Overcoming drug resistance in metastatic castration-resistant prostate cancer with novel combination of TGF-β receptor inhibitor LY2157299 and enzalutamide: a randomized multi-site phase II study

Johns Hopkins Protocol #: J1557, IRB00065746

NCT#: NCT02452008

Principal Investigator: Channing Paller, MD
Sidney Kimmel Comprehensive Cancer Center, The Skip Viragh Building, 201 N. Broadway, Room 9162 Baltimore, MD 21287 Phone: (410) 955-8239 (Office) or 202-660-6322 (Clinic), Fax: 410-367-2668, Email: cpaller1@jhmi.edu

Co-Investigators:
Michael Carducci, MD (Carducci@jhmi.edu)
Samuel Denmeade, MD (denmesa@jhmi.edu)
Vickie Sinbaldi, NP (sinibvi@jhmi.edu
Mark Markowski, MD (mmarko12@jhmi.edu)
Catherine H. Marshall, MD (chm@jhmi.edu)
Yasser Ged, MD (yged1@jhmi.edu)
Jun Luo, PhD (jluo2@jhmi.edu)

Biostatistics:
Marianna Zahurak (zahurma@jjhmi.edu)

Lead Coordinator: Serina King (Multicenter GU Trials Coordinator)
201 N Broadway, Room 9132, Baltimore, MD 21287
Phone: (410) 614-6139 Fax: (410) 367-2668
Email: sking18@jhmi.edu

Research Nurse: Irina Rifkind, RN (irifin1@jhmi.edu)

Regulatory: Jennifer Uram, PhD/Rose Parkinson, PhD
(juram1@jhmi.edu/rparkin1@jhmi.edu)

IND Sponsor: **Channing Paller, MD**

IND: IND 125694

Lilly Supplied Agent: Transforming Growth Factor-Beta Receptor I Kinase Inhibitor LY2157299 Monohydrate (Galunisertib)

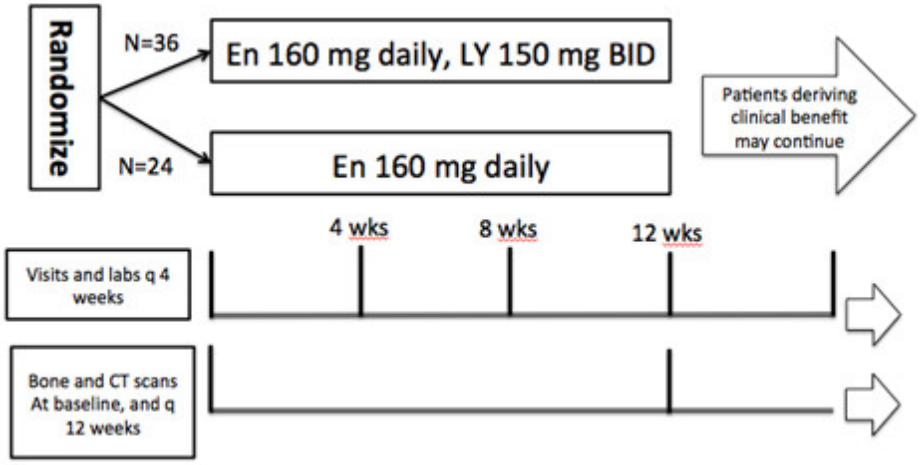
Commercial Agent: Enzalutamide

Date of Issue: Version 10.0/ Amendment 9 dated January 23. 2024

SCHEMA

Part 1: Safety Run-In
The first six patients will be continuously monitored through Cycle 1 of treatment in the safety run-in portion of the study.

Part 2: Phase 2 Randomized Trial



Arm 1: Enzalutamide and LY 2157299
Arm 2: Enzalutamide
LY2157299 (LY) is given orally twice daily 14 days on/14 days off in a 28-day cycle
Enzalutamide (En) is given orally once daily continuously.

Primary Objective
To compare radiographic progression-free survival (rPFS) in chemotherapy-naïve, post-abiraterone-treated metastatic castration-resistant prostate cancer (mCRPC) patients treated with enzalutamide and LY2157299 versus enzalutamide alone.

Secondary Objectives
1. To compare the prostate-specific antigen (PSA) response rate and duration of benefit of chemotherapy-naïve, post-abiraterone mCRPC patients treated with a combination of enzalutamide and LY2157299 vs. enzalutamide alone.
2. To summarize and compare overall survival in chemotherapy-naïve, post-abiraterone mCRPC patients treated with a combination of enzalutamide and LY2157299 vs. enzalutamide alone.
3. To assess safety and tolerability the combination of enzalutamide and LY2157299 as measured by CTCAE v 4.03 criteria.
4. To assess the pharmacokinetics of LY2157299 in the presence of enzalutamide during the safety run-in phase.

Exploratory Objectives: Correlates

1. To measure correlates that may, individually or as a group, be predictive of response or lack of response to the combination of enzalutamide and LY2157299: AR-V7 splice variant status, C-reactive protein (CRP), TGF-β, and SMAD2.

2. To quantify and compare the function of the peripheral $CD8^+$ and $CD4^+$ cells from patients in each of the two treatment groups.

3. To evaluate and compare the serum cytokine profile from patients in each of the two treatment groups (including but not limited to IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, TNF-α, and IFNγ).

4. To evaluate and compare the generation of novel antibody specificities to prostate-associated antigens in the serum of patients in each group, after the receipt of protocol therapy.

5. To evaluate markers of epithelial to mesenchymal transition in tumor biopsies pre- and post-therapy: E-cadherin (CDH1), N-cadherin (CDH2), TGF-β1, vimentin (VIM), ZEB1, and SMAD2

TABLE OF CONTENTS

SCHEMA 2
TABLE OF CONTENTS 4
1. Objectives 6
1.1. Primary Objective 6
1.2. Secondary Objectives 6
1.3. Exploratory Objectives: Correlates 6
1.4. Study Design 7
2. Background 9
2.1. General Summary of the Trial 9
2.2. Rationale for the Study 11
2.3. Correlates 11
3. Patient Selection 13
3.1. Inclusion Criteria 13
3.2. Exclusion Criteria 15
3.3. Inclusion of Minorities and Women and Children 17
4. Registration 18
4.1. General Guidelines 18
4.2. Registration Process 18
4.3. Randomization 18
5. Treatment 19
5.1. Agent Administration 19
5.2. Concomitant Medication and Supportive Care Guidelines 19
5.3. Contraception 19
5.4. Instructions for Missed Doses 20
5.5. Adverse Events 20
5.6. Duration of Treatment 22
5.7. Dose Limiting Toxicity (DLT) Criteria 23
5.8. Criteria for Removal from Study 23
5.9. Safety Follow-up Visit 25
5.10. Duration of Follow-Up 25
6. Dosing Delays/Dose Modifications 25
6.1. Dosing Delays 25
6.2. Dose Modifications 26

7. Adverse Events: Reporting Requirements 26
7.1. Definitions 27
7.2. Relationship 28
7.3. Expectedness 29
7.4. Handling of Expedited Safety Reports 29
7.5. Reporting 29
8. Pharmaceutical Information 32
8.1. Enzalutamide 32
8.2. LY2157299 (Galunisertib) 33
9. Correlative Studies 36
9.1. AR-V7 Liquid Biopsy Procedure 36
9.2. TGF-β Procedure 37
9.3. Peripheral Blood Mononuclear Cells 37
9.4. Serum and Plasma Samples 38
9.5. Tumor Tissue Studies 38
9.6. Pharmacokinetic (PK) study: Johns Hopkins Site Only 39
10. Study Calendar 41
11. Data Reporting/Regulatory Requirements 45
11.1. Data Management 45
11.2. Safety Meetings 46
11.3. Monitoring 46
12. Statistical Considerations 48
13. References 53
Appendices 58
Appendix A: Performance Status Criteria 59
Appendix B: Concomitant Medications to Use with Caution 60
Appendix C: Medication Diaries 61
Appendix D: Serious Adverse Event Reporting Form 63

1. Objectives

1.1. Primary Objective

To compare radiographic progression-free survival (rPFS) in chemotherapy-naïve, post-abiraterone-treated metastatic castration-resistant prostate cancer (mCRPC) patients treated with enzalutamide and LY2157299 versus enzalutamide alone.

1.2. Secondary Objectives

1. To compare the prostate-specific antigen (PSA) response rate of chemotherapy-naïve, post-abiraterone mCRPC patients treated with a combination of enzalutamide and LY2157299 vs. enzalutamide alone.
2. To summarize and compare overall survival in chemotherapy-naïve, post-abiraterone mCRPC patients treated with a combination of enzalutamide and LY2157299 vs. enzalutamide alone.
3. To assess safety and tolerability the combination of enzalutamide and LY2157299 as measured by CTCAE v 4.03 criteria.
4. To assess the pharmacokinetics of LY2157299 in the presence of enzalutamide during the safety run-in phase.

