explore the potential benefit of CRLX101 in combination with enzalutamide in participants with progressive mCRPC following prior enzalutamide treatment. Participants meeting eligibility criteria will be enrolled into a single cohort to receive standard of care enzalutamide at the FDA-approved dose (160 mg orally once daily) in combination with CRLX101 administered as a via IV infusion if given every 2 weeks as monotherapy). The duration of each cycle will be 28 days. **Please note**, participants taking Enzalutamide at the time of enrollment will not be required to stop.

Blood and urine will be collected at multiple time points for PK, PD analyses as described in Section **5.1**. For logistical purposes, participants may be admitted to the inpatient unit for frequent serial sample collections on select cycles.

Toxicity will be graded according to CTCAE version 5.0. Participants will be treated until radiographic progression on scans using RECIST v1.1(62) and PCWG3(2, 63) criteria.

The primary endpoint of this trial will be treatment response (defined as ≥50% PSA decline or stable disease at 5 months), with secondary endpoints including duration of response (defined as sustained >30% decline in PSA), overall response and changes in measurable disease using RECIST v1.1 and PCWG3 criteria.

3.1.1 Lead-In Component

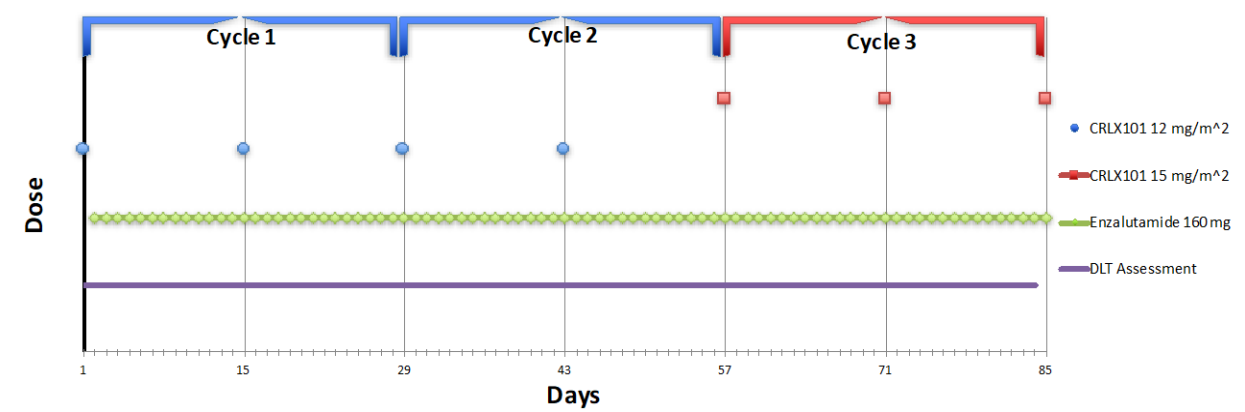

Figure 6: Lead-In Dosing Schema and DLT Assessment

For the first 3 to 6 participants, there will be a lead-in period of two cycles to ensure the safety and tolerability of CRLX101 in combination with enzalutamide. CRLX101 will be started at a dose of 12 mg/m^2, administered via IV infusion on Day 1. Enzalutamide will be administered orally at a dose of 160 mg once daily starting on Day 2 (C1D2) once the 24-hour pharmacokinetic sample timepoint has been collected (at least 24 hours after the end of the CRLX101 infusion). After the first dose on C1D2, Enzalutamide will be taken once daily for the full duration of the study. Participants will receive CRLX101 12 mg/m^2 via IV infusion on Cycle 1 Day 15 (C1D15), Cycle 2 Day 1 (C2D1), and Cycle 2 Day 15 (C2D15). On Cycle 3 Day 1 (C3D1), and Cycle 3 Day 15 (C3D15), CRLX101 will be administered at the previously established MTD (15 mg/m^2 administered via IV infusion). Participants will be assessed from Day 1 to Day 56 (C1D1 to C2D28) for dose limiting toxicities of the CRLX101 12 mg/m^2 dose and from Day 57 to 84 (C3D1 to C3D28) for dose limiting toxicities of the CRLX101 15 mg/m^2 dose (Enzalutamide will be at approximate steady state concentration following Day 29 [C2D1]). Following the DLT assessment period (at the start of Cycle 4), each participant will continue to receive CRLX101 at the participant's specific maximum tolerated dose via IV infusion every 2 weeks (Days 1 and 15) for every subsequent cycle.

For the purposes of this study, re-assessment of the CRLX101 RP2D will be determined based on DLTs deemed likely attributable to CRLX101. DLTs are outlined in Section **3.1.1.1** and the dose modification procedures of CRLX101 are outlined in **3.3.2**. Adverse events deemed attributable to enzalutamide will not influence the on-trial treatment of subsequent participants and will be managed according to the dose modification protocol outlined in Section **3.3.3**. Final determination of the drug attributable to an adverse event is at the discretion of the Principal Investigator or designee.

If none of the three participants experience a dose limiting toxicity during the lead-in phase (Day 1-84), subsequent participants enrolled on trial with receive CRLX101 at a dose of 15 mg/m^2 via IV infusion every 2 weeks.

If one of three participants experience intolerable toxicity during the lead-in phase, an additional 3 participants will be enrolled with no adjustments to the lead-in dosing schema. If no further toxicity is observed (or a total of 1 in 6 participants experience dose-limiting toxicity),

subsequent participants enrolled on study will receive CRLX101 at a dose of 15 mg/m^2 every 2 weeks. If 2 of 6 participants experience a dose limiting toxicity, the dose at which the DLT occurred will determine further action:

- If both participants experience a DLT attributable to CRLX101 at a dose of 15 mg/m^2 or only one participant experiences a DLT at 12 mg/m^2, subsequent participants will receive CRLX101 at 12 mg/m^2 every 2 weeks.
- If both participants experience a DLT attributable to CRLX101 at a dose of 12 mg/m^2, participants will be given one dose of CRLX101 at 9 mg/m^2.
    - If further toxicity is observed with the 9 mg/m^2 dose, an amendment to modify dosing strategies will be considered. If necessary, the RP2D will be determined at the discretion of the Principal Investigator or designee after factoring in participant specific characteristics and the nature of the adverse event.

Based on limited potential for overlapping toxicity and well-characterized pharmacokinetics for both enzalutamide and CRLX101, it is anticipated 3 participants will participate in the lead-in phase. An accrual ceiling of 6 participants for the lead-in phase will be used to account for possible dose adjustments based on DLTs.

3.1.1.1 Dose Limiting Toxicity

Dose-limiting toxicity (DLT) is defined as an adverse event that is related (possibly, probably, or definitely) to the administration of CRLX101. DLTs (toxicities occurring within the first 84 days of treatment after the first intravenous infusion of CRLX101) will be defined using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The following events will be considered DLTs if deemed drug-related:

- Grade ≥3 neutropenia complicated by fever ≥38.5°C (i.e. febrile neutropenia and/or documented infection.
- Grade 4 neutropenia that does not improve within 5 days *
- Grade 4 thrombocytopenia that does not resolve within 5 days* or any grade 3-4 thrombocytopenia complicated with hemorrhage;
- Grade 4 anemia that does not resolve within 7 days despite optimal therapy (withholding study drug and red blood cell transfusion);
- Any grade 3-4 non-hematologic toxicity (except fatigue/asthenia < 2 weeks in duration; mucositis in subjects who have not received optimal therapy for mucositis; vomiting or diarrhea lasts less than 72 hours whether treated with an optimal anti-emetic or anti-diarrheal regimen or not; or alkaline phosphatase changes)
- Failure to receive at least 75% of study drug doses during cycle 1 due to toxicity.
- Laboratory results of 3xULN elevation of transaminases and concomitant 2xULN total bilirubin without an alternative etiology
- Any toxicity resulting in greater than two weeks of dose delay.

*Note: In the event of a Grade 4 neutropenia or thrombocytopenia, a full blood count must be performed no more than 5 days after the onset of the event to determine if a DLT has occurred. The subject will be closely monitored until the resolution to Grade 3 or less.

3.1.2 Dosing Schema following Lead-In Component

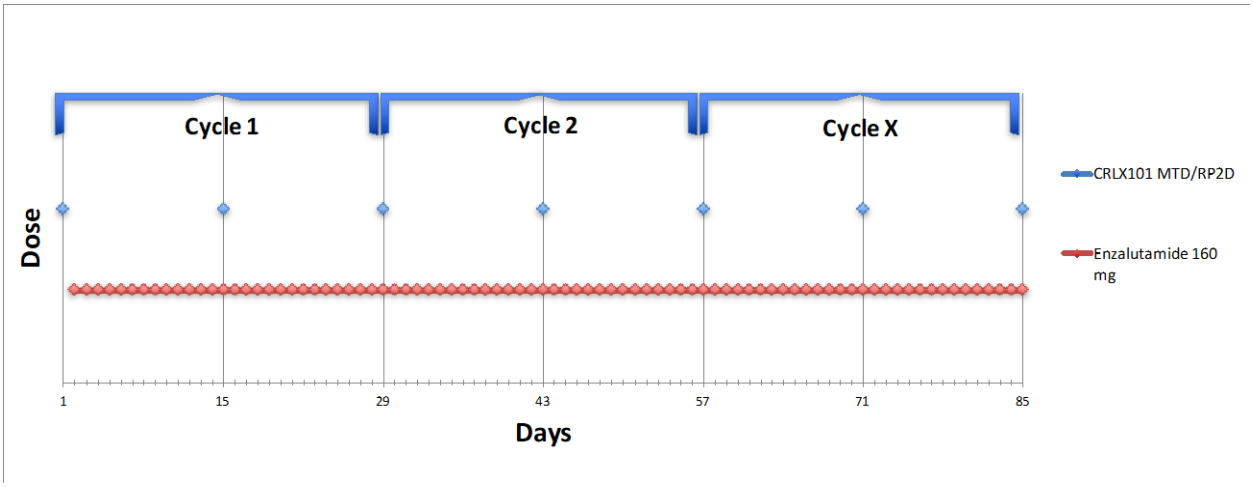

Figure 7: Dosing schema following the lead-in component.

Following the DLT assessment during the lead-in component and validation of the RP2D, participants enrolled thereafter will not participate in the lead-in period. Participants will receive CRLX101 at the RP2D established during the lead-in component via IV infusion on Cycle 1 Day 1 (C1D1). Enzalutamide will be administered orally at a dose of 160 mg once daily beginning on Cycle 1 Day 2 (C1D2), at least 24 hours after the end of the infusion of CRLX101. After the first dose on C1D2, Enzalutamide will be taken once daily for the full duration of the study. Participants will receive CRLX101 at the established RP2D via IV infusion on C1D15 and on Days 1 and 15 of each subsequent cycle.