1.3. Exploratory Objectives: Correlates

1. To measure correlates that may, individually or as a group, be predictive of response or lack of response to the combination of enzalutamide and LY2157299:

    - AR-V7 splice variant status
    - C-reactive protein (CRP)
    - TGF-β
    - SMAD2

2. To quantify and compare the function of the peripheral $CD8^+$ and $CD4^+$ cells from patients in each of the two treatment groups.
3. To evaluate and compare the serum cytokine profile from patients in each of the two treatment groups (including but not limited to IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, TNF-α, and IFNγ).
4. To evaluate and compare the generation of novel antibody specificities to prostate-associated antigens in the serum of patients in each group, after the receipt of protocol therapy.
5. To evaluate markers of epithelial to mesenchymal transition in tumor biopsies pre- and post-therapy:

    - E-cadherin (CDH1)
    - N-cadherin (CDH2)
    - TGFB1
    - vimentin (VIM)

- ZEB1
- SMAD2

1.4. Study Design

This is a multi-center, open-label, randomized, Phase 2 study to evaluate the safety and clinical activity of enzalutamide in combination with LY2157299 versus enzalutamide alone in mCRPC patients who are chemotherapy-naïve and have progressed on abiraterone therapy per Prostate Cancer Working Group PSA or radiographic criteria. The primary endpoint of the trial is rPFS. The rPFS of subjects treated with enzalutamide in combination with LY2157299 (Arm 1) will be compared to subjects treated with enzalutamide alone (Arm 2). Subjects will be enrolled and randomized 3:2 to the two treatment arms for a total of 60 subjects treated (36 in Arm 1; 24 in Arm 2).

In order to assure the safety of the combination of enzalutamide and LY2157299, the first six patients will be continuously monitored through Cycle 1 of treatment in the safety run-in portion of the study. The primary DLTs of concern for this safety monitoring are listed in **Section 5.7.2**. In the safety run-in, no more than one patient will be accrued in any seven day period.

The study will consist of a screening period (within 28 days of first dose, inclusive of a randomization period of up to 7 days of first dose), a treatment period per Table 1, and a follow-up period.

Table 1: Treatment Schedule

Arm	Enzalutamide	LY2157299
1	Days 1-28 (160 mg QD)	Days 1-14 (150 mg BID)
2	Days 1-28 (160 mg QD)	None

Dose reductions are allowed per **Section 6**. Enrollment will continue until 60 subjects have received at least one dose of study treatment. Enzalutamide will be provided as standard of care while LY2157299 will be provided by the trial sponsor.

Immune and clinical responses will be evaluated through the following methods at baseline and during treatment: (1) liquid and metastatic tumor biopsies, (2) PBMCs, (3) serum and plasma, and (4) computed tomography (CT) scans (magnetic resonance imaging [MRI], if CT contraindicated) will be obtained for assessment of activity and correlative studies. Tumor assessments will be made using RECIST 1.1 and Prostate Cancer Working Group criteria.

All subjects may continue in the treatment period until discontinuation due to unacceptable toxicity, lack of clinical benefit as determined by the investigator, or termination of the study by the sponsor. After a patient is discontinued from treatment, a mandatory Safety Follow-Up Visit should be performed approximately four weeks after the last dose of study medication (or within seven days prior to initiation of a new anti-cancer treatment, whichever comes first). Subjects will be considered in the treatment period until four weeks after the last dose of study drug.

The patient will be monitored for adverse events up to the mandatory Safety Follow-Up Visit or to resolution of drug-related toxicities to Grade 0-1, whichever occurs later. Serious adverse events that occur within 4 weeks of the end of treatment or before initiation of a new antineoplastic treatment should also be followed up and recorded. Patients who are discontinued from the study due to an unacceptable drug-related adverse event will be followed until the resolution of the event to Grade 0-1 or stabilization, or until initiation of a new therapy for their cancer, whichever occurs first.

Patients who discontinued study therapy without documented disease progression should continue to be monitored for disease status by radiologic imaging. Disease monitoring should continue every six months, until disease progression, or two years, whichever occurs first.

All patients who discontinue treatment should be contacted every six months to monitor overall survival. Information of other cancer therapies after discontinuation from the study treatment will be collected.

2. Background

2.1. General Summary of the Trial

More than 29,000 men died from prostate cancer in the United States in 2013. [1 1] In the early stages of advanced prostate cancer, the disease can be suppressed by gonadotropin-releasing hormone (GnRH) androgen-deprivation therapy (ADT). Eventually, however, most patients experience resistance to androgen-deprivation-induced apoptosis[2] and are treated with abiraterone as one standard of care. Once the patient develops resistance to abiraterone, no standard of care has been defined. In an attempt to avoid toxicities associated with chemotherapy, many physicians prescribe enzalutamide, although the drug has been approved by the FDA only in the post-chemotherapy setting. As of September 2014, Enzalutamide has been approved in the prechemotherapy setting. Their decision to use enzalutamide is supported by recently-reported results from a Phase III study (PREVAIL) of enzalutamide in chemotherapy-naïve (and abiraterone-naïve) mCRPC patients, which was halted when a planned interim analysis showed a 30% decline in the risk of death and an 81% decline in the risk of radiographic progression.[3] These results allowed approval of enzalutamide before chemotherapy per label change. This could affect accrual in the long run because it would alter the standard of care. It is our practice to use abiraterone as first line in over half of our patients and thus we do not think accrual will be a problem. Post-abiraterone treatment, however, resistance to enzalutamide arises quickly, and the response to treatment is muted.[4-7] A retrospective study of 28 chemotherapy-naïve mCRPC patients reported that the percentage of patients experiencing PSA response (defined as $\geq$50% decline in PSA) when treated with enzalutamide after abiraterone treatment is less than half (36% vs 78%) that reported for abiraterone-naïve patients in the PREVAIL trial.[4] Epithelial-mesenchymal transition (EMT) is one mechanism of resistance,[8] and transforming growth factor (TGF)-β is a potent inducer of EMT.[9] In addition, TGF-β is a potent immunosuppressive agent.[10,11] LY2157299 specifically inhibits the kinase activity of the TGF-β receptor type I (TGF-β RI) and its downstream signaling pathway. Combining the inhibition of TGF-β with enzalutamide treatment is a novel approach that may delay resistance to enzalutamide by inhibiting tumor spread and improving the host's antitumor immune response in the growing population of mCRPC patients who have progressed on abiraterone treatment.

2.1.1. The Changing Treatment Landscape for Metastatic Castration-resistant Prostate cancer

Over the past three years, the mCRPC treatment landscape has been transformed by FDA approvals of the androgen biosynthesis inhibitor, abiraterone,[12,13] and the androgen receptor signaling inhibitor, enzalutamide.[14] While these drugs extend median overall survival by several months, resistance inevitably develops.[15] What is perhaps most daunting is that prostate cancer that is resistant to these next generation androgen-ablating agents is potentially resistant to chemotherapy, as well.[16] Therefore, the treatment of prostate cancer that progresses after treatment with abiraterone acetate or enzalutamide represents a major unmet medical need.

2.1.2. The Role of TGF-β in Overcoming Resistance to Enzalutamide

Rapidly growing clinical evidence suggests that persistent and/or aberrant AR signaling drives resistance to enzalutamide and progression to recurrent disease,[17] TGF-β protein accelerates prostate tumorigenesis via selective effects on EMT,[18] and TGF-β expression is significantly elevated in prostate cancer tissue.[19] Thus, inhibition of TGF-β signaling is a proposed strategy for overcoming resistance to enzalutamide. TGF-β has tumor-inhibitory activity in the early stages of tumorigenesis, but it promotes tumor migration and invasion in late stages towards metastasis.[20,21] Mechanistically, TGF-β impairs growth by inhibiting proliferation and inducing apoptosis through two transmembrane serine/threonine kinase receptors, type I and type II receptors (TβRI and TβRII). Once this pathway is disrupted, TβRI becomes a potent tumor promoter and the resistant tumor cells secrete high levels of TGF-β.[22] TGF-β levels are significantly higher in prostate cancer (71.2% vs 19.0%) than in adjacent tissue;[19] metastatic prostate tumors exhibit increased TGF-β expression[23] that is associated with enhanced invasion and metastasis in experimental and pre-clinical model systems.[24] TGF-β has been established as a promoter of tumor progression to metastasis in the context of the tumor microenvironment. Specifically, TGF-β is a potent inducer of EMT that drives metastatic progression,[9] including prostate tumor metastasis;[18] TGF-β is also a potent inhibitor of both adaptive and innate immunity.[10,11]

The TGF-β signaling pathway and the AR signaling pathway crosstalk at multiple levels. The most pronounced connection between the two pathways in prostate cancer is the activation of TGF-β signals following androgen withdrawal, leading to elevated expression of TGF-β receptors TβRI and TβRII.[25] EMT emerges as a significant contributor to prostate cancer metastatic progression and therapeutic resistance.[26] Aberrant TGF-β signaling accelerates prostate tumorigenesis in the TRAMP mouse model via selective effects on EMT.[18] Moreover, a functional interaction between TGF-β1 and AR signaling has been causally implicated in hepatocarcinogenesis.[27]

Pre-clinical evidence indicates a requirement of a threshold of AR activity to induce prostate cancer cell EMT and invasive behavior.[28] The emerging EMT landscape in prostate cancer involves the governing by TGF-β signaling and AR-contribution towards aggressive disease and therapeutic resistance. Navigating the EMT-MET cycles via selective inhibition of TGF-β signaling (in combination targeting the androgen/AR axis) may represent a powerful combination strategy for effectively overcoming anti-androgen resistance in advanced disease. This research may lead to optimization of the TGF-β receptor I kinase inhibitor, LY2157299, for further clinical utility for the treatment of mCRPC towards actions disrupting EMT in an androgen/AR co-dependent manner.