Following establishment of the RP2D, enrollment on this study will continue until 8 participants are evaluable for the primary endpoint. If 3 or more participants achieve response, then additional participants will be enrolled until a total of 21 participants have been evaluated for the primary endpoint. The accrual ceiling is set for 30 participants, to account for participants that are inevaluable or participants that do not tolerate treatment in the lead-in phase. For more details, refer to Section 8.

3.2 Drug Administration

3.2.1 CRLX101

CRLX101 will be diluted with 5% dextrose for injection (D5W) to a total volume of 500 mL and should be infused intravenously over 60-75 minutes (See **13.1**). **Nothing else should be added to the bag.**

The CRLX101 infusion should begin immediately after preparation and diluted CRLX101 infusion solution not used within 6 hours should be destroyed following institutional practices.

Hypersensitivity reaction (HSR) have been observed in a small number of subjects treated with CRLX101. Most HSR associated with CRLX101 are mild and moderate, reversible upon study drug interruption. In general, subjects restarted CRLX101 infusion at a slower infusion rate without recurrence. In addition to the mandatory premedication to prevent HSR, the duration of CRLX101 infusion must be prolonged from 1 hour to 2 hours (+/- 10 minutes) in participants who have experienced a previous infusion-related reaction.

3.2.1.1 Hydration and Premedication Prior to CRLX101 Treatment

Subjects will receive IV hydration infused approximately over two hours with up to 1,000 mL of 0.9% sodium chloride solution before and after the administration of CRLX101 to reduce risk of cystitis adverse reaction. The administration rate and volume should follow standard site procedures and may be adjusted by the Principal Investigator or designee based on patient-specific factors. Participants should be encouraged to adequately hydrate throughout the cycle, especially during the first 48 hours following the infusion of CRLX101.

Subjects should be pre-medicated with the following drug classes 30-90 minutes prior to start of CRLX101 infusion to reduce likelihood of hypersensitivity adverse reactions:

- a corticosteroid (dexamethasone 20 mg IV or PO)
- an antihistamine (diphenhydramine 50 mg IV or PO)
- an H2 antagonist (famotidine 20 mg IV or PO; 30-60 minutes prior to start of CRLX101)
- an anti-emetic, (e.g., a 5-HT3 receptor antagonist)

At the discretion of the Principal Investigator or designee, a 650 mg dose of Acetaminophen PO may also be given.

The drug recommendations can be adjusted per local institutional guidelines with approval from the Principal Investigator or designee.

The example medications, route of administration, and dose indicated should be the medication-of-choice if clinically feasible. It is acknowledged that the premedication regimen may be altered for participant safety during the study (for example, if a participant experiences a hypersensitivity/infusion reaction related to study drug, the premedication regimen may be altered for subsequent cycle dosing based on the Principal Investigator or designee's discretion).

3.2.1.2 Sodium Bicarbonate Administration with CRLX101 Treatment

Participants will undergo an experimental urine alkalinization protocol in addition to IV hydration on C2D15 (See Section **5.1.1.2**). Participants will receive sodium bicarbonate orally pre- and post-CRLX101 infusion on C2D15. Details about recommended dosing are outlined below (**Table 1**) and in Section **5.1.1**. All other doses of CRLX101 will be given with IV hydration as described above. At the discretion of the Principal Investigator or designee, the strength, frequency, and/or schedule of oral sodium bicarbonate dosing may be modified on a participant by participant basis.

Table **1:** Recommended Oral Alkalinization Pre- and Post-CRLX101 Infusion

Timing	**Dose, route, and frequency**

Starting from 48 hours prior to CRLX101 infusion	Take sodium bicarbonate by mouth, 650mg x2, 4 times a day (QID), for two days before CRLX101 infusion
On the day of CRLX101 infusion	Take sodium bicarbonate by mouth, 650mg x2, 4 times a day (QID), for one day
After CRLX101 infusion	Take sodium bicarbonate by mouth, 650mg x2, 4 times a day (QID), for one day after CRLX101 infusion

At the discretion of Principal Investigator or designee, participants who are prone, or likely, to develop cystitis may be recommended to receive either oral or intravenous sodium bicarbonate pre and/or post CRLX101 administration.

3.2.2 Enzalutamide

All participants will receive enzalutamide 160 mg orally, daily starting cycle 1 day 2 (C1D2). Enzalutamide may be administered with or without food. It is recommended that enzalutamide be administered daily in the evenings, however, daily administration time of enzalutamide is based on participant preference.

3.3 Dose Modifications

3.3.1 General Rules for Dosing Delays/Dose Modifications and Management of Toxicities

- In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).
- Once a participant has a dose reduction for toxicity, the dose will not be increased.
- A maximum of two dose reductions are permitted
- If either agent is discontinued due to toxicity, the participant may continue on the other agent.
- Participants continuing to experience toxicity at the off-treatment visit will be contacted for additional assessments until the toxicity has resolved or is deemed irreversible. When logistically feasible, willing participants will remain on the study to have additional assessments.
- For AEs that are unrelated to the study drugs, dose may be held for up to 28 days at the discretion of the Principal Investigator or designee.
- The associate investigator and/or PI will determine whether one or both drugs are responsible for an observed toxicity and will manage that toxicity as described below.

3.3.2 CRLX101 Dose Modification

3.3.2.1 General Recommendations

The lead-in dosing schema is designed to ensure against significant drug-drug interactions between CRLX101 and enzalutamide. If dose modifications are necessary, the general approach to dose modification is listed in **Table 2**. Such dose modifications should be followed according to the dose level in which the participant experiences an undesirable adverse event (See Section

3.1.1 for Lead-in schema rules). Adverse events should be treated with the appropriate maximum intervention and dose reductions should be clearly documented in participant notes.

Table **2**: Guidelines for CRLX101 Dosing Reductions

	CRLX101 Dosage Schedule		
	Initial Dosing	First dose reduction	Second dose reduction
Dose Level 1	12 mg/m^2	9 mg/m^2	6 mg/m^2
Dose Level 2	15 mg/m^2	12 mg/m^2	9 mg/m^2

3.3.2.2 CRLX101-Related Hematologic Toxicities

Table **3:** Dose Modification and Management of Hematologic Adverse Events (CRLX101)

Cycle Day	**Grade or count (cells/mm^3)**	**Dose Delay or Reduction for Hematological Toxicity (Phase 2 stage and after Cycle 1 of a new dose level in lead-in stage)**
Neutropenia		
1 or 15	Grade 2 (<1500)	• For first neutropenic event[a], delay CRLX101 dose until ≤ Grade 1, resume CRLX101 with no dose change • For second neutropenic event[a], delay CRLX101 until ≤ Grade 1, resume CRLX101 with no dose change, add G-CSF[b] support • For third neutropenic event[a], delay dose until ≤ Grade 1, resume CRLX101 with the dose reduced by one dose level, continue G-CSF[b] support
	≥ Grade 3 (<1000)	• For first neutropenic event[a], delay CRLX101 dose until ≤ Grade 1, resume CRLX101 with no dose change, add G-CSF[b] support • For second neutropenic event[a], delay CRLX101 until ≤ Grade 1, resume CRLX101 with dose reduced by one dose level, continue G-CSF[b] support • For third neutropenic event[a], delay dose until ≤ Grade 1, resume CRLX101 with the dose reduced by one further dose level, continue G-CSF[b] support
Thrombocytopenia		
1 or 15	<100,000	• Delay CRLX101 dose until ≥ 100,000

Cycle Day	**Grade or count (cells/mm^3)**	**Dose Delay or Reduction for Hematological Toxicity (Phase 2 stage and after Cycle 1 of a new dose level in lead-in stage)**
		• Resume CRLX101 with no dose change
	Grade 2 (<75,000) and Grade 3 (<50,000)	• Delay dose forCRLX101 until ≥ 100,000 • Resume CRLX101 with no dose change (the dose may be reduced one dose level or kept at the same dose at the discretion of the Principal Investigator or designee) if grade 2 or grade 3 recurs, • Hold dose for CRLX101 until ≥ 100,000 • Resume CRLX101 with dose reduced by one dose level
	Grade 4	• Hold dose for CRLX101 until ≥ 100,000 • Resume CRLX101 with dose reduced by one dose level • Do not make up missing dose
Anemia		
1 or 15	≥ Grade 3 (<8g/dL)	• Delay dose for CRLX101, and consider RBC transfusion(s) based on clinical criteria • After improved to ≤ Grade 2, resume CRLX101 with no dose change (the dose may be reduced one dose level or kept at the same dose at the discretion of the Principal Investigator or designee) • Consider EPO support per guidelines If Grade 3 recurs, • Delay dose for CRLX101, and consider RBC transfusion(s) based on clinical criteria • After improved to ≤ Grade 2, resume CRLX101 with dose reduced by one dose level • Consider EPO support per guidelines

Cycle Day	**Grade or count (cells/mm^3)**	**Dose Delay or Reduction for Hematological Toxicity (Phase 2 stage and after Cycle 1 of a new dose level in lead-in stage)**
	Grade 4	• Delay dose for CRLX101, and consider RBC transfusion(s) based on clinical criteria • After improved to ≤ Grade 2, resume CRLX101 with dose reduced by one dose level • Consider EPO support per guidelines.
[a] A neutropenic event is defined as ≥ Grade 2 neutropenia on D1 or ≥ Grade 3 neutropenia on Day 15 [b] G-CSF (filgrastim or equivalent, pegfilgrastim is also permitted) should not start until at least 24 hours after discontinuation of chemotherapy. Discontinue G-CSF once ANC is ≥1000/mm^3. Wait at least 24 hours after G-CSF is discontinued before resuming chemotherapy.		

- Treatment may be delayed for a maximum of 28 days after holding the treatment for toxicities that develop and do not resolve as defined above (exemptions: lymphopenia, or leukopenia in the absence of grade 3 or higher neutropenia).
- Bi-weekly blood counts will be obtained throughout the study. If any evaluation demonstrates grade ≥ 3 neutropenia or grade ≥ 2 thrombocytopenia, a repeat hematology assessment will be obtained 2-4 days later.
- Management of prolonged hematological toxicities while on study treatment

If a participant develops prolonged hematological toxicity such as:

- ≥2 week interruption/delay in study treatment due to CTC grade 3 or worse anemia and/or development of blood transfusion dependence
- ≥3 week interruption/delay in study treatment due to CTC grade 3 or worse neutropenia (ANC < 1 x 109/L)
- ≥3 week interruption/delay in study treatment due to CTC grade 3 or worse thrombocytopenia (Platelets < 50 x 109/L)

Weekly differential blood counts including reticulocytes (calculate reticulocyte index (RI), RI = reticulocyte count x hematocrit (Hct)/normal Hct; a value of 45 is usually used for normal Hct) and peripheral blood smear could be considered (may be done at an outside facility). If any blood parameters remain clinically abnormal after 4 weeks further investigations could be considered including hematology evaluation. Bone marrow analysis and/or blood cytogenic analysis could be considered at this stage according to standard hematological practice.