Eli Lilly and Company's novel TGF-β Receptor I Kinase Inhibitor, LY2157299 monohydrate (Galunisertib), has demonstrated safety and efficacy in the treatment of hepatocellular carcinoma patients,[29] but has not yet been tested in men with mCRPC. Dr. Michael Carducci led the first-in-human Phase 1 trial of the TGF-β receptor I kinase inhibitor, LY2157299, in solid tumors.[30,31] LY2157299 is administered orally 150 mg BID and has been well tolerated in Phase Ib and Phase II trials in glioma[32,33] and in hepatocellular carcinoma.[34]

2.2. Rationale for the Study

Abiraterone is currently approved both pre- and post-chemotherapy, while enzalutamide is approved only post-chemotherapy in the treatment of men with mCRPC. To avoid toxicities associated with chemotherapy, and in light of the promising results of the PREVAIL trial of pre-chemotherapy enzalutamide, chemotherapy-naïve patients who progress on abiraterone are often offered enzalutamide[35] (on clinical trials or if allowed by insurance). If enzalutamide is approved by the FDA for chemotherapy-naïve mCRPC patients, the use of enzalutamide will increase both before and after abiraterone. The purpose of the proposed clinical trial is to determine whether the response of chemotherapy-naïve men to enzalutamide post abiraterone can be enhanced by combining enzalutamide with the TGF-β inhibitor LY2157299.

The muted response to enzalutamide when administered post abiraterone progression is stark. In abiraterone-naïve mCRPC patients treated with enzalutamide, 78% of chemotherapy-naïve and 54% of post-chemotherapy patients experienced PSA declines of $\geq$50%.[14,36] Following progression on abiraterone, however, retrospective studies show that only about 13% to 36% of mCRPC patients experience PSA declines of $\geq$50% when treated with enzalutamide.[4-6,35] Similar levels of reduced response were seen in radiographic progression-free survival in post-chemotherapy mCRPC patients – from 8.3 months in abiraterone-naïve patients[14] to 4 months post abiraterone.[6] We propose to test the efficacy of LY2157299 in combination with enzalutamide in chemotherapy-naïve mCRPC patients following progression on abiraterone, using radiographic progression-free survival as the primary endpoint and PSA response as a secondary endpoint. We will compare the combination treatment arm to a small, adequate control arm of enzalutamide alone.

2.3. Correlates

2.3.1. AR-V7

We anticipate employing AR splice variant 7 (AR-V7) as a correlative biomarker because AR-V7 represents an emerging mechanism of therapeutic resistance to enzalutamide and other potent AR-directed therapies.[28,37] AR-V7 lacks the AR ligand-binding domain (LBD), the intended target of nearly all forms of AR-directed therapies.[38] Ongoing work by our group has generated evidence implicating the presence of AR-V7 in circulating tumor cells (CTCs) from patients with mCRPC with primary resistance to enzalutamide.[39]

We will evaluate AR-V7 expression as a potential biomarker to identify patients who may have greater benefit from TGF-β inhibition. AR-V7 positivity is defined as detection of the AR-V7 transcript by means of a quantitative-RT-PCR assay at 36 or fewer PCR cycles, corresponding to detection of one or more copies of AR-V7 complementary DNA (cDNA). Our hypothesis is that patients who have AR-V7-associated primary resistance to enzalutamide will experience a greater relative increase in rPFS from the combination of LY2157299 and enzalutamide through blocking EMT and decreasing immunosuppression than patients whose AR-V7 status predicts they will benefit more from enzalutamide.

Blood samples will be collected from peripheral veins for analysis of CTCs. This will serve as a "liquid" biopsy. Reliable isolation, detection, and analysis of CTCs, often existing at a

frequency of only a few tumor cells per mL blood (range: zero to hundreds) in patients with metastatic prostate cancer, is analytically challenging. The AlereTM CTC AdnaTest will be utilized for molecular analysis of CTCs in this project. The test uses the "combinations of combinations" principle to isolate and analyze CTCs. For CTC isolation, the ProstateCancerSelect kit will be used. The goal is to enrich circulating prostate tumor cells in the blood using magnetic beads coated with three different antibodies, one of which is against the epithelial antigen EpCAM (the other two are proprietary).

2.3.2. C-reactive Protein Levels in Serum

C-reactive protein (CRP) has been shown to be an adverse prognostic marker for men with mCRPC.[40] In addition, high levels of CRP in blood serum are found to be positively correlated with plasma TGF-β1 of metastatic prostate cancer patients.[41] More than 50% of mCRPC patients have elevated CRP levels (> 5 ml/L).[42] To develop hypotheses about patients most likely to benefit from LY2157299, we will correlate rPFS and PSA response to baseline CRP levels.

2.3.3. Markers of Epithelial-Mesenchymal Transition

Because metastatic prostate tumors exhibit increased TGF-β expression[23] that is associated with EMT and enhanced invasion and metastasis,[24] we will determine whether there are differences in TGF-β, E-cadherin, N-cadherin, SMAD2, vimentin, and ZEB1 levels between patients treated with the combination of enzalutamide and LY2157299 and those treated with enzalutamide alone. The markers will be measured in tumor cells from biopsies taken prior to initiation and at 12 weeks. Blood will be used to measure TGF-β levels and levels of phosphorylated SMAD2 in peripheral blood mononuclear cells.[41] Unpublished data from the laboratory of Dr. Jun Luo suggest that markers of EMT are unregulated in AR-V7$^+$ patients.

2.3.4. Immune Function Markers

T Lymphocyte Number/Function

Perhaps the best-documented immunological effects of TGF-β are those on T cell function and number. TGF-β clearly inhibits antigen-specific and non-specific T cell proliferation, and by inhibiting secretion of Il-7 from follicular cells in lymph nodes may drive a CD4$^+$ predominant lymphopenia.[43] Although elevated TGF-β levels have been well documented in prostate cancer patients, to date a correlation between elevated TGF-β and CD4$^+$ T cell number and/or function has not been examined. Such data would be important, as antigen-specific immunotherapy approaches are likely critically dependent on adequate T cell number/function, and blocking TGF-β could prove an immediately tractable approach to augment immunotherapy efficacy in men with mCRPC. To quantify and compare the function of the peripheral CD8$^+$ and CD4$^+$ cells from patients in each of the two treatment groups, we will use a standard tritiated thymidine (^{3}H-thymidine) incorporation assay as previously described.[44]

Pro-inflammatory Cytokines

Multiple groups, including our own, have suggested that prostate cancer arises in a context of chronic inflammation,[45] and this hypothesis is supported by multiple studies showing that

cytokine levels may correlate with recurrence and outcome.[46] Interestingly, this influence may extend even into the advanced disease setting, where TGF-β levels correlate with the outcome of docetaxel treatment.[47] This trial provides an ideal setting to test whether baseline cytokine levels correlate with the outcome to treatment, whether changes in cytokine levels correlate with outcome, and, most critically, whether blocking TGF-β signaling with LY2157299 alters cytokine levels.[41] We will evaluate serum cytokine profile from patients in each of the two treatment groups (including but not limited to IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, TNF-α, and IFNγ), as previously described.[48]

Adaptive Immune Response

Several studies suggest that early prostate cancer lesions may be recognized by the adaptive immune system, a finding supported by the detection of prostate-associated antibodies in the sera of prostate cancer patients in multiple studies.[49-51] Several of these studies focused on the use of serum antibody levels (or serum antibody profiles) as a potential tool for prostate cancer diagnosis, while others suggested that antibody induction may be associated with treatment. Immunologically, the generation of high-affinity Immunoglobulin G (IgG) antibodies is dependent on antigen recognition by $CD4^+$ (helper) T cells and $CD4^+$ T cell activation, and helper function is clearly inhibited by TGF-β. We thus hypothesize that TGF-β blockade will release this repression, leading to the expansion and/or induction of new prostate-cancer directed antibodies. Patient sera will be cryopreserved in both arms. As previously described,[52] a high-content protein microarray platform (ProtoArray® v5.0, Life Technologies Corporation) will be used to measure IgG levels against an array of secondary antigens in pre-treatment (baseline) and post-treatment sera. Levels of antigen-specific IgG will be quantified using a fluorescently labeled secondary antibody against IgG. A "response" will be defined as a 2x or > increase in levels as compared to baseline. Reactivity to candidate antigens will be independently confirmed using the Luminex xMAP platform, a bead-based, flow-based technology that avoids the need to generate and validate a separate ELISA assay for each candidate antigen.

2.3.5. Pharmacokinetics

There is a potential for a drug-drug interaction since enzalutamide is a strong CYP3A4 inducer and a moderate CYP2C19 inducer and LY2157299 is a substrate of both isozymes. However, in a Phase I clinical trial, patients taking enzyme-inducing anticonvulsants (CYP3A4) and proton pump inhibitors did not significantly alter the pharmacokinetics of LY2157299. The pharmacokinetics of LY2157299 in the presence of enzalutamide will be assessed during the safety run-in phase only to ensure that the exposure is similar to historical controls.

3. Patient Selection

3.1. Inclusion Criteria

Patients are eligible to be included in the study only if they meet ***all*** of the following criteria:

[1] Have metastatic castration-resistant prostate cancer, are chemo-naïve for mCRPC (however, six cycles of docetaxel are allowed in hormone-sensitive disease, per ECOG3805 data), and have progressed on abiraterone treatment (patients may have had prior therapy including sipuleucel-T, radium-223, abiraterone, ketoconazole, and/or Tak-700). Progression on abiraterone is defined as:

- Radiologic progression by RECIST 1.1 or PCWG2 criteria, or
- PSA progression on abiraterone:
  - For responders to abiraterone: 25% or greater increase and an absolute increase of 2 ng/mL or more from the nadir, confirmed by a second value obtained 2 or more weeks later
  - For non-responders to abiraterone: 25% increase above baseline with an increase in absolute value of 2 ng/mL or more after 12 weeks of treatment

[2] Have a pathological diagnosis of prostate carcinoma

[3] Patients may be receiving continuous hormonal ablation with surgical or medical castration with baseline testosterone <50ng/dL

[4] Patient may be receiving bone targeted agents.

[5] Must have had prior Abiraterone treatment.

[6] Have evaluable disease by RECIST 1.1 or PCWG2 criteria.

[7] Patient's acceptance to have a tumor biopsy of an accessible lesion at baseline and on treatment if the lesion can be biopsied with acceptable clinical risk (as judged by the investigator).

[8] Have ECOG performance status 0-2 (**Appendix A**).

[9] Have estimated life expectancy > 3 months.

[10] Be able to take oral medication

[11] Have normal organ and marrow function as defined below:

- Absolute neutrophil count	$\geq$ 1500/mm³
- Platelets	$\geq$ 100,000/mm³
- Hemoglobin	$\geq$ 9 g/dL
- Total bilirubin	$\leq$ 1.5 ULN
- AST(SGOT)/ALT(SGPT)	$\leq$ 2.5 x ULN (<5X ULN if with known liver metastases provided bilirubin is normal)
- Creatinine	$\leq$ 2.0 x ULN (for patients with >1.5 x ULN, calculated or measured creatinine clearance must be = 40 mL/minute (Cockcroft-Gault)).

[12] Men of reproductive potential and those who are surgically sterilized (i.e., postvasectomy) must agree to practice effective barrier contraception that has an expected failure rate of < 1% during and for 6 months after discontinuation of study treatment.

- If condoms are used as a barrier contraceptive, a spermicidal agent should be added to ensure that pregnancy does not occur.

[13] Have the ability to understand, and have given written informed consent before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care.

3.2. Exclusion Criteria

Patients are ineligible to be included in the study if they:

[1] Have had known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for CNS involvement for at least one week prior to trial treatment. Patients with primary brain tumors are not eligible. However, as patients are completing abiraterone therapy, they will be allowed to continue up to 10 mg/day of prednisone.

[2] Have had prior chemotherapy for metastatic disease in castration-resistant prostate cancer (prior chemotherapy for hormone-sensitive disease, more than six months prior to registration, is acceptable).

[3] Have had had surgery within four weeks of dosing of investigational agent, excluding minor procedures (dental work, skin biopsy, etc.), celiac plexus block, and biliary stent placement.

[4] Have had palliative radiation or biological cancer therapy within 2 weeks prior to the first dose of study drug.

[5] Have had second-line hormonal therapy (abiraterone, ketoconazole, and/or Tak-700, etc.) within two weeks prior to the first dose of study drug. Patients require a two-week wash out. Patients may continue standard supportive dosing of prednisone and hydrocortisone for abiraterone and ketoconazole, respectively, as needed.

[6] Have received other investigational drugs within 14 days prior to enrollment.

[7] Is expected to require any other form of systemic or localized antineoplastic therapy while on study.

[8] Have received systemic corticosteroids (used in the management of cancer or non-cancer-related illnesses) within one week prior to first dose. Note: Systemic steroid therapy is allowed for subjects on replacement therapy as long as prednisone ≤ 10 mg or its steroid equivalent, and those patients should continue at the same dose through the trial.

[9] Have had prior enzalutamide, ARN-509, or galeterone therapy.

[10] Are taking strong or moderate CYP3A4 or CYP2C8 inducers, or CYP3A4, CYP2C9 and CYP2C19 substrates with a narrow therapeutic index (a full list of drugs to be used with caution can be found in **Appendix B**). Patients may switch to an alternative any time prior to Day 1 of trial drug administration. For patients taking strong CYP2C8 inhibitors, see dose modification in **Section 6.2**.

[11] Have moderate or severe cardiovascular disease:

- Has the presence of cardiac disease, including a myocardial infarction within six months prior to study entry, unstable angina pectoris, New York Heart Association Class III/IV congestive heart failure, or uncontrolled hypertension.

- Has documented clinically significant ECG abnormalities (not responding to medical treatments) or not clinically stable for at least 6 months.

- Has major abnormalities documented by ECHO with Doppler (for example, moderate or severe heart valve function defect) that is not stable for at least 6 months. Note: Left ventricular [LV] ejection fraction <50% is allowed only if clinically stable for at least 6 months (evaluation based on the institutional lower limit of normal).

- Has predisposing conditions that are consistent with development of aneurysms of the ascending aorta or aortic stress (for example, family history of aneurysms, Marfan-Syndrome, bicuspid aortic valve, evidence of damage to the large vessels of the heart documented by CT scan/MRI with contrast)

[12] Have a history of a seizure

[13] Have uncontrolled intercurrent illness, including but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

[14] Have a history of any autoimmune disease. Patients with a history of inflammatory bowel disease, including ulcerative colitis and Crohn's Disease, are excluded from this study, as are patients with a history of symptomatic disease (e.g., rheumatoid arthritis, systemic progressive sclerosis [scleroderma], systemic lupus erythematosus, or autoimmune vasculitis [e.g., Wegener's Granulomatosis]); CNS or motor

neuropathy considered of autoimmune origin (e.g., Guillain-Barre Syndrome and Myasthenia Gravis, multiple sclerosis). Patients with thyroid disease will be allowed. Autoimmune diagnoses not listed here must be approved by the protocol chair.

[15] Have a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies), hepatitis B, or hepatitis C infection.

3.3. Inclusion of Minorities and Women and Children

Since prostate cancer is only a disease of men, only male patients will be enrolled. Men of all ages and ethnic groups are eligible for this trial. The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins serves a prostate cancer population in which approximately 25% of these patients are African Americans. We anticipate that enrollment in this study will be reflective of our usual patient population. Minority patients who meet the eligibility requirements will be actively recruited to participate in this study. This study was designed to encourage minority accrual, but was not designed to measure differences in racial or ethnic populations.

4. Registration

4.1. General Guidelines

Potential study subjects will be identified and screened by the study team. Eligible patients will be consented prior to treatment initiation. Eligible, consented patients will be entered on study centrally at the Sidney Kimmel Comprehensive Cancer Center at the Johns Hopkins University by the Lead Study Coordinator. Patient names and identifying information will only be linked in the CRMS (Clinical Research Management System) database, which is accessible through a password-protected encrypted university server. All sites should call Serina King, the Lead Study Coordinator at (410) 614-6139 or email sking18@jhmi.edu to verify slot availabilities.

Following randomization, patients should begin protocol treatment within 10 business days. Issues that would cause treatment delays should be discussed with Dr. Paller. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

4.2. Registration Process

To register an eligible, consented patient for the trial, the following documents should be completed by the Research Nurse or Study Coordinator and faxed (410-367-2668) or emailed (sking18@jhmi.edu) to the Lead Study Coordinator, Serina King:

- Fax cover sheet
- Registration form
- Signed patient consent form
- HIPAA authorization form
- Eligibility Screening Checklist
- Copy of required screening tests and scans

The Research Nurse or Study Coordinator at the participating site will then call (410-614-6139) or e-mail (sking18@jhmi.edu) the Lead Study Coordinator (Serina King) to verify eligibility. To complete the registration process, the Lead Study Coordinator will:

- Assign a patient study number
- Register the patient on the study
- Fax or e-mail the patient study number to the participating site
- Call the research nurse or data manager at the participating site and verbally confirm registration.

4.3. Randomization

Subjects will be randomized by pharmacy to Arm 1 (enzalutamide with LY2157299) or Arm 2 (enzalutamide alone) in a 3:2 fashion.

5. Treatment

5.1. Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in **Section 5.5**. Appropriate dose modifications and delays are described in **Section 6.2**. No investigational or commercial agents or therapies other than those described below, and LHRH agonists or antagonists, may be administered with the intent to treat the subject's malignancy. The drug regimen for the trial is shown in Table 2.

Table 2: Regimen Description

Regimen Description				
Agent	**Dose**	**Route**	**Schedule**	**Cycle length**
Enzalutamide*	160 mg	Oral	Once daily, days 1-28	28 days (4 weeks)
LY2157299**	150 mg	Oral	Twice daily, days 1-14, off days 15-28	

* Enzalutamide: four 40 mg capsules orally, with or without food, daily. The capsules are to be swallowed whole (do not chew, dissolve, or open the capsules). Administration should be in accordance with the package insert.[53]
** LY2157299 Acetate: one 150 mg tablet orally twice daily 14 days on and 14 days off. The tablets are to be swallowed whole.

Each patient will maintain a medication diary of each dose of medications to assess compliance. The medication diary, which will be reviewed on day 1, +/- 3 days, of each cycle, is found in **Appendix C**.

5.2. Concomitant Medication and Supportive Care Guidelines

a) Because there is a potential for interaction of enzalutamide with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes as described in the Enzalutamide Package Insert.[53] See Exclusion Criteria in **Section 3.2** and **Appendix B** for additional guidance.

b) No interactions have been seen between LY2157299 and other drugs. No formal drug-drug studies have been undertaken.[54]

c) In case participants develop nausea/vomiting/diarrhea or myelosuppression, supportive medications will be prescribed as per institutional guidelines.

d) Careful monitoring for embolic events should be instituted while patients are receiving LY2157299. If a patient develops an embolism, standard anticoagulation therapy should be initiated and the study drug discontinued.

5.3. Contraception

Based on their mechanism of action, LY2157299 and enzalutamide can cause fetal harm when administered to a pregnant woman. Therefore all efforts must be made to prevent pregnancy of the female partner of any study participant. Patients enrolled in this study must agree to use an adequate method of contraception starting with Visit 1 through 6 months after the last dose of study drug, if applicable.