Development of a confirmed myelodysplastic syndrome or other clonal blood disorder should be reported as an SAE. Study treatment should be discontinued if diagnosis of myelodysplastic syndrome is confirmed.

3.3.2.2.1 Neutropenia

In the lead-in stage, subjects will not receive growth factors prior to C1D1 and prophylactic use of growth factors is prohibited during the first 3 cycles (Days 1-84) or until neutropenia is identified as a DLT (at discretion of the Principal Investigator or designee), whichever comes first. Growth factors can be used during a drug hold to assist the recovery and in subsequent cycles after a drug hold. Please note that G-CSF should not be used within at least 24 hours of the last dose of CRLX101 treatment.

In the Phase 2 stage, subjects may receive growth factors prophylactically at the discretion of the Principal Investigator or designee.

3.3.2.2.2 Thrombocytopenia

Thrombocytopenia will be treated conservatively. In the absence of bleeding or a necessary invasive procedure, platelet transfusions should be given for a platelet count ≤ 10,000/mcL. If invasive procedure(s) is (are) planned, or the participant develops bleeding, platelet transfusions should be administered in accordance with the standard of practice, usually maintaining a platelet count above 50,000/mcL

3.3.2.2.3 Anemia

Symptomatic anemia should be treated with red blood cell transfusion and is recommended if the hemoglobin falls below 8 g/dL or the participant is symptomatic. Erythropoietin (EPO) may also be given per clinical guidelines.

3.3.2.3 CRLX101-Related Non-Hematologic Adverse Events

Table 4: Dose Modification and Management of Non-Hematologic Adverse Events (CRLX101)

Observation	Action
Grade 1 or 2 AEs resolves promptly (within 48 hours) with supportive care	Maintain dose level (DL)
Any ≥ 3 non-hematologic*	Hold study drug(s) causing the toxicity for up to 28 days until toxicity resolves to ≤ Grade 1. Treatment with CRLX101 may be restarted at one DL lower, as per the dose reduction guidelines. Lab abnormalities that can be readily corrected (e.g. electrolyte abnormalities) do not require a dose reduction unless by discretion of the PI or designee.
Grade 3 or 4 non-hematologic AE related to drugs that does not	Remove participant from CRLX101 therapy.

Observation	Action
resolve to grade 1 or less within 28 days despite maximum supportive care after treating participant at the lowest reduced DL	
Toxicities requiring more than 2 dose reductions	Remove participant from CRLX101 therapy.

* Except fatigue/asthenia < 2 weeks in duration; mucositis in subjects who have not received optimal therapy for mucositis; vomiting or diarrhea lasting less than 72 hours whether treated with an optimal anti-emetic or anti-diarrheal regimen or not; or alkaline phosphatase changes

3.3.2.4 Management of the hypersensitivity/infusion reactions

Table **5**: Dosing modification and management of hypersensitivity reactions (CRLX101)

Grade 1 or 2	Stop infusion immediately, continue hydration fluids and provide medications as indicated per institutional guidelines If symptoms resolve within 1-2 hours, at Principal Investigator or designee's discretion may re-start study drug administration at slower rate (i.e. 2x slower rate) and increase rate slowly to complete administration of full dose OR Hold dose administration on day of event, and resume treatment at next scheduled day at the slower rate. Additionally, if an HSR occurs upon re-administration at the slower infusion rate, the participant will be removed from study.
Grade 3 or 4	Stop infusion immediately and administer medical support as indicated per institutional guidelines. Hold dose administration on day of event Review with study Principal Investigator or designee to determine whether to discontinue from study drug treatment. Confirm that rate of administration was appropriate and pre-meds were given as suggested.

If opting to resume CRLX101 infusion following Grade 1 or 2 HSR, administration of CRLX101 should be over 120 minutes (+/- 10 minutes) (using the following infusion schedule) with additional pre-medications (dexamethasone 20 mg PO or IV, famotidine 20 mg IV or PO, acetaminophen 650 mg PO, ondansetron 16 mg PO or IV, and diphenhydramine 50 mg PO or IV).

CRLX101 in 500 mL 5% dextrose IV infusion:

- 15 mL/hr for 15 minutes, then increase to
- 30 mL/hr for 15 minutes, then increase to
- 60 mL/hr for 15 minutes, then increase to
- 120 mL/hr for 15 minutes, then increase to
- 240 mL/hr for 15 minutes, then increase to
- 480 mL/hr for 15 minutes, then increase to
- 528 mL/hr for 30 minutes to complete infusion

Please note: a gradual increase in rate may be used in place of incremental rate increases. Infusion rates may also be adjusted at the discretion of the investigator.

If a subject has a history of infusion related reactions to other drug treatments, or has many environmental allergies (e.g. food, pollen), the total infusion time may be slowed during the first CRLX101 infusion and for all subsequent infusions, at the discretion of the Principal Investigator or designee.

3.3.3 Enzalutamide Dose Modification

If a participant experiences a ≥ Grade 3 non-hematologic toxicity (except hypertension) attributable to enzalutamide, withhold dosing for up to 1 month or until symptoms improve to ≤ Grade 2, then resume at the same dose if clinically appropriate. If toxicity recurs and/or in the judgment of the investigator dose reduction is appropriate, lower dose levels (120 mg/DL-1 or 80 mg/DL-2), may be considered. Enzalutamide may be discontinued if toxicity recurs and dose modification is no longer clinically appropriate.

3.3.3.1 Enzalutamide Dose Delay

Enzalutamide may be continued within 28 days if an appointment is missed due to scheduling or logistical issues (e.g. vacation, weather) during the treatment period. If more than 28 days have elapsed, participant will need to be seen to assess continuation of treatment procedure.

3.4 STUDY CALENDAR

On study assessment can be performed within ±7 days of the specified time, unless otherwise indicated. CRLX101 can be given within ±2 days of the specified time, unless otherwise indicated.

Table **6:** Study Calendar

Procedure	*[1]Screening/ Baseline*	*Cycle 1*			*Cycle 2*		*Subsequent Cycles*		*[2]Disease Progression*	*[3]End of Treatment*	*[4]Long Term Follow-Up*
		Day 1	*Day 2*	*Day 15*	*Day 1*	*Day 15*	*Day 1*	*Day 15*			
History and PE	*X*	*X*		*X*	*X*	*X*	*X*	*X*	*X*	*X*	
Vital signs	*X*	*X*		*X*	*X*	*X*	*X*	*X*	*X*	*X*	
ECOG Performance Score	*X*										
[5]Pathologic Confirmation of Dx	*X*										
[6]CBC/Diff	*X*	*X*		*X*	*X*	*X*	*X*	*X*	***X***		
[7]Acute Care Panel w/ Mineral Panel, Hepatic Panel, LDH, CK, Uric Acid, Total Protein	*[8]X*	*X*		*X*	*X*	*X*	*X*	*X*	***X***		
[9]Urinalysis	*[10]X*	*X*				*X*					
Serum PSA	*X*	*X*		*X*	*X*	*X*	*X*	*X*	*X*		
Serum Testosterone	*X*	*X*			*X*		*X*		*X*		
[11]EKG	*X*	*X*		*X*	*X*						
Tumor Assessment: CT scan (chest, abdomen & pelvis), [99]Tc bone scintography	*X*						*[12]X*		*X*		

Procedure	*[1]Screening/ Baseline*	*Cycle 1*			*Cycle 2*		*Subsequent Cycles*		*[2]Disease Progression*	*[3]End of Treatment*	*[4]Long Term Follow-Up*
		Day 1	*Day 2*	*Day 15*	*Day 1*	*Day 15*	*Day 1*	*Day 15*			
CRLX101		*X*		*X*	*X*	*X*	*X*	*X*			
Enzalutamide			*X*	→	→	→	→	→			
[13]Plasma PK		*X*				*X*					
[13]Urine PK		*X*				*X*					
[14]VEGF Biomarker, CTC, and Whole Blood Collection		*X*				*X*	*[15]X*		*X*		
[14]NanoString Sample Collection		*X*				*X*			*X*		
Adverse Events		*X*		*X*	*X*	*X*	*X*	*X*	*X*	*X*	
Concomitant Medications	*X*	*X*		*X*	*X*	*X*	*X*	*X*	*X*	*X*	
[16]APTT, INR	*X*										
Survival Phone Call											*X*

[1]Baseline procedures are performed within 2 weeks of initiation of study therapy. Screening labs may also be used as baseline/Day 1 labs if performed within two weeks of study initiation. Height and weight must be done within 14 days of the first study dose.

[2]To be completed on the day disease progression is noted according to PCWG3 and/or RECIST criteria. May be completed within 30 days or at end of treatment visit if logistically necessary and feasible. Subject may also be contacted by phone to assess adverse events if unwilling or unable to come to NIH."

[3]To be completed within 30 days after the last dose of study therapy, presuming logistically feasible. The study team may contact the participant by phone to assess adverse events if unwilling or unable to come to NIH.

[4]After the end of treatment visit the participant will receive a phone call every 6 months to assess for survival until expiration.

[5]Pathologic confirmation should be obtained at any point prior to enrollment. If no pathological specimen is available, participants may enroll with a pathologist's report showing the histological diagnosis of prostate cancer and clinical course consistent with disease. Participant may begin therapy based on evidence of disease progression if pathologic confirmation is not available at the time of study enrollment.

[6]Includes Neutrophils, Lymphs, Monos, Eos, Basos, WBC, RBC, Hemoglobin, Hematocrit, RBC Indices, MCV, RDW, Platelets, and RBC Indices. Results should be available prior to administration of study drugs. If absolute differentials are not available, percentage differentials are acceptable.

[7]Acute Care Panel: Sodium (Na), Potassium (K), Chloride (Cl) Total CO2 (Bicarbonate), Creatinine, random Glucose, urea or blood urea nitrogen [BUN], eGFR; Hepatic Panel: Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin; Mineral Panel: Albumin, Calcium, Magnesium, Phosphorous; Lactate Dehydrogenase (LDH); Creatine Kinase (CK); Uric Acid; Total Protein;

[8]Only the Acute Care and Hepatic panels are required at screening

[9]Additional urinalysis will be performed as clinically indicated in the investigator's judgment.

[10]To be done within 7 days prior enrollment/treatment.

[11]Must be completed within 14 days prior to the first study dose of CRLX10. Please note: EKGs are required within 60-180 minutes post-infusion on C1D1, C1D15 and C2D1 of CRLX101 for participants with any conduction abnormality on baseline ECG.

[12]Tumor Assessments will be performed prior to or on C3D1, C6D1, C9D1, and every 3rd cycle thereafter until evidence of disease progression. Primary endpoint assessment will be conducted following tumor assessment on C6D1.

[13]See Section **5.1.1** for complete details about PK biospecimen collection time points on C1D1 and C2D15. The exact time of the draw should be recorded.

[14]Samples are to be collected prior to treatment on the specified days. For samples collected prior to dosing on C1D1, the sample may be collected during baseline assessment or any time following baseline assessment but prior dosing on C1D1. Following disease progression, samples should be collected within 30 days, if logistically feasible.

[15]After sample collection on C2D15, samples will be collected prior to C6D1 and at disease progression for VEGF measurement, CTC analysis, and whole blood collection.

[16]Coagulation [activated partial thromboplastin time (APTT) and international normalized ratio (INR)] will be performed at baseline and if clinically indicated unless the participant is receiving warfarin. Participants taking warfarin may participate in this study;

however, it is recommended that prothrombin time (INR and APTT) be monitored carefully at least once per week for the first month, then monthly if the INR is stable.

3.5 COST AND COMPENSATION

3.5.1 Costs

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

3.5.2 Compensation

Participants will not be compensated on this study.

3.5.3 Reimbursement

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

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete an end of treatment visit approximately 30 days after the last dose of study therapy.

3.6.1 Criteria for removal from protocol therapy

- Progressive disease
- Participant requests to be withdrawn from active therapy
- Unacceptable toxicity as defined in Section **3.3**
- Toxicity related dose delay lasting longer than 28 days in which case participant will be removed from the drug causing the toxicity
- Investigator discretion
- Requirement for any drug deemed to cause an significant drug-drug interaction that could impact the study treatment (at discretion of the PI and/or Associate Investigator and as discussed in Section **4.1**)

3.6.2 Off-Study Criteria

- Participant requests to be withdrawn from study
- Death
- PI decision to end the study
- Participant lost to follow up

- Investigator's discretion

3.6.3 Lost to Follow-up

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

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

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

4 CONCOMITANT MEDICATIONS/MEASURES

Participants will be on concomitant androgen suppression therapy with a GnRH agonist or GnRH antagonist (unless they have had a prior orchiectomy). Participants may also be on concomitant drugs to prevent bone loss, including bisphosphonates and denosumab.

Other supportive care with blood components, antibiotics, analgesics, general medical therapy, etc., will be delivered as required. Use caution when co-administering medication which may lower the seizure threshold.

4.1 GENERAL GUIDELINES

All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) taken within 28 days of starting study treatment through the 30 day end of treatment visit should be reported on the CRF.

While participants on protocol treatment, all medications required for the health of the participant are allowed with the following exceptions:

- Concurrent chemotherapy or secondary hormonal treatment outside of the investigational treatment
- Concurrent cancer immunotherapy (e.g. therapeutic cancer vaccines)
- Concurrent anti-cancer radionuclides

Concomitant use of herbal supplements should be discouraged and/or avoided.

Palliative radiotherapy is allowed for pre-existing small areas of painful metastases (e.g. bone) that are uncontrolled with local or systemic analgesics as long as no evidence of disease progression is present and the metastatic site treated is not a target lesion.

4.1.1 Seizure threshold lowering drugs

- Use caution when co-administering medications which may lower the seizure threshold (e.g. atypical antipsychotic drugs, phenothiazines, bupropion, aminophylline/theophylline, tricyclic and tetracyclic antidepressants, lithium, pimozide, venlafaxine).

4.1.2 Cytochrome P450 and P-glycoprotein

- Co-administration of enzalutamide with strong or moderate CYP2C8 inducers (e.g., rifampin) may alter the plasma exposure of enzalutamide. Moderate CYP2C8 inducers maybe used concomitantly with enzalutamide at the Principal Investigator or designee's discretion. If co-administration of enzalutamide with a strong CYP2C8 inhibitor cannot be avoided, reduce the dose of enzalutamide to 80 mg per day.
- Co-administration of enzalutamide with strong CYP3A4 inducers (e.g., carbamazepine, phenobarbital, phenytoin, rifabutin, rifampin, rifapentine) may decrease the plasma exposure of enzalutamide and should be avoided if possible. Selection of a concomitant medication with no or minimal CYP3A4 induction potential is recommended.
- Moderate CYP3A4 inducers (e.g., bosentan, efavirenz, etravirine, modafinil, nafcillin) and St. John's Wort may also reduce the plasma exposure of enzalutamide and should be avoided if possible.
- Concomitant use of enzalutamide with narrow therapeutic index drugs that are metabolized by CYP3A4 (e.g., alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus and tacrolimus), CYP2C9 (e.g., phenytoin, warfarin) should be avoided, as enzalutamide may decrease their exposure.
- Grapefruit, Seville oranges, and starfruit affect P450 and PgP activity. Concomitant use should be avoided.

4.2 SUPPORTIVE CARE

Participant should receive general concomitant and supportive care medications based on best medical practice.

The use of any natural/herbal products or other "folk remedies" should be discouraged.

The preferred anticoagulant therapy is subcutaneous heparin or low molecular weight heparin. A participant may continue warfarin (with increased INR monitoring) or other anticoagulant agents under close supervision and at the discretion of the Principal Investigator or designee.

4.2.1 Concomitant medications

All medications (prescriptions or over-the-counter medications) continued at the start of the trial or started during the study or until 30 days from the end of the last protocol treatment and different from the study medication must be documented.

4.2.2 Palliative radiotherapy

Palliative radiotherapy may be used for the treatment of pain at the site of bony metastases that were present at baseline, provided the Principal Investigator or designee does not feel that these are indicative of clinical disease progression during the study period.

CRLX101 should be discontinued for a minimum of 3 days before the participant undergoes palliative radiation treatment. CRLX101 should be restarted at least 14 days but no more than 21 days after palliative radiation, as long as any bone marrow toxicity has recovered ≤ Grade 1.

Consistent with the PREVAIL trial which demonstrated improved survival in mCRPC and led to FDA approval in the chemotherapy-naïve subgroup, palliative radiation is allowed to be given in combination with enzalutamide.

4.2.3 Administration of other anti-cancer agents

Participants must not receive any other concurrent anti-cancer therapy, including investigational agents, while on study treatment. Participants may continue the use of bisphosphonates for bone disease provided the dose is stable before and during the study.

4.2.4 Medications that may NOT be administered

No other chemotherapy, immunotherapy, hormonal therapy (with the exception of LHRH agonists/antagonists that will be continued while on study) or other novel agent is to be permitted while the participant is receiving study medication.

Live virus and bacterial vaccines should not be administered whilst the participant is receiving CRLX101 and during the 30-day follow-up period. An increased risk of infection by the administration of live virus and bacterial vaccines has been observed with conventional chemotherapy drugs.

5 BIOSPECIMEN COLLECTION

5.1 Correlative Studies for Research/Pharmacokinetic Studies

Pharmacokinetic analyses of camptothecin will assessed at two points during the study treatment. Plasma camptothecin concentrations will be measured before and after initiation of enzalutamide treatment to determine the impact of enzalutamide on CRLX101/camptothecin pharmacokinetics. Urine camptothecin concentrations will also be measured with and without the co-administration of sodium bicarbonate to assess the impact of urine alkalinization on the physicochemical characteristics of camptothecin accumulating in the bladder.

Multiple pharmacodynamic biomarkers will be assessed prior to study treatment and at multiple time points following treatment. The inhibition of HIF-1α will be measured indirectly via the circulating plasma protein biomarker, VEGF, a prominent downstream target and marker of tumor angiogenesis. The presence of acquired resistance will be assessed via the enumeration of CTCs and the measurement of androgen receptor splice variation in CTCs. The presence of CTCs with high phenotypic genomic instability will also be measured before and after initiation of combination therapy.

Biomarkers of DNA damage will be measured using a novel NanoString platform aimed to measure gene expression of 192 gene markers. Evaluation of the response to a DNA damaging agent (camptothecin) may help generate hypotheses for future studies.

Table 7**:** Correlative Studies Summary

Sample	**Assay**	**Time Points**	**Type of Tube/ Sample (sample amount)**	**Location of specimen analysis**
Plasma	Pharmacokinetics	Refer to PK sampling outlined in Section 5.1.1	Refer to PK sampling outlined in Section 5.1.1	Figg Lab
Urine	Pharmacokinetics	Refer to PK sampling outlined in Section 5.1.1	Refer to PK sampling outlined in Section 5.1.1	Figg Lab
Whole Blood	Circulating Plasma Proteins (VEGF)	Pre-treatment on C1D1, C2D15, C6D1, and at disease progression	Purple top EDTA (one 6 mL each)	Cao Lab
	Nanostring DNA damage response panel (192 genes)	Pre-treatment on C1D1, C2D15, and at disease progression	PAXgene RNA (one 2.5 mL each)	Trepel Lab
Circulating Tumor Cells (CTCs) and AR Analysis	Enumeration Androgen Receptor (AR) Analysis Genomic Instability γ-H2AX Expression (if feasible)	Pre-treatment on C1D1, C2D15, C6D1and at disease progression	one 10 mL Streck Cell-Free DNA and one 2.5 mL PAXgene RNA	EPIC Sciences and Figg Lab

5.1.1 Pharmacokinetics

Table 8**:** Plasma Pharmacokinetic Sample Collection with CRLX101 Infusion. Note: Samples are to be approximately drawn around these time points, with the collection times noted on the tubes.

Sample	**Cycle**	**Day**	**PK Collection with CRLX101 Infusion**
1	**1**	**1**	**Predose**

Sample	Cycle	Day	PK Collection with CRLX101 Infusion
2	**1**	**1**	**Mid-infusion (~30 minutes into infusion)**
3	**1**	**1**	**End of Infusion (EOI)**
4	**1**	**1**	**1 hr post EOI**
5	**1**	**1**	**2 hr post EOI**
6	**1**	**1**	**3 hr post EOI**
7	**1**	**1**	**4 hr post EOI**
8	**1**	**1**	**8 hr post EOI**
9	**1**	**1**	**12 hr post EOI**
10	**1**	**2**	**24 hr post EOI**
11	**2**	**15**	**Predose**
12	**2**	**15**	**Mid-infusion (~30 minutes into infusion)**
13	**2**	**15**	**End of infusion (EOI)**
14	**2**	**15**	**1 hr post EOI**
15	**2**	**15**	**2 hr post EOI**
16	**2**	**15**	**3 hr post EOI**
17	**2**	**15**	**4 hr post EOI**
18	**2**	**15**	**8 hr post EOI**
19	**2**	**15**	**12 hr post EOI**
20	**2**	**16**	**24 hr post EOI**

5.1.1.1 Plasma PK

CRLX101 Pharmacokinetics

To identify a drug-drug interaction between CRLX101, camptothecin, and enzalutamide on clinically-relevant doses, blood samples for the determination of camptothecin plasma concentrations will be obtained from each participant on cycle 1 day 1 prior to CRLX101 administration, when CRLX101 will be administered alone. Blood will be collected into a 6 mL

sodium heparin tube (BD, Franklin Lakes, NJ) at the following time points: pre-infusion, mid-infusion, immediately at end of infusion (EOI), then 1, 2, 3 4, 8, 12, and 24 hr post-EOI. After the 24 hour time point, the first dose of enzalutamide will be administered.