The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

Patients should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study the patients must adhere to the contraception requirement (described above) for the duration of the study. If there is any question that a patient will not reliably comply with the requirements for contraception, that patient should not be entered into the study.

5.4. Instructions for Missed Doses

Enzalutamide: Patients should try to take enzalutamide at a consistent time each day. However, if a dose is delayed, patients may take it any time that day. If a daily dose is missed, patients are to take the normal dose on the following day. If more than two consecutive days are missed, the study doctor or study team must be informed and the missed doses will be recorded in the patient's diary, with cause of delay, if available. Refer to **Section 6** for dose continuation after delays.

LY2157299: Patients should take LY2157299 twice daily on a regular schedule, at least 5 hours apart. Any missed doses should not be made up. If more than two consecutive days are missed, the study doctor or study team must be informed and the missed doses will be recorded in the patient's diary, with cause of delay, if available. Refer to **Section 6** for dose continuation after delays.

5.5. Adverse Events

5.5.1. LY2157299

- The most common treatment-emergent adverse events (TEAEs) possibly related to the LY2157299 monotherapy were asthenia (3 of 39 patients and 1 of 5 patients in two separate studies) and nausea (2 of 39 patients). All other events occurred in 1 patient each. Of potential interest, and which may be related to the mechanism of the inhibition of the TGF-β pathway, are the cases of gastritis (n=1), diarrhea (n=1), anal inflammation (n=1), and pulmonary embolism (n=1). The most common TEAEs (≥10%), regardless of causality, for the LY2157299 monotherapy were asthenia (34.1%), headache (25.0%), lymphopenia (18.2%), and hyperglycemia (18.2%). The incidence of hyperglycemia was thought to be related to steroid intake in the patients

on prior trials. Patients on this trial will not be on high doses of steroids (See Inclusion/Exclusion Criteria).

- Cardiotoxicity: In preclinical studies, dose-progressive incidence of vascular degeneration to the base of the aorta in the dog and rat was observed. These vascular changes led to transmural aneurysms and mortality in the mid- and high-dose rats. Due to the possibility of aneurysm formation, patients with a predisposition of developing aneurysms are excluded from this trial. Reversible decreases in blood pressure and increases in heart rate and arterial pulse pressure were observed in dogs but no compound-related changes in QT intervals, arrhythmias, or altered waveforms identified were observed during this animal study. Because of these cardiac findings in animals, careful cardiac monitoring, including assessment of heart rate, blood pressure, and cardiac function, is being performed in patients. The assessment for cardiotoxicity also includes regular measurements of laboratory markers for cardiac damage (troponin-I, B-type natriuretic peptide, cystatin C, and high-sensitivity C-reactive protein). No clinically significant valvulopathies or aneurisms have been reported or observed at dosages of up to 300 mg/day given on an intermittent schedule (14 days on and 14 days off). If moderate or severe cardiac insufficiency signs are observed, LY2157299 should be immediately stopped, the cause of the cardiac insufficiency must be identified, and if necessary, appropriate treatment initiated.[55]

- Thromboembolic events: Careful monitoring and awareness for thromboembolic events should be instituted while patients are receiving LY2157299. If a patient develops an embolism, standard anticoagulation therapy should be initiated and the study drug discontinued. Thromboembolic events are common in cancer and vary depending on cancer type.[56] Emboli detected during the administration of LY2157299 were successfully treated with low-molecular heparins and hence LY2157299 was not found to diminish such treatment interventions.

- Impaired Immune Function: Administering LY2157299 may result in thrombocytopenia, anemia, leucopenia, lymphopenia, a reduction in natural killer (NK) cells, and other changes that may increase a patient's risk for infections or inflammatory changes. Autoimmune reactions are also possible and patient immune functions should be carefully monitored.

Once trial entry criteria were adjusted to exclude patients likely to experience thromboembolic events or impaired immune function (as the entry criteria have been for this trial), these issues were not seen.

5.5.2. Enzalutamide

- Common side effects associated with enzalutamide (≥ 5%): 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.

- Enzalutamide may cause dizziness, mental impairment, paresthesia, hypoesthesia, and falls.

- Enzalutamide has been associated with an increased risk of seizure.

5.5.3. LY2157299 and Enzalutamide Interactions

- As both drugs have been associated with fatigue, monitoring for fatigue should be instituted.

- LY2157299 was not found to be a significant inducer of overall cytochrome P450 (CYP) content in rat and dog *in vivo* studies.

- LY2157299 is a substrate of CYP2C19 and CYP3A4. In a Phase I clinical trial, patients taking enzyme-inducing anticonvulsants (CYP3A4) and proton pump inhibitors did not significantly alter the pharmacokinetics of LY2157299.

- Enzalutamide is a strong CYP3A4 inducer and a moderate CYP2C9 and CYP2C19 inducer.

- There are no known interactions with other medications,.

5.6. Duration of Treatment

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- The patient or legal representative (such as a parent or legal guardian) withdraws consent for treatment

- Disease progression as described in **Section 5.8.1**

- Intercurrent illness that prevents further administration of treatment

- Unacceptable adverse event(s) as described in **Section 5.8.2**

- Therapy delay for more than two weeks (and the patient does not recover within 60 days)

- Requirement of a dose reduction of enzalutamide below 80 mg daily

- Noncompliance with trial treatment or procedure requirements

- Patient is lost to follow-up

- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

- Termination by sponsor.

5.7. Dose Limiting Toxicity (DLT) Criteria

A dose-limiting toxicity (DLT) is defined as toxicity related to study drug occurring during the first 28 days (1 Cycle) after administration of the first dose that meets any of the following criteria:

- For hematologic toxicity, the only adverse events that will be considered dose limiting are:
    - Grade 4 anemia
    - Grade 4 neutropenia that lasts > than 7 days or febrile neutropenia
    - ≥ grade 3 thrombocytopenia that lasts > than 7 days or has clinically significant bleeding
- For non-hematologic toxicity, the only adverse events that will be considered dose limiting are:
    - Any grade 4 toxicity excluding asymptomatic electrolyte aberrations
    - Any grade 3 toxicity except Grade 3 nausea, vomiting or diarrhea if it resolves to <= grade 2 within 48 hours of maximal supportive care,
    - Grade 3 or greater seizure

5.8. Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in **Section 5.6** apply. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

5.8.1. Disease Progression

Progression will be evaluated on the basis of biochemical, radiographic, or symptomatic progression based on modified PCWG2 criteria.[57] Determinations of disease progression will be made by the investigator.

Within the first 12 weeks of treatment (primary treatment phase), progression will be defined as follows:

1. Radiographic progression defined by at least one of the following:

- Soft tissue disease progression by modified RECIST criteria v.1.1 (baseline LN size must be ≥ 2.0 cm to be considered target or evaluable lesion)
- Development of two or more new bone lesions not consistent with tumor flare per PCWG2.

2. Symptomatic or clinical progression defined by one of the following:

- Development of a skeletal related event (SRE) defined as pathologic fracture, spinal cord or nerve root compression, palliative radiation to bone, or surgery to bone
- Worsening pain due to CRPC unable to be controlled by non-narcotic or narcotic medications
- Sustained worsening of ECOG status resulting from CRPC (e.g., 2 to 3)
- Treating physician decides to initiate new systemic anti-cancer therapy

We will encourage investigators to continue study treatment until radiographically confirmed disease progression requires initiation of new systemic antineoplastic therapy. In addition we will measure PSA levels but do not plan to use PSA progression (based on Prostate Cancer Working Group 2 criteria[57]) as a reason for cessation of treatment.

5.8.2. Unacceptable Adverse Event(s)

Permanent discontinuation of LY2157299 and/or enzalutamide should be considered for any of the following:

- Grade 4 hematologic or grade 3 or 4 non-hematologic toxicity not resolving or improving to ≤ Grade 1 or clinically asymptomatic grade 2 within 2 weeks. Dosing delays and reductions are described in **Section 6.2**.
- Seizure
- Thromboembolic event
- Severe immune-mediated reactions involving any organ (e.g., nephritis, pneumonitis, pancreatitis, non-infectious myocarditis)
- Moderate or severe heart valve toxicities or new aneurysms

In case toxicity does not resolve or improve to ≤ Grade 1 within two weeks after the last dose of the study drug (and the patient does not recover within 60 days), study therapy discontinuation should be considered after discussion with the Sponsor. With Sponsor agreement, patients still at Grade 2 may continue in the study only if asymptomatic and controlled. One dosing delay due to the same toxicity will be permitted. In the event of a second occurrence of the same toxicity that would require dosing delay, study therapy will be discontinued permanently.

5.9. Safety Follow-up Visit

After a patient is discontinued from treatment, a mandatory Safety Follow-Up Visit should be performed approximately four weeks after the last dose of study medication (or within seven days prior to initiation of a new anti-cancer treatment, whichever comes first). Procedures and assessments performed at this visit and beyond should follow the respective guidelines described in **Section 10**. The patient will be monitored for adverse events up to the mandatory Safety Follow-Up Visit or to resolution of drug-related toxicities to Grade 0-1, whichever occurs later. Serious adverse events (SAEs) that occur within four weeks of the end of treatment or before initiation of a new antineoplastic treatment should also be followed and recorded.

5.10. Duration of Follow-Up

Patients who are discontinued from the study due to an unacceptable drug-related adverse event (AE) will be followed until the resolution of the AE to Grade 0-1 or stabilization or until initiation of a new therapy for their cancer, whichever occurs first.

Patients who discontinued study therapy without documented disease progression should continue to be monitored for disease status by radiologic imaging. Disease monitoring should continue to be assessed every six months, until disease progression or two years, whichever occurs first.