On cycle 2 day 15 (C2D15), after being on the daily combination of CRLX101 and enzalutamide for at least 4 weeks to establish steady state, participants will have blood drawn at pre-infusion, mid-infusion, EOI, then 1, 2, 3, 4, 8, 12, and 24 hr post EOI. Comparisons of CRLX101 and camptothecin exposure between cycle 2 (with enzalutamide) and first-dose (alone) will determine the extent of a drug-drug interaction (if any) with enzalutamide.

Note: PK samples should be drawn approximately around the specified time points, with the collection times noted on the tubes.

Enzalutamide Pharmacokinetics

Blood will be collected during the visit on C2D15 prior to the participant's scheduled dose of enzalutamide and the infusion of CRLX101 to measure enzalutamide trough plasma concentrations. Blood will be collected into a 6 mL sodium heparin tube (BD, Franklin Lakes, NJ). Enzalutamide steady-state trough concentrations during cycle 2 dosing will be compared to relevant literature to determine the extent of a drug-drug interaction with CRLX101 and camptothecin.

Bioanalytical Measurements and Assay Methodology

Bioanalytical measurements of unconjugated camptothecin, conjugated camptothecin (measured indirectly), enzalutamide, and enzalutamide metabolites (e.g. N-desmethyl enzalutamide) will be conducted on an ultra HPLC-MSMS system using an assay developed and validated by the Clinical Pharmacology Program.

The PK sampling will also be used to monitor camptothecin and enzalutamide exposure metrics in order to correlate to pharmacodynamic endpoints, clinical response, toxicity, and pharmacogenetic analyses.

5.1.1.2 Urinary Clearance of CRLX101/camptothecin

To identify a mechanism of camptothecin-induced cystitis on clinically relevant doses, urine samples for the determination of camptothecin species (e.g. closed lactone vs. open carboxylate form) and nanoparticle concentrations will be obtained from each participant on C1D1 and C2D15, with or without the administration of sodium bicarbonate to alkalinize urine accumulating in the bladder.

Urine samples (dependent upon whether the participant needs to void) will be collected when feasible in willing participants at the discretion of the Principal Investigator from pre-infusion through 24 hours post-EOI. The nursing staff will be supplied with urine collection tubes for the duration of urine PK collection time frame. Each time the participant needs to void, the participant will be asked to notify the nurse, who will collect a urine sample, if feasible, and then record the date/time on each collection tube. Each collected sample will be kept separately and should be obtained using the "clean catch" method (total urinary excretion is not of importance).

On C1D1, participants will receive IV fluids pre- and post-infusion following the currently established hydration protocol (HP) for CRLX101. On C2D15, participants will follow the hydration protocol with the additional administration of oral sodium bicarbonate (HP+SB).

Comparisons of CRLX101 and camptothecin urinary accumulation between the HP and HP+SB protocols will be used to help characterize an underlying mechanism of cystitis and determine a potential method of prophylaxis for participants at high risk for camptothecin-induced cystitis.

Prior to C2D15, participants will be given instructions on how to administer sodium bicarbonate before their scheduled clinic appointment visit. **Table 9** provides recommendations for the administration of oral sodium bicarbonate on C2D15. The oral sodium bicarbonate dosing schedule may be modified at the discretion of the Principal Investigator or designee.

Table **9:** Recommended Urine Alkalinization Procedure On C2D15

Timing	**Dose, route, and frequency**
Starting from 48 hours prior to CRLX101 infusion	Take sodium bicarbonate by mouth, 650mg x2, 4 times a day (QID), for two days before CRLX101 infusion
On the day of CRLX101 infusion	Take sodium bicarbonate by mouth, 650mg x2, 4 times a day (QID), for one day
**Within Two Hours Prior to CRLX101 Infusion*	If feasible, Obtain a Urine Sample for Analysis
After CRLX101 infusion	Take sodium bicarbonate by mouth, 650mg x2, 4 times a day (QID), for one day after CRLX101 infusion

Note: Doses may be reduced based on age (e.g. over 60) or based on renal function at the discretion of the Investigator. Dosing frequency, dose strength and dosing schedule may also be modified at the discretion of the Principal Investigator or designee. In the case of poor compliance to oral sodium bicarbonate administration for the experimental procedure, collection of urine samples will be done when feasible in willing participants, at the discretion of the principal investigator or designee

Urine collection will not be a requirement in participants with incontinence, conditions preventing urine collection by standard techniques, or other conditions at the discretion of the Principal Investigator or designee. If a participant with incontinence is willing to participate, a sterile catheter inserted into the bladder will be placed on C1D1 and C2D15 to facilitate urine collection.

Bioanalytical Measurements and Assay Methodology

Bioanalytical measurements of CRLX101 and camptothecin accumulation and pH dependent properties of CRLX101 and camptothecin (e.g. ratio of closed lactone form vs. open carboxylate from, release of camptothecin from CRLX101) will be conducted on an HPLC-FLD system using an assay developed and validated by the Clinical Pharmacology Program.

The urine sampling will also be used to monitor camptothecin exposure metrics specific to the bladder in order to correlate to toxicity and clinical response.

5.2 FIGG LAB SAMPLES

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

5.2.1 Plasma and Urine PK Sample Collection

All samples will be immediately placed on wet ice and refrigerated. The date and the exact time of each blood draw and urine samples should be recorded on the sample tube and the PK sheet provided.

5.2.2 Plasma Protein Measurement (VEGF)

VEGF protein expression has been shown to be reduced following the down-regulation of HIF-1α in preclinical assessments *in vitro*. As a downstream target of HIF-1α and a biomarker of angiogenesis, VEGF will serve as a surrogate biomarker for the efficacy of CRLX101. One 6 mL EDTA plasma tube (BD Franklin Lakes, NJ) will be collected from each participant prior to drug administration on C1D1 (baseline), C2D15, C6D1, and at disease progression to measure VEGF for anti-angiogenic activity.

The analyses will be performed with assays developed and validated in Dr. Liang Cao's laboratory using electrochemiluminescence technology that provides ultra-high sensitivity and very large signal dynamic range. The VEGF assay utilized by Dr. Liang Cao's laboratory has been used in previous studies evaluating anti-angiogenic therapies.(64) Purified protein standards will be used for generating standard curves for concentration determination.

Immediately after collection, invert the blood tube 8-10 times. Place the tube on wet ice and then store at 4°C in the refrigerator. The date and exact time of each blood draw should be recorded on the tube. Contact Dr. Figg's lab for sample pick-up.

5.2.3 Circulating Tumor Cells (CTCs) and AR Analysis

Circulating tumor cells (CTCs), often prevalent in mCRPC, present a readily accessible 'liquid biopsy'. Methods to purify and analyze CTCs have been developed and widely utilized to help determine if biomarkers could be correlated with treatment response and/or disease progression. Of particular interest, the enumeration of CTCs and AR splice variant analyses have been shown to be potential clinical biomarkers of disease recurrence and treatment resistance.(65) The enumeration of CTCs with genomic instability has been shown to be a potential biomarker of treatment resistance and susceptibility to DNA damaging agents (e.g. camptothecin).(59, 60) Previously, the expression of γ-H2AX in biopsied tumor tissue has also been used to confirm the activity of CRLX101 (CRLX101 Investigator's Brochure); this study will assess this biomarker in CTCs if feasible.

Peripheral blood will be collected prior to treatment on C1D1 (baseline), C2D15, C6D1, and at disease progression. Two tubes of blood will be collected: one 10 mL Streck Cell-Free DNA (brown-black top) tubes and one 2.5 mL PAXgene tube.

5.2.4 EPIC Sciences

The blood collected in one Streck Cell-Free DNA tube will be shipped via FedEx Priority Overnight to Epic Sciences. These samples will be sent in ambient shippers provided by Epic Sciences to keep samples at room temperature. CTC detection and analysis (e.g. enumeration, AR expression, phenotypic genomic instability) will be assessed using the Epic Sciences platform technology. If feasible, detection of AR-V7 in CTCs may also be examined using the Epic AR-V7 test. Additionally, the expression of γ-H2AX in CTCs may be examined if feasible. Samples will be shipped to the following address:

Epic Sciences
Attn: Joseph Schonhoft
9381 Judicial Drive, Ste 200
San Diego, CA 92121
Phone: 858-263-0694

Assuming this study treats at least 24 participants, at least 6 of these evaluable participants will have high phenotypic genomic instability CTCs at baseline as determined by the Epic Sciences algorithm. Differences in clinical response will be examined between participants who are positive for high phenotypic genomic instability CTCs in comparison to participants who are negative.

5.2.5 AR Analysis

Blood will be collected in a PAXgene tube (PreAnalytix; 2.5 mL peripheral blood per tube) per the manufacturer's instructions. Briefly, immediately after blood collection, gently invert the PAXgene Blood RNA 8-10 times. Store the PAXgene Blood RNA Tube upright at room temperature (18-25°C). Contact Figg Lab for PAXgene tube.

The blood collected in the PAXgene tube will be retained in the Figg Laboratory. AR/ARV and AR-regulated gene (e.g., PSA) expression levels will be assayed from RNA isolated using PAXgene Blood RNA Kit according to the manufacturer's instructions.

5.3 WHOLE BLOOD- MULTIPLEXED GENE EXPRESSION APPROACH TO PROFILING DNA DAMAGE RESPONSE SIGNATURES – TREPEL LAB

Camptothecin induces DNA damage via topoisomerase I inhibition. DNA damage response transcriptional signatures will be evaluated by the Trepel Lab using the NanoString nCounter platform (NanoString Technologies, Seattle, WA). The 192 gene DNA Damage and Repair panel will be used in this study. Peripheral blood will be collected in a PAXgene tube (PreAnalytix; 2.5 mL peripheral blood per tube) per the manufacturer's instructions. RNA will be isolated using the PAXgene Blood RNA Kit according to the manufacturer's instructions. Specimens for peripheral gene signature analysis will be collected prior to treatment on C1D1 (baseline), C2D15, and at disease progression to look for correlates of clinical response. Contact Figg Lab for PAXgene tubes.

5.4 SAMPLE STORAGE, TRACKING AND DISPOSITION

All samples (except samples processed via the Trepel Lab [NanoString] and EPIC Sciences [CTCs]) will be sent to and stored in Dr. Figg's Lab.

Samples will be ordered in CRIS and tracked through Clinical Trial Data management system. Should a CRIS screen not be available, CRIS downtime procedures will be followed . Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required. Any transfer of materials to other NIH or non-NIH investigators will occur following NIH Intramural Research Program guidelines. If the subject withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

5.4.1 Clinical Pharmacology Program (Figg Lab)

Upon arrival in the Clinical Pharmacology Program, blood samples will be centrifuged and the plasma transferred into cryovials for storage at -80° C until the time of analysis. Urine samples will be aliquoted into cryovials, diluted with an equal volume of methanol and stored at -80°C until the time of analysis. In addition, samples will be barcoded.

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

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

Barcoded samples are stored in bar-coded boxes in locked freezers at either -20 C or -80 C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services in Frederick, MD. Samples will be stored until requested by a researcher named on the protocol.

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

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material. If, at any time, a participant withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the participant, if

so requested). The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

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

5.4.2 Molecular Targets Core (Dr. Cao's Lab)

Biospecimens will be collected and processed using validated SOPs that will ensure both specimen quality and participant confidentiality pursuant to informed consent provisions. To ensure participant confidentiality, only containers used for the initial specimen collections will be labeled with participant identifiers. Coded, linked labels will be applied to all subsequent specimen containers. When specimens are processed and aliquoted, no participant information will be included on the new containers. Samples are labeled with printed consecutive numbers, frozen on dry ice, and stored in a -80°C freezer located at the NCI Research Facility. The freezer is locked and monitored 24/7 for temperature control. Samples will be tracked using a limited access Excel database.

Access to the inventory system and associated documents will be restricted to appropriate personnel only. Requests to use specimens stored in the repository must be approved by the IRB. SOPs ensure that any changes in the informed consent made by a participant and relayed to the PI will be reflected in the inventory system to ensure that specimens are destroyed as appropriate. All laboratory personnel will be trained to adhere to SOPs and will be monitored for high-quality performance. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

5.4.3 Trepel Lab

Tracking and disposition of samples will conform to the NCI CCR Biospecimen Guidelines.

All samples will be barcoded and data entered and stored in the Labmatrix system utilized by the NIH Clinical Center. This is a secure system with access limited to defined personnel.

Labmatrix creates a unique barcode ID for every sample which cannot be traced back to subjects without Labmatrix access. The data recorded for each sample includes the subject ID, name, trial name/protocol number, date/time drawn, as well as box and freezer location. Subject demographics associated with the clinical center participant number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.). Access to personally identifiable information (PII) is limited to the PI and associate investigators.

An additional layer of encryption will be added for samples undergoing genetic analysis in the Trepel lab where a separate clinically annotated unique sample ID will be generated linked with the sample ID in Labmatrix. As additional clinical information is generated and linked to the unique participant ID, it is also electronically linked via Labmatrix to the sample ID. The Trepel lab will proceed with sample analysis and record data under the unique sample ID.

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C or in liquid nitrogen according to stability requirements. These freezers are located onsite, and access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in the Labmatrix System. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the NCI. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Samples will be stored in a freezer at either -4°C or -70°C behind a door locked after working hours. Samples will be tracked by a designated member of the laboratory who is responsible for notifying the PI about requests for use of the material, for allocating the material to other members of the laboratory, for recording the disposition of the allocated material.

5.4.4 Future Use/IRB Reporting/Protocol Completion/Sample Destruction

Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study, including gene/protein expression and germline analysis. However, this research may only be done if the risks of the new questions were covered in the consent document and the proposed research has undergone prospective IRB review and approval.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**. If the subject withdraws consent the participants data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system, C3D, and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

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

Document AEs from the first study intervention, Study Day 1, through 30 days after the last dose of study therapy. Beyond 30 days after the last of study therapy, only adverse events which are serious and related to the study intervention need to be recorded.

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

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms

- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the participant's outcome.

As the toxicity profile of Enzalutamide and CRLX101 is well defined and published, expected grade 1 clinical adverse events will not be recorded in the database.

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

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

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6.2 Data Sharing Plans

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

_X__Coded, linked data in an NIH-funded or approved public repository.

_X_ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

_X_ Coded, linked or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

_X__ An NIH-funded or approved public repository. Insert name or names:: ClinicalTrials.gov.

_X_ BTRIS (automatic for activities in the Clinical Center)

_X_ Approved outside collaborators under appropriate individual agreements.

_X_ Publication and/or public presentations.

When will the data be shared?

_X_ Before publication.

_X_ At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

The present protocol, which ascertains transcriptomic data from 192 genes, does not apply to the GDS policy. The GDS policy states that RNA sequencing data from 100-sample threshold need to be shared. This protocol will not meet the 100 sample threshold.

6.3 RESPONSE CRITERIA

Restaging bone scans and/or CT scan of chest, abdomen and pelvis will be obtained at prior to Cycle 3 and **every 3 cycles thereafter.**

Progression will be evaluated in this study using the international criteria proposed by the Prostate Cancer Working Group 3 (PCWG3) guideline(2) and/or the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).(62)

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.

Changes in PSA and measurable lesions will be analyzed for efficacy according to PCWG3 recommendations. The recommended PSA progressions criteria will not be applied to the study as the criteria are arbitrarily proposed and do not necessarily reflect overall disease status. PSA values will be captured at each visit and PSA declines and progression will be followed. PSA is not sufficient in the evaluation of disease progression in this participant population. This is consistent with the recent recommendations by the Prostate Cancer Clinical Trials Working Group 2. Progression will be determined by radiographic evidence as discussed below or by clinical symptoms (symptomatic clinical progression).

6.3.1 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:

- By chest x-ray: ≥20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under: as ≥10 mm
    - Scan slice thickness >5 mm: double the slice thickness
- With calipers on clinical exam: ≥10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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, and abdominal masses (not followed by CT or MRI), are considered as 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.

6.3.2 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks 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 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 slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for 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.

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.

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 date 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 at 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.

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.

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.

6.3.3 Response Criteria

6.3.3.1 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 of 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 of diameters while on study.

6.3.3.2 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 participant 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).

6.3.3.3 Metastatic Bone Lesions

Disease progression is considered if a minimum of two new lesions is observed on bone scan. New lesions seen on first re-staging (at 3 months) may represent disease that was not detected on the baseline scan. In these circumstances, the first re-staging scan then will serve as the baseline comparison for future scans. Participant can be removed at first re-staging at the discretion of the investigator if the clinical scenario is most consistent with disease progression and not "flare" on bone scan. This is consistent with Prostate Cancer Working Group 3 recommendations.(63)

6.3.3.4 Clinical Disease Progression

- The need for chemotherapy or other change in therapy based on increased cancer related symptoms
- Worsening ECOG PS to a PS of 3 or 4 based on cancer (not treatment) related symptoms
- Clinical disease progression can also be determined based on the clinical discretion of the investigator

6.3.3.5 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 participant's best response assignment will depend on the achievement of both measurement and confirmation criteria.

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

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)

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

6.3.4 Duration of Response

The duration of response is defined by time of sustained >30% decline in PSA from baseline.

6.3.5 Overall Survival

Overall Survival is defined as the duration of time from the start of treatment to the time of death..

6.3.6 Response Review

Tumor measurements will be performed in consultation with the Center for Cancer Research Radiology and Imaging Sciences processing service.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each participant while on the study. The descriptions and grading scales found in the revised NCI

Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS/ DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

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

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

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

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

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

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

7.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly regular basis when participants are being actively treated on the trial to discuss each participant. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior participants.

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

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

8 SPONSOR SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

8.1.2 Serious Adverse Event (SAE)

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

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

8.1.3 Life Threatening

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

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

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

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

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

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

SAEs will be:

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

8.3 Reporting of Serious Adverse Events

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

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

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

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

8.4 Safety Reporting Criteria To The Pharmaceutical Collaborators

All events listed below must be reported in the defined timelines to OSROSafety@mail.nih.gov .

8.4.1 To be sent by OSRO Safety

8.4.1.1 Ellipses Pharma Reporting Requirements - Expedited Reporting

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient begins taking study drug and until 30 days after the patient has stopped study treatment will be reported by the Sponsor to Ellipses Pharma via their safety vendor, Simbec-Orion, within 1-2 business days of learning of its occurrence. The MedWatch 3500a form or equivalent will be used to report all SAEs.

Any SAE experienced after this 30 day period should only be reported to Ellipses Pharma if the investigator suspects a causal relationship to the study drug. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 1-2 business days of the sponsor receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered separately as a new event. The investigator must assess and record the relationship of each SAE to each specific study drug (if there is more than one study drug).

Ellipses safety vendor, Simbec-Orion:

Email address: pharmacovigilance@simbecorion.com

Fax no: +1 609 514 1000

Back-up SAE fax: +1 609 452 7888

8.4.1.2 Routine Reporting

The Sponsor will also send the FDA annual report to Ellipses Pharma as agreed upon in the collaborative agreement.

8.4.2 To Be Reported by the Research team

8.4.2.1 Adverse Events of Special Interest (AESI) associated with CRLX101

For all subjects, the following adverse events are categorized as Adverse Events of Special Interest (AESI) associated with CRLX101:

- Infusion-related hypersensitivity reaction(s) of any grade
- Any newly developed cystitis (including Grade 1) in subjects without cystitis at baseline

Any occurrence of hypersensitivity/allergic reactions and/or cystitis a patient experiences associated with CRLX101 must be reported to Ellipses Pharma within 1-2 business days of learning of its occurrence using the OSRO Serious Adverse Event Form.

AESIs will be sent to Ellipses Pharma via their safety vendor, Simbec-Orion by email to: pharmacovigilance@simbecorion.com

Or alternatively by fax to: +1 609 514 1000 or back-up fax: +1 609 452 7888 .

8.5 REPORTING PREGNANCY

8.5.1 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 120 days following the last dose of study drug.

Pregnancy of the patient's partners is not considered to be an adverse event. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality) should if possible be followed up and documented.

The outcome of any conception occurring from the date of the first dose until 120 days after the last dose of study drug should be followed up and documented.

8.6 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

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

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

9 CLINICAL MONITORING

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary and secondary study endpoints; adherence to the protocol, regulations, ICH E6, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESES

10.1.1 Primary efficacy endpoints

The primary efficacy endpoint is response to therapy as defined by a composite endpoint.

The primary objective is to evaluate the anti-tumor activity of CRLX101 at the recommended phase II dose (RP2D) combined with enzalutamide with respect to treatment response, defined as ≥50% PSA decline or stable disease on imaging following 5 months of treatment in participants with progressive mCRPC following enzalutamide treatment.

10.1.2 Secondary Efficacy endpoints

The secondary efficacy endpoints are duration of response, overall survival, and safety.

The secondary objectives are to evaluate the duration of response as defined by a sustained >30% decline in PSA, overall survival, and changes in measurable disease as determined by Response Evaluation Criteria in Solid Tumors (RECIST) and Prostate Cancer Working Group 3 (PCWG3) for the treatment combination; and to validate the recommended phase 2 dose (RP2D) of CRLX101 in combination with enzalutamide.

10.2 SAMPLE SIZE DETERMINATION

For the first 3 to 6 participants, there will be a lead-in period of two cycles to ensure the safety and tolerability of CRLX101 in combination with enzalutamide. CRLX101 will be started at a dose of 12 mg/m^2, administered via IV infusion on Day 1. Based on limited potential for overlapping toxicity and well-characterized pharmacokinetics for both enzalutamide and CRLX101, it is anticipated 3 participants will participate in the lead-in phase. An accrual ceiling of 6 participants for the lead-in phase will be used to account for possible dose adjustments based on DLTs.