All patients who discontinue treatment should be contacted every six months to monitor overall survival. Information of other cancer therapies after discontinuation from the study treatment will be collected.

6. Dosing Delays/Dose Modifications

6.1. Dosing Delays

All scheduled cycles occur over a 28-day period. If necessary, treatment may be delayed for up to two weeks. If treatment is delayed more than two weeks (and the patient does not recover within 60 days), the Sponsor must be contacted for further instructions on continued treatment. Additional delays or modifications to the treatment schedule must be approved by the IND Sponsor. When the delay is due to toxicity, then the next cycle may begin with a dose reduction.

LY2157299 will be withheld for the following drug-related toxicities:

- Absolute neutrophil count (ANC) <500/uL for longer than seven days
- ANC <1000/uL with a single temperature of >101ºF/38.3ºC or a sustained temperature of >100.4ºF/38ºC for more than one hour
- Platelet count < 25 x 10^3/uL or < 50 x 10^3/uL with clinically significant bleeding
- Hemoglobin < 8 g/dL

- Absolute lymphocyte count (ALC) < 200/uL

Enzalutamide and/or LY2157299 will be withheld for the following drug-related toxicities:

- Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 or 4 non-hematologic toxicity

- Any AE, laboratory abnormality, or intercurrent illness that, in the judgment of the investigator, warrants delaying the dose of study medication.

In case toxicity does not resolve to Grade 0-1 within two weeks after the last dose (and the patient does not recover within 60 days), trial treatment should be discontinued after consultation with the Sponsor (with the exception of alopecia, fatigue, skin rash, nausea, vomiting, constipation, or diarrhea that can be controlled with treatment).

6.2. Dose Modifications

If patient experiences grade 3 or 4 toxicities (individually defined in **Section 6.1**), prolonged (> 2 weeks), symptomatic grade 2 toxicities, or an intolerable side effect, withhold dosing for one week or until symptoms improve to ≤ grade 1. Then resume therapy at the next lower dosing level per Table 3.

Table 3: Dose Reductions

Dose Level	Dose of Enzalutamide	Dose of LY2157299
0 (starting dose)	160 mg PO daily x 28 days	150 mg PO BID x 14 days
-1	120 mg PO daily x 28 days	150 mg PO BID x 14 days
-2	80 mg PO daily x 28 days	150 mg PO BID x 14 days
-3	80 mg PO daily x 28 days	80 mg PO BID x 14 days

If a dose reduction of enzalutamide below 80 mg daily and/or LY2157299 below 80 mg twice daily is required, patients will be removed from protocol treatment. Dose re-escalations to the previous dose level are not permitted.

Co-administration of strong CYP2C8 inhibitors: The concomitant use of strong CYP2C8 inhibitors should be avoided if possible. If patients must be co-administered a strong CYP2C8 inhibitor, reduce the enzalutamide dose to 80 mg once daily. If co-administration of the strong inhibitor is discontinued, the enzalutamide dose should be returned to the dose used prior to initiation of the strong CYP2C8 inhibitor.

7. Adverse Events: Reporting Requirements

This study will use the descriptions and grading scales found in the revised CTCAE version 4.0 for AE reporting that can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Information about all AEs, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected, recorded, and followed up as appropriate.

All AEs and SAEs experienced by subjects will be collected and reported from the first dose of the investigational agent, throughout the study, and will only be followed for four weeks unless related to the investigational agent. All SAEs will be collected for four weeks after the last dose of the investigational agent.

Subjects who have an ongoing AE related to the study procedures and/or medication(s) may continue to be periodically contacted by a member of the study staff until the event is resolved or determined to be irreversible by the investigator.

Laboratory abnormalities: Laboratory abnormalities present at the screening visit will be recorded as pre-treatment signs and symptoms. After study treatment administration, all Grade 3 and 4 clinical laboratory results that represent an increase in severity from baseline will be reported as AEs. A Grade 1 or 2 clinical laboratory abnormality should be reported as an AE only if it is considered clinically significant by the investigator.

7.1. Definitions

7.1.1 Adverse Event

An AE is defined as any undesirable sign, symptom, or medical condition occurring after starting the study drug (or therapy), even if the event is not considered to be related to the study. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver), or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). Medical conditions/diseases present before starting the study treatment are only considered AEs if they worsen after starting the study treatment (any procedures specified in the protocol). AEs occurring before starting the study treatment but after signing the informed consent form will not be recorded. Abnormal laboratory values or test results constitute AEs only if they induce clinical signs or symptoms or require therapy.

7.1.2 Serious Adverse Event

A SAE is an undesirable sign, symptom, or medical condition that:

- Results in death
- Is life threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below for exceptions) for >24 hours

- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect (note: reports of congenital anomalies/birth defects must also be reported on the Pregnancy Supplemental Form)
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above). Examples of such events include but are not limited to intensive treatment in an emergency room or at home for allergic bronchospasm; and blood dyscrasias or convulsions that do not result in hospitalization.
- Is a new cancer (that is not a condition of the study)
- Is associated with an overdose

Events ***not*** considered to be SAEs are hospitalizations for:

- Admissions as per protocol for a planned medical/surgical procedure or to facilitate a procedure
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission for purpose other than remedying ill health state and that was planned prior to entry into the study. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative).
- Admissions for monitoring of treatment-related infusion reactions that do not otherwise meet the criteria for a SAE.

7.2. Relationship

The relationship of an AE to the administration of the study drug is to be assessed by the investigator according to the following definitions:

- No (unrelated, not related, no relation): The time course between the administration of the study drug and the occurrence or worsening of the adverse event rules out a causal relationship, and another cause (concomitant drugs, therapies, complications, etc.) is suspected.

- Yes (related): The time course between the administration of the study drug and the occurrence or worsening of the adverse event is consistent with a causal relationship and no other cause (concomitant drugs, therapies, complications, etc.) can be identified.

The following factors should also be considered:

- The temporal sequence from study drug administration: The event should occur after the study drug is given. The length of time from study drug exposure to event should be evaluated in the clinical context of the event.

- Underlying, concomitant, intercurrent diseases: Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- Concomitant medication: The other medications the subject is taking or the treatment the subject receives should be examined to determine whether any of them might be recognized to cause the event in question.
- Known response pattern for this class of study drug: Clinical and/or preclinical data may indicate whether a particular response is likely to be a class effect.
- Exposure to physical and/or mental stresses: The exposure to stress might induce adverse changes in the recipient and provide a logical and better explanation for the event.
- The pharmacology and pharmacokinetics of the study drug: The known pharmacologic properties (absorption, distribution, metabolism, and excretion) of the study drug should be considered.

7.3. Expectedness

Unexpected adverse event: An AE that varies in nature, intensity, or frequency from information on the investigational drug/agent provided in the product IB, package insert, or safety reports. Any AE that is not included in the IB consent is considered "unexpected."

Expected (known) adverse event: An AE that has been reported in the IB. An AE is considered "expected" only if it is included in the IB document as a risk.

7.4. Handling of Expedited Safety Reports

In accordance with local regulations, the IND Sponsor will notify investigators of all SAEs that are unexpected (i.e., not previously described in the IB), and related to the investigational agent. This notification will be in the form of an expedited safety report (ESR) that is to be faxed to the investigators and the study coordinators. Upon receiving such notices, the investigator must review and retain the notice with the IB and, where required by local regulations, submit the ESR to the appropriate IRB. The investigator and IRB will determine if the informed consent requires revision. The investigator should also comply with IRB procedures for reporting any other safety information.

7.5. Reporting

7.5.1. General

All AEs (both expected and unexpected) will be captured on the appropriate study-specific CRFs.

In addition, all SAEs, regardless of causality to study drug and/or administration device, will be reported promptly to the IND sponsor(via Lead Study Coordinator, Serina King) (fax: 410-614-7287, attn.: Serina King); e-mail: sking18@jhmi.edu) and Lilly (fax: 866-

644-1697), within 24 hours of recognition of the event (**Appendix D**). If this falls on a weekend or holiday, an email notification is acceptable but must be followed by an SAE reporting form on the next business day.

Although pregnancy and lactation are not considered AEs, it is the responsibility of investigators or their designees to report the pregnancy of a male patient's female partner within 4 weeks days of completing the trial. All pregnancies of female partners of patients must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage, and stillbirth must be reported as SAEs (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported to the IND sponsor and Lilly.

7.5.2. Institutional Review Board

Participating sites will be responsible for reporting to their Institutional Review Board (IRB). All SAEs will be reported to the IRB per institutional standards within three business days of recognition of the AE if the event is related and expected, related and unexpected, or related and fatal or life-threatening due to administration of the investigational product. If the SAE is unrelated to administration of the investigational agents, then it should be reported to the IRB within 15 days of recognition of the SAE. Follow-up information will be submitted to the IRB and IBC as soon as relevant information is available.

7.5.3. Food and Drug Administration

All reporting to the Food and Drug Administration (FDA) will be completed by the IND Sponsor.

7.5.3.1 Expedited IND Safety Reports:

Seven Calendar-Day Telephone or Fax Report:
The IND Sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the investigational agent. Such reports are to be telephoned or faxed (301-827-9796) to the FDA within seven calendar days of first learning of the event. Follow-up information will be submitted to the FDA as soon as relevant information is available.

15 Calendar-Day Written Report:
The IND Sponsor is required to notify the FDA of any SAE that is unexpected and related to the investigational agent in a written IND Safety Report.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new

report should contain comments on the significance of the new event in light of the previous related reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA within 15 calendar days of first learning of the event. Follow-up information will be submitted to the FDA as soon as relevant information is available.

7.5.3.2 IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the IND Sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the adverse events and progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.33, for a list of the elements required for the annual report. All IND annual reports will be submitted to the FDA by the IND Sponsor.

8. Pharmaceutical Information

8.1. Enzalutamide

8.1.1. Agent Accountability

The sponsor/investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution, and usage of investigational products in accordance with the protocol and any applicable laws and regulations. Enzalutamide is commercially supplied.

8.1.2. Mode of Action

Enzalutamide is an androgen receptor inhibitor that acts on different steps in the androgen receptor signaling pathway. Enzalutamide has been shown to competitively inhibit androgen binding to androgen receptors and inhibit androgen receptor nuclear translocation and interaction with DNA. A major metabolite, N-desmethyl enzalutamide, exhibited similar *in vitro* activity to enzalutamide. Enzalutamide decreased proliferation and induced cell death of prostate cancer cells *in vitro,* and decreased tumor volume in a mouse prostate cancer xenograft model.

8.1.3. Description

The chemical name is 4-{3-[4-cyano-3-(trifluoromethyl)phenyl]-5,5-dimethyl-4-oxo-2-sulfanylideneimidazolidin-1-yl}-2-fluoro-N-methylbenzamide. The molecular weight is 464.44 and molecular formula is C21H16F4N4O2S.

Enzalutamide 40 mg capsules are white to off-white oblong soft gelatin capsules imprinted in black ink with ENZ.

Enzalutamide 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.

8.1.4. Preparation

Enzalutamide is a hazardous agent. Use appropriate precautions for handling and disposal in accordance with institutional policies.

8.1.5. Storage

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).

8.1.6. Stability

Do not use enzalutamide after the expiration date.

8.1.7. Route of Administration

Enzalutamide can be taken with or without food. Swallow capsules whole. Do not chew, dissolve, or open the capsules.

8.1.8. Patient Care Implications

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.

Co-administration of enzalutamide with a strong CYP2C8 inhibitor (e.g. gemfibrozil) should be avoided if possible. IF co-administration of enzalutamide with strong CYP2C8 inhibitor cannot be avoided, reduce the dose of enzalutamide. See **Section 6.2** for dose reduction instructions for patients taking strong CYP2C8 inhibitors.

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.

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), and 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., S-mephenytoin) should be avoided, as enzalutamide may decrease their exposure. If co-administration with warfarin cannot be avoided, conduct additional INR monitoring.

8.1.9. Returns and Reconciliation

Not applicable.

8.2. LY2157299 (Galunisertib)

8.2.1. Agent Accountability

The sponsor/investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution, and

usage of investigational product in accordance with the protocol and any applicable laws and regulations.

8.2.2. Mode of Action

Transforming Growth Factor-Beta (TGF-β) has been recognized as a potent mediator of tumor growth in advanced forms of cancer.[58] Because of this, TGF-β inhibition is currently being investigated as a treatment option in patients with advanced metastatic cancer.[22] LY2157299 monohydrate (hereafter referred to as LY2157299) is a small molecule that inhibits the TGF-β receptor Type 1 (RI) kinase activity. This mechanism of action is determined by changes in phosphorylated SMAD2 (pSMAD) levels. Its antitumor activity was demonstrated in three different *in vivo* tumor animal models: two breast cancer models, MX1 and 4T1; and a non-small cell lung cancer (NSCLC) model, Calu6.

8.2.3. Description

LY2157299 monohydrate is supplied for clinical trial use as a film-coated tablet. Each tablet contains LY2157299 monohydrate equivalent to 80 and 150 mg of the base compound. The inactive ingredients in the drug product are: Tablet core: mannitol, microcrystalline cellulose, croscarmellose sodium, colloidal silicon dioxide, magnesium stearate; Coating: lactose monohydrate, hydroxypropyl methylcellulose, titanium dioxide, triacetin, iron oxide red and iron oxide yellow; and talc as polishing aid.

8.2.4. Preparation

There are no special precautions. However, if direct contact of the oral solution occurs with the skin or eyes, wash skin thoroughly with soap and water; rinse eyes with plain water.

8.2.5. Storage

The drug product is stable when stored at room temperature.

8.2.6. Stability

Do not use LY2157299 after the expiration date.

8.2.7. Route of Administration

Oral. The tablets should not be crushed or dissolved.

8.2.8. Patient Care Implications

LY2157299 is contraindicated in patients who have shown hypersensitivity to any of its components. LY2157299 is also contraindicated in patients with:

- Cardiac disease, including a myocardial infarction within six months prior to study entry, unstable angina pectoris, New York Heart Association (NYHA) Class III/IV congestive heart failure, or uncontrolled hypertension, or
- Major cardiac abnormalities

- Patients with a predisposition for developing aneurysms should be excluded from receiving LY2157299 (for example, family history of aneurysms, Marfan-Syndrome, bicuspid aortic valve, or evidence of damage to the large vessels of the heart)

Embolic events: careful monitoring for embolic events should be instituted while patients are receiving LY2157299. If a patient develops an embolism, standard anticoagulation therapy should be initiated and the study drug discontinued.

Impaired Immune Function: Administering LY2157299 may result in thrombocytopenia, anemia, leucopenia, lymphopenia, a reduction in natural killer (NK) cells, and other changes that may increase a patient's risk for infections or inflammatory changes. Autoimmune reactions are also possible and patient immune functions should be carefully monitored.

8.2.9. Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies, the amount dispensed to and returned by the patients, and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational products will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

9. Correlative Studies

Sample collection, storage, and shipment instructions will be provided in the Procedures Manual.

9.1. AR-V7 Liquid Biopsy Procedure

Blood will be collected at baseline, (day 1, cycle 1 prior to drug administration), day 1 of cycle 4, and at End of Treatment/Disease Progression visit.

In select patients, it may be feasible to interrogate AR mutations using excess cDNA samples. If there is adequate CTC-enriched cDNA remaining after qRT-PCR analysis for AR-V7, cDNA will be subjected to AR mutation analysis, with a focus on known gain-of-function mutations in the ligand-binding domain.

1. Blood draws should be scheduled on Monday-Thursday, or, in the case of holidays, at least two days prior to the holidays, to allow overnight shipping and receiving the next business day without interruption by holidays or weekends.

2. Blood samples will be collected in a 8.5 mL BD Vacutainer ACD Solution A tubes (BD ACD-A) (yellow top). Product #s for the BD ACD-A tubes in the US is 364606.

3. Ensure that at least 8.5 mL of blood is drawn into each tube. Avoid low volume to minimize agitation during shipping.

4. After blood draw, invert the tubes gently 180 degree and back 3-4 times.

5. Inset tube(s) into Aqui-Pak™ Absorbent Pouches (https://www.therapak.com/catalog/absorbents). Insert Aqui-Pak™ with tubes into a biohazard bag.

6. Store samples at 4-8ºC in a refrigerator if a cold box (see below) is not immediately available (maximum exposure time to ambient temperature should be limited to 2 hours), but do NOT put the tube on ice. Do NOT leave the samples overnight in a refrigerator. Ship the same day before close of business (see below).

7. Active the FEDEX cold box (http://images.fedex.com/us/healthcare/pdf/Cold_Shipping_Info_Sheet.pdf.) and place the biohazard bag inside the box. Top the bag with bubble wraps to ensure snug fit in the shipping box. The sample requisition sheet should be included.

8. Ship by FEDEX to the recipient address above the same day of blood draw. Notify the recipient of the tracking number on the day of shipment by an email with a subject title identifying the study.

Shipping address (overnight Monday-Thursday only):

Dr. Jun Luo
Assistant Professor of Oncology
The Johns Hopkins Hospital
415A Marburg Building
600 N Wolf St. Baltimore, MD 21287-2101
443-287-5625

Any AR-V7 sample shipping notification and question, please contact the Lead Study Coordinator Serina King, sking18@jhmi.edu, and copy Dr. Luo, jluo2@jhmi.edu.

9.2. TGF-β Procedure

Blood will be collected at baseline (day 1, cycle 1 prior to drug administration), day 1 of cycles 2 and 4, and at End of Treatment/Disease Progression visit. In collecting blood, after the tourniquet is released, a single discard tube of approximately 10 ml blood will be drawn to minimize potential effects of localized platelet activation caused by the tourniquet. Subsequently, and to further reduce pre-analytical variation in TGF-β1 measurements caused by *ex vivo* platelet activation, blood will be drawn into a Vacutainer tube containing citrate solution, theophylline, adenosine, and dipyridamole (CTAD) (Becton Dickinson, Franklin Lakes, NJ, USA). The CTAD tube will be processed as recommended by the manufacturer and plasma will be stored at -70^{o}C.

Upon request, samples will be shipped on dry ice to (overnight Monday-Thursday only):

Johns Hopkins University
Lab address to be determined at a later date and updated in separate lab manual.

9.3. Peripheral Blood Mononuclear Cells

Peripheral Blood Mononuclear Cells (PBMCs) will be collected to measure post-treatment changes in $CD4^{+}$ and $CD8^{+}$ T cell counts and function that will be correlated with clinical responses. PBMCs will be collected at baseline (day 1, cycle 1 prior to drug administration), day 1 of cycles 2 and 4, and at End of Treatment/Disease Progression visit. Whole blood will be collected in four 8ml CPT tubes at the designated time points and processed using standard laboratory procedures within 4 hours of collection. PBMC are isolated and stored frozen until use.

Upon request, samples will be shipped on dry ice to (overnight Monday-Thursday only):

Johns Hopkins University
Lab address to be determined at a later date and updated in separate lab manual.