Following the 3- to 6-participant lead-in cohort, this study will comprise a single arm cohort of participants with mCRPC eligible for treatment following prior disease progression while on enzalutamide therapy. Based on data derived from a retrospective cohort analysis, the estimated mPFS of participants with mCRPC who failed two prior therapies (all participants received docetaxel first line, followed by either cabazitaxel, enzalutamide, or abiraterone acetate) and were receiving third line of treatment (cabazitaxel, enzalutamide or abiraterone acetate) was 4 months (n = 260 participant; IQR – 3 to 8 months). The ORR (defined as the percentage of participants with a PSA decline of ≥50%) of this participant population was 24%. The reported data for participants receiving a 4th line of treatment (n = 38 participants) showed a mPFS of 5 months (IQR - 3 to 12 months) and an ORR of 16%. The most common therapy sequence of the third line treatment cohort was docetaxel followed by abiraterone acetate or enzalutamide then cabazitaxel (n = 110 participants), which demonstrated an ORR of 28% with a mPFS of 5 months (IQR of 3 to 9 months).(66)

The goal is to determine if the addition of CRLX101 with enzalutamide following prior enzalutamide failure could improve current response rates. Response to therapy will be assessed as a composite endpoint (biochemical response defined as 50% decline in PSA or clinical response defined as stable disease on bone scan after 5 months); attaining either of these

endpoints will be considered a success. To see at least minimal potential improvement beyond ~35% of participants, the goal is to rule out a 25% cohort specific response (success) rate and target a 50% success rate.

Following the three- to six-participant lead-in cohort, the trial will be conducted using an optimal two-stage phase II trial design to rule out an unacceptably low success rate of 25% ($p_0 = 0.25$) in favor of an improved success rate of 50% ($p_1 = 0.50$). With an alpha = 0.10 (probability of accepting a poor treatment = 0.10) and beta = 0.20 (probability of rejecting a good treatment = 0.20), this trial will enroll 8 evaluable participants. If 0 to 2 of the 8 participants have success, no further clinical evaluation will be warranted for this combination treatment. If 3 or more of the 8 evaluable participants have success, then accrual will continue until a total of 21 evaluable participants have been treated in the two-stage portion of the trial. If there are 3 to 7 participants with a success, this will be considered inadequate for further investigation while if there are 8 or more participants out of 21 with a success, then this will be considered worthy of further investigation for subsequent trials. Under the null hypothesis (25% success rate), the probability of early termination is 67.9%

Participants who receive at least one dose of the study treatment will be evaluable for safety. Participants who receive at least one dose of CRLX101 and have a follow-up imaging study will be evaluable for efficacy in an overall study report but the participants in the two-stage portion of the trial will be the primary cohort for efficacy analysis. The maximum number of evaluable participants enrolled in the lead-in component will be 6 participants (anticipation to enroll 3 participants). With up to 6 participants explored in the lead-in portion, and up to 21 participants in the two-stage portion of the trial, up to 27 evaluable participants would need to be enrolled onto the trial. To allow for a small number of inevaluable participants, the accrual ceiling will be set at 30 participants.

10.3 Populations for analysis

Modified intention to treat: all participants who receive at least one dose of each of the two agents will be included in the statistical analyses performed.

10.4 Statistical analyses

10.4.1 General approach

For participants in the primary efficacy cohort, the proportion of evaluable participants who experience a success will be reported along with a confidence interval. Safety will be evaluated by tabulating and describing the DLTs in the first 6 participants of the lead-in arm.

10.4.2 Analysis of the primary endpoints

The fraction of evaluable participants who experience a success as defined above will be determined and reported along with 80% and 95% two-sided confidence intervals.

10.4.3 Analysis of the secondary endpoints

Duration of response and overall survival will be estimated using a Kaplan-Meier curve based on all evaluable participants included in the two-stage cohort of the trial. The validation of the RP2D of CRLX101 in combination with enzalutamide will be done by evaluating the toxicity

and safety data obtained and the RP2D and confirming that the number of DLTs does not 1/6 in participants enrolled during the lead-in phase.

10.4.4 Safety Analyses

Safety of the agent will be assessed by determining the grade of adverse events noted in each participant, and reporting the fraction with grade 3 and grade 4 adverse events. Participants who receive at least one dose of the study treatments will be evaluable for safety. Safety data will be presented in a summary. Results from the safety evaluation in the initial 3-6 participant safety cohort will be reported separately from safety data from participants included in the efficacy cohort. The safety data will consist of the reporting of all adverse events, and may also include reporting vital signs, physical examination data, and laboratory safety data.

10.4.5 Baseline Descriptive Statistics

Limited demographic and clinical characteristics of all participants will be reported.

10.4.6 Planned interim analyses

An evaluation of the safety data based on the initial 3-6 participant lead-in cohort will be performed prior to enrollment of participants in the efficacy cohort. As indicated in the sample size determination section, an interim evaluation of the number of participants who experienced a success in the first stage of the two-stage optimal design will be performed to ensure that enrollment to the second stage is warranted.

10.4.7 Exploratory analyses

The following are the intended exploratory objectives:

- To evaluate the pharmacokinetic profile of CRLX101 (both total drug and released camptothecin) and enzalutamide in plasma;
- To evaluate underlying mechanisms of camptothecin-induced cystitis;
- To evaluate the pharmacodynamic activity of CRLX101 and enzalutamide using surrogate biomarkers to measure acquired treatment resistance mediated by angiogenesis and androgen receptor signaling (e.g. VEGF, CTCs, AR-V7);
- To evaluate the activity of CRLX101 and enzalutamide with respect to the prevalence of circulating tumor cells with high genomic instability;
- To explore possible correlations between clinical response and biomarkers of DNA damage response (e.g. PAR up-regulation, DNA damage response panel).

Any exploratory evaluations which generate quantitative measures will be done using descriptive statistics including confidence intervals when appropriate. Any statistical tests performed for evaluation of exploratory objectives will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENTS

11.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

11.1.1 Ellipses Pharma

The investigational study agent, CRLX101 is provided by the company, Ellipses Pharma under MTA # 45025-18 executed 10/10/2018, under CRADA # 03089.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

Subjects treated on this study will be individuals with metastatic castration resistant prostate cancer, which has recurred (or persisted) after appropriate treatment. Men of any race or ethnic group will be eligible for this study. Eligibility assessment will be based solely on the participant's medical status. Women will not be eligible as the disease under study is not present in this population. Recruitment of participants onto this study will be through standard CCR mechanisms. No special recruitment efforts will be conducted.

12.2 PARTICIPATION OF CHILDREN

Individuals under the age of 18 will not be eligible to participate in this study because they are unlikely to have prostate cancer, and because of unknown toxicities of CRLX101 in combination with enzalutamide in the pediatric participant.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **12.4**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study.

Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

12.4 PLEASE SEE SECTION **12.6.1** FOR CONSENT PROCEDURE. EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The potential benefit to a participant that goes onto study is a reduction in the bulk of their tumor and improvement in their bony lesions, which may or may not have favorable impact on symptoms and/or survival. Potential risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document. The procedure for protecting against or minimizing risks will be to medically evaluate participants on a regular basis.

12.5 RISKS/BENEFITS ANALYSIS

For participants with castration resistant prostate cancer, median survival is in the range of 12-18 months. Enzalutamide has shown activity against prostate cancer in a clinical setting. CRLX101 has demonstrated clinical activity to down-regulate HIF-1α in ovarian cancer and *in vitro*

assessments examining the down-regulation of HIF-1α in combination with enzalutamide have demonstrated a synergistic effect, decreasing angiogenesis and androgen receptor signaling in LNCaP cell models. Preclinical assessments (*in vitro* and *in vivo*) examining this treatment combination are currently ongoing. Although possible toxicities from the proposed therapy are serious, given the nature of the underlying disease, they are reasonable. Additionally, we do not anticipate toxicities significantly more severe than those observed with other approved agents. For these reasons, the risk/benefit ratio of this protocol is favorable; therefore, this protocol involves greater than minimal risk to all participants on study, but presents the potential for direct benefit to all individual subjects enrolled on study.

NOTE: This same risks/benefits analysis applies to all study populations included in this clinical study.

12.5.1 Risks related to Imaging

CT scans and TC-99 scas often use a contrast agent. There is a small risk of having a reaction to the contrast and most often include nausea, pain in the vein where the contrast is given, headache, metallic and/or bitter taste in the mouth and a warm, flushing feeling. Rarely, some people have more severe allergic reactions to the contrast which may include skin rashes, shortness of breath, wheezing or low blood pressure.

12.5.2 Risks from Radiation Exposure

The procedures for performing the CT scans (chest/abdomen/pelvis) and Tc-99 whole body scintography scans will follow clinical policies, no special procedures apply to these additional assessments for research purposes. In summary, subjects may receive additional radiation exposure in one year from up to four (4) CT scans of the chest, abdomen, and pelvis, and four (4) Tc-99 whole body scintography scans. Radiation exposure is associated with an increased risk of cancer.

12.6 Consent Process and Documentation

The informed consent document will be provided to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. If a prolonged period of time expires during the decision making process (several weeks, as an example), it may be necessary to reassess the participant for protocol eligibility. A signed informed consent document will be obtained prior to entry onto the study.

Re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When re-consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

12.6.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.6**.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 Study Discontinuation and Closure

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

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

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

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

13.2 Quality Assurance and Quality Control

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

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

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

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

13.3 Conflict of Interest Policy

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

13.4 Confidentiality and Privacy

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

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

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

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

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

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

14 PHARMACEUTICAL INFORMATION

14.1 CRLX101 (#138099)

CRLX101 is a polymer drug conjugate composed of 20 (S)-camptothecin conjugated to a biocompatible polymer. The compound self assembles into soluble nanoparticles composed of several molecules when dissolved in aqueous solution. Camptothecin (CPT) is an antineoplastic that inhibits topoisomerase I resulting in cell death during the S phase of the cell cycle. CRLX101 nanoparticles are believed to be taken up by tumor cells followed by release of the active pharmaceutical ingredient 20(S)-camptothecin.

14.1.1 Source

The CRLX101 is provided by the company, Ellipses Pharma, the manufacturer of drug under a Collaborative Agreement [Cooperative Research and Development Agreement (CRADA)].

14.1.2 Toxicity

Please see Section **1.2.5**.

14.1.3 Handling and Dispensing

CRLX101 is a sterile injectable and must be handled under appropriate controls, conditions, and aseptic techniques to maintain product sterility. CRLX101, a cytotoxic, should be prepared in a class II biological safety cabinet using standard precautions for the safe handling of antineoplastic agents. Latex gloves are recommended. It must be dispensed only from official study sites by authorized personnel according to local regulations, and stored in a secure area according to local regulations. It is the responsibility of the Investigator to ensure that study drug is only dispensed to eligible study participants.

14.1.4 Formulation and preparation

CRLX101 will be provided at no cost to the study participant. It is supplied as a lyophilized cake in: a 30 mL single-use vial containing 35 mg of CPT equivalents (approximately 350 mg of polymer drug conjugate).

Vials of CRLX101 should be removed from the refrigerator and warmed to room temperature for approximately one hour prior to preparation. Concentrated CRLX101 solution is prepared by adding 14 mL of USP Sterile Water for Injection (SWFI) to each 35 mg vial. The required volume of SWFI is to be aseptically withdrawn into a sterile calibrated syringe and the syringe needle inserted through the vial septum. The SWFI should be slowly added along the inside of the vial wall and not directly onto the lyophilized product cake to minimize foaming. The product is dissolved by gentle swirling (do not shake) until a clear homogenous solution is achieved. The majority of the product cake will dissolve quickly, however complete dissolution to a clear, homogeneous solution will take longer and may require up to 30 minutes. The CRLX101 concentrated solution should be inspected visually for particulate matter prior to further preparation. Visually inspect for completion of reconstitution every 2-3 minutes. Reconstitution is complete when (i) foam head has dissipated down to a thin bubble ring along inner wall of vial, (ii) solids in solution and foam head are no longer visible, and (iii) translucent

polymer "vapors" are no longer visible in solution and the solution is clear and uniform in appearance. Complete reconstitution of the vial may take up to 30 minutes.

Each milliliter of concentrated solution contains 2.5 mg of camptothecin equivalents. Concentrated CRLX101 solution is diluted to the recommended dose with 5% dextrose in water for injection (D5W) to a total volume of 500 mL. After determining the amount of CRLX101 concentrated solution required for dosing, an equivalent volume is aseptically withdrawn from a 500 mL infusion bag or bottle of D5W with a calibrated syringe. The measured volume of concentrated CRLX101 solution is then withdrawn with a calibrated syringe and injected into the prepared 500 mL D5W infusion bag or bottle. The solution is thoroughly mixed by gentle manual rotation.

14.1.5 Stability and Storage

CRLX101 vials should be stored at refrigerated conditions (2°-8°C) and should only be accessible to authorized individuals. CRLX101 is stable for at least 3 years when stored at refrigerated conditions (2°-8°C). The reconstituted solution is stable for 24 hours when stored at room temperature. Any unused product solution from the reconstituted vials should be destroyed per the site's standard operating procedures.

Formulated CRLX101 (i.e., IV bag) should be used within 6 hours of reconstituting the drug product vials.

14.1.6 Dosage and Administration procedures

See Sections **3.2** and **3.3** for the dose levels of CRLX101 to be evaluated in this study. Each dose should be administered by IV infusion over 60-75 minutes on Day 1 and Day 15 of each cycle. See Section **13.1.4** for how to prepare each dose for administration.

See Section **3.3.2.4** for management of any CLRX101 infusion related/hypersensitivity reactions.

The second and subsequent dose of CRLX101 may be delayed if related AEs have not resolved to grade 1 or better. If a dose of CRLX101 is delayed, then the subsequent dose should be administered 2 weeks later to avoid significant carry-over of unconjugated plasma CPT from one dose to the next (See Section **3.3**). However the maximum delay for AEs to resolve is 28 days beyond which the participant will be taken off treatment.

14.1.7 Incompatibilities

No known incompatibilities at this time.

14.1.8 Premedication and Hydration

The premedication prior to CRLX101 treatment and pre and post hydration and additional prevention measurements to prevent hypersensitivity/infusion reaction have been described in Section **3.3.2.4**.

14.1.9 Return and Retention of CRLX101

Any used study drug vials, or partially used vials, remaining after trial is complete will be destroyed per institution drug destruction policy. Unused study drug vials may be destroyed in the same manner or returned if requested to Ellipses Pharma.

14.2 ENZALUTAMIDE

Enzalutamide (XTANDI ©) is an androgen receptor inhibitor. The chemical name is 4-(3-[4-cyano-3-(trifluoromethyl)phenyl]-5,5-dimethyl-4-oxo-2-sulfanylideneimidazolidin-1-yl)-2-fluoro-*N*-methylbenzamide.

Please see FDA-approved package insert for Enzalutamide for complete agent information.

14.2.1 Source

Enzalutamide will be obtained from commercial sources and dispensed by the institutional pharmacy.

14.2.2 Toxicity

Seizures

In the randomized clinical trial, 7 of 800 (0.9%) participants treated with enzalutamide 160 mg once daily experienced a seizure. No seizures occurred in participants treated with placebo. Seizures occurred from 31 to 603 days after initiation of enzalutamide. Participants experiencing seizure were permanently discontinued from therapy and all seizures resolved. There is no clinical trial experience re-administering enzalutamide to participants who experienced seizures. The safety of enzalutamide in participants with predisposing factors for seizure is not known because these participants were excluded from the trial. These exclusion criteria included a history of seizure, underlying brain injury with loss of consciousness, transient ischemic attack within the past 12 months, cerebral vascular accident, brain metastases, brain arteriovenous malformation or the use of concomitant medications that may lower the seizure threshold. Because of the risk of seizure associated with enzalutamide use, participants should be advised of the risk of engaging in any activity where sudden loss of consciousness could cause serious harm to themselves or others.

Other adverse events

The most common adverse drug reactions (≥5%) reported in participants receiving enzalutamide in the randomized clinical trial were asthenia/fatigue, back pain, diarrhea, arthralgia, hot flush, peripheral edema, musculoskeletal pain, headache, upper respiratory infection, muscular weakness, dizziness, insomnia, lower respiratory infection, spinal cord compression, and cauda equina syndrome, hematuria, paresthesia, anxiety, and hypertension. Grade 3 and higher adverse reactions were reported among 47% of enzalutamide-treated participants and 53% of placebo-treated participants. Discontinuations due to adverse events were reported for 16% of enzalutamide-treated participants and 18% of placebo-treated participants. The most common adverse reaction leading to treatment discontinuation was seizure, which occurred in 0.9% of the enzalutamide-treated participants compared to none (0%) of the placebo-treated participants.

Laboratory Abnormalities

In the randomized clinical trial, Grade 1-4 neutropenia occurred in 15% of participants on enzalutamide (1% Grade 3-4) and in 6% of participants on placebo (no Grade 3-4). The incidence of Grade 1-4 thrombocytopenia was similar in both arms; 0.5% of participants on enzalutamide and 1% on placebo experienced Grade 3-4 thrombocytopenia. Grade 1-4 elevations in ALT occurred in 10% of participants on enzalutamide (0.3% Grade 3-4) and 18% of

participants on placebo (0.5% Grade 3-4). Grade 1-4 elevations in bilirubin occurred in 3% of participants on enzalutamide and 2% of participants on placebo.

Infections

In the randomized clinical trial, 1.0% of participants treated with enzalutamide compared to 0.3% of participants on placebo died from infections or sepsis. Infection-related serious adverse events were reported in approximately 6% of the participants on both treatment arms.

Falls and Fall-reported Injuries

In the randomized clinical trial, 1.6% of participants treated with enzalutamide were reported to have Grade 1 or 2 hallucinations compared to 0.3% of participants on placebo. Of the participants with hallucinations, the majority were on opioid-containing medications at the time of the event. Hallucinations were visual, tactile, or undefined.

14.2.3 Formulation and Preparation

Enzalutamide is a white crystalline non-hygroscopic solid. It is practically insoluble in water. XTANDI is provided as liquid-filled soft gelatin capsules for oral administration. Each capsule contains 40 mg of enzalutamide as a solution in caprylocaproyl polyoxylglycerides. The inactive ingredients are caprylocaproyl polyoxylglycerides, butylated hydroxyanisole, butylated hydroxytoluene, gelatin, sorbitol sorbitan solution, glycerin, purified water, titanium dioxide, and black iron oxide.

14.2.4 Storage and Stability

Store enzalutamide capsules at 20°C to 25°C (68°F to 77°F) in a dry place and keep the container tightly closed. Excursions permitted from 15°C to 30°C (59°F to 86°F).

The company providing the IND drug will also provide the expiration date for each lot allocated to this study.

14.2.5 Dosage and Administration Procedures

Enzalutamide 160 mg (four 40 mg capsules) administered orally once daily. Swallow capsules whole. Enzalutamide can be taken with or without food.

14.2.6 Incompatibilities and Drug-Drug Interactions

Drugs that Inhibit or Induce CYP2C8

Co-administration of a strong CYP2C8 inhibitor (gemfibrozil) increased the composite area under the plasma concentration-time curve (AUC) of enzalutamide plus N-desmethyl enzalutamide in healthy volunteers. Co-administration of enzalutamide with strong CYP2C8 inhibitors should be avoided if possible. If co-administration of enzalutamide with a strong CYP2C8 inhibitor cannot be avoided, reduce the dose of enzalutamide. The effects of CYP2C8 inducers on the pharmacokinetics of enzalutamide have not been evaluated *in vivo*.

Co-administration of enzalutamide with strong or moderate CYP2C8 inducers (e.g., rifampin) may alter the plasma exposure of enzalutamide and should be avoided if possible. Selection of a concomitant medication with no or minimal CYP2C8 induction potential is recommended.

Drugs that Inhibit or Induce CYP3A4

Co-administration of a strong CYP3A4 inhibitor (itraconazole) increased the composite AUC of enzalutamide plus N-desmethyl enzalutamide by 1.3 fold in healthy volunteers. The effects of CYP3A4 inducers on the pharmacokinetics of enzalutamide have not been evaluated *in vivo*.

Co-administration of enzalutamide with strong CYP3A4 inducers (e.g., carbamazepine, phenobarbital, phenytoin, rifabutin, rifampin, rifapentine) may decrease the plasma exposure of enzalutamide and should be avoided if possible. Selection of a concomitant medication with no or minimal CYP3A4 induction potential is recommended. Moderate CYP3A4 inducers (e.g., bosentan, efavirenz, etravirine, modafinil, nafcillin) and St. John's Wort may also reduce the plasma exposure of enzalutamide and should be avoided if possible.

Effect of Enzalutamide on Drug Metabolizing Enzymes

Enzalutamide is a strong CYP3A4 inducer and a moderate CYP2C9 and CYP2C19 inducer in humans. At steady state, enzalutamide reduced the plasma exposure to midazolam (CYP3A4 substrate), warfarin (CYP2C9 substrate), omeprazole (CYP2C19 substrate). Concomitant use of enzalutamide with narrow therapeutic index drugs that are metabolized by CYP3A4 (e.g., alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, and tacrolimus), CYP2C9 (e.g., phenytoin, warfarin) and CYP2C19 (e.g., proton pump inhibitors and clopidogrel) should be avoided as enzalutamide may decrease their exposure. If co-administration with warfarin cannot be avoided, conduct additional INR monitoring.

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16 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.