9.4. Serum and Plasma Samples

Blood will be collected at baseline (day 1, cycle 1 prior to drug administration), day 1 of cycles 2 and 4, and at End of Treatment/Disease Progression visit to identify potential therapeutic targets, biomarkers, and predictors of response and autoimmune toxicity through proteomic approaches. Whole blood will be collected in a 10 milliliter Serum Separator Tube (SST tube) at the designated time points and processed within 1 hour of collection using standard laboratory procedures. Using a pipette, aliquots of 1 mL of serum should be transferred to cryogenic vials and stored at -80ºC.

We will also collect whole blood in two 10 mL plasma preparation tubes with EDTA (PPT, BD Vacutainer, Franklin Lakes, NJ) and gently swirl tubes to mix blood with EDTA. Within two hours of collection, the sample will be processed using standard procedures for plasma separation. Plasma will be divided into 1 mL aliquots and stored at -80ºC. Pellets from this separation procedure will be washed in PBS and then divided into aliquots and also stored at -80ºC.

Upon request, samples will be shipped on dry ice to (overnight Monday-Thursday only):

Johns Hopkins University
Lab address to be determined at a later date and updated in separate lab manual.

9.5. Tumor Tissue Studies

Tumor samples will be collected from a site of active metastatic disease at baseline and 12 weeks (4-6 cores per time point) if a patient's tumor is thought to be reasonably safe and easy to biopsy. This will serve as a tissue biopsy. This will usually be done under image-guidance (e.g., CT/MRI- guided or ultrasound-guided biopsy). Preference should be given to a soft tissue metastasis over a bone metastasis, if both are available. In addition, when obtaining serial biopsies from the same patient, the same type of lesion should be biopsied each time, if possible. Patients may opt out of tissue biopsy collection. At least four standard core biopsies should be obtained from a metastatic site (soft tissue, lymph node, or bone). Fine needle aspiration (FNA) biopsy is unlikely to yield adequate tumor tissue for molecular analysis, and should only be attempted when core biopsy is not feasible. Approximately half of the collected tissue will be submitted as formalin-fixed paraffin-embedded (FFPE) tissue according to standard procedures. The other half will be snap frozen in liquid nitrogen. To minimize tissue autolysis, a dedicated tissue technician will be called/paged to the biopsy room to initiate processing steps, including flash freezing in liquid nitrogen and FFPE preparation, which should be done within 30 minutes of biopsy collection. All samples will be evaluated for adequacy and assessment of tumor-to-normal ratio by the urologic pathologist on the study (Dr. George Netto or local pathologist). When needed, macro or micro dissection of tumor component from intervening stroma will be performed under the supervision of the study pathologist. Frozen specimens will be stored in a –80ºC freezer in the laboratory of Jun Luo,

PhD. FFPE specimens will be processed according to standard FFPE procedures in the Pathology Department. Downstream processing steps and molecular studies will be conducted in the lab of Jun Luo, PhD.

Where there is sufficient tissue, we will also perform whole-exome sequencing using next-generation sequencing on DNA from tumors and matched normal tissue in order to delineate which genetic determinants define which subset of patients will respond to therapy.

Special Considerations for Bone Biopsies

In patients with metastatic bone lesions only, the guidelines BELOW should be considered when obtaining a bone biopsy.[59] These guidelines have been established by a Johns Hopkins musculoskeletal pathologist with over 30 years of experience in interpreting bone biopsies.

- Bone biopsies should preferably be obtained using CT guidance.
- Osteolytic lesions should be targeted preferentially over osteoblastic lesions.
- Metastases in the pelvic bones and long bones (e.g., femurs, humeri) should be targeted preferentially over vertebral or rib metastases.
- At least FOUR cores from different zones of the lesion should be obtained.

Samples will be shipped to (overnight Monday-Thursday only):

Dr. Jun Luo
Assistant Professor of Oncology
The Johns Hopkins Hospital
415A Marburg Building
600 N Wolf St.
Baltimore, MD 21287-2101
443-287-5625

9.6. Pharmacokinetic (PK) study: Johns Hopkins Site Only

The following plasma sampling times are required for day 1 for six patients in the Safety Run-In phase: pretreatment prior to Cycle 1 Day 1 and then Cycle 1 Day 14 pretreatment, 30, 60 minutes, and 1.5, 2, 3, 4, 6, 8, and 24 hours. A 7-hour sample may be done instead of the 8-hour sample if necessary. The analytical method development/implementation and pharmacokinetic analysis will be performed by Dr. Michelle Rudek of Johns Hopkins University for this study. The time of the enzalutamide and galunisertib doses, when the last meal was consumed, and the sample collection times will be recorded. Patients will be instructed to take the pills on an empty stomach (one hour before food or 2 hours after food).

9.6.1 Blood sample collection and preparation

Blood will be obtained from a peripheral vein at baseline.
Instructions for collection and shipment of samples:

- Collect 4 mL whole blood in a green top Vacutainer tube containing sodium heparin anticoagulant
- Centrifuge the sample to 1,000 x g for 10 minutes at 4°C within 30 minutes of collection
- Samples will be transferred to 3.6 mL Nunc internal thread round bottom cryotubes (NUNC 366524) with bar-coded labels attached. Samples will be immediately stored at -70°C or below until transfer to central PK lab on dry ice.

Extra plasma samples will be frozen at -80^0C, and saved for possible future studies.

All specimens should be correctly labeled with patient initials, study-specific subject ID number, protocol number, and date of collection. NOTE: In the event that baseline specimens are available but the patient is not successfully registered to the protocol, do not submit the patient's specimens. Specimens should be shipped on dry ice and must be shipped for express/overnight delivery.

Address shipments and any question regarding specimen processing to:

Analytical Pharmacology Core Laboratory
Attn: Galunisertib/Enzalutamide Study J1557
1650 Orleans St., CRB1 Rm 184
Baltimore, MD 21231
Phone: 410-502-7192 or 410-955-1129
Fax: 410-502-0895

10. Study Calendar

Required Studies[1, 20]	Pre-study	R	C1 D1	C1 D14 or D15	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12 and beyond[17]	End of Treatment/ Progression	30-day FU[18]	Long FU
Visit Windows (days)[2]	-21 to -7	-7 to 0	± 3		± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3		± 7	± 28
ELIGIBILITY																		
Inclusion/exclusion review	X																	
Informed consent	X																	
Randomization		X																
Enzalutamide dispensing			X		X	X	X	X	X	X	X	X	X	X	X			
LY2157299 dispensing			X		X	X	X	X	X	X	X	X	X	X	X			
PHYSICAL																		
Medical and Cancer History	X																	
Physical Exam[5]	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	
Vital Signs, Weight, and ECOG[3]	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	
Disease Assessment																		X[4]
AE/Toxicity Notation			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Concurrent meds	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	
LABORATORY																		
Hematology, Chemistry[6,9]	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	
PT, PTT[7, 9]	X				X													
Troponin I, BNP, hsCRP, Cystatin C[9, 19 post screening, cardiac level labs are for arm 1 only]	X		X		X												X	
Serum testosterone	X																	
TSH, ACTH[8,9]			X				X			X			X		X[17]		X	

Required Studies[1, 20]	Pre-study	R	C1 D1	C1 D14 or D15	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12 and beyond[17]	End of Treatment/ Progression	30-day FU[18]	Long FU
PSA[9]	X		X		X	X	X			X			X		X[17]	X		
Urinalysis[9]	X																	
ECG	X								X									
ECHO with Doppler[10] post screening, ECHOs are for arm 1 only	X					X						X			X		X	
CORRELATIVE SAMPLES																		
AR-V7 Liquid Biopsy[11]		X					X									X		
TGFβ [12]			X		X		X									X		
Whole Blood (PBMC)[13]			X		X		X									X		
Serum and Plasma Samples[14]			X		X		X									X		
PK Sampling – JHU only[15]			X	X														
Optional Tumor Biopsy	X						X											
X-RAYS AND SCANS																		
Radiologic Evaluation/ RECIST 1.1[16]	X						X			X			X		X[17]	X		

[1] **All studies will be scheduled for day 1 of the cycle unless otherwise noted.**

[2] Longer delays to be approved by the Principal Investigator and study Sponsor.

[3] Vital signs include temperature, blood pressure, respiration rate, heart rate, pain score, and ECOG – Eastern Cooperative Oncology Group performance status. Also height is collected pre-study only study.

[4] Disease assessment will be done after completion of the trial, by phone, every six months for three years, annually thereafter. Gather date of death, date of radiographic and/or PSA progression, start and stop dates for other therapies.

[5] After screening, physical exams may be limited as indicated by symptoms.

[6] Clinical Hematology: CBC with differential ANC, ALC, AEC, and platelet count; Serum Chemistry: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, ALT, AST, alkaline phosphatase, total bilirubin, total protein, albumin, calcium.

[7] If enzalutamide is co-administered with warfarin (CYP2C9 substrate), conduct additional INR monitoring

[8] TSH (with reflex to free T3 and free T4 if abnormal), adenocorticotropic hormone (ACTH) collected in the morning (with reflex to cortisol if abnormal) every 3 cycles.

[9] Labs may be collected within a window of up to three days prior to scheduled visit/dosing.

[10]ECHO should be performed at (baseline-both arms, **post screening-for arm 1 only)**: after Cycle 2, every six months thereafter, and at the Off-Study Visit. If there were no clinically significant cardiac findings at the last cardiac assessment conducted within the last 30 days and the patient has started another treatment, the 30-day follow-up visit ECHO will not be performed. If the patient has clinically significant cardiac findings at the 30-day follow-up visit, ECHO will be repeated every 2 months for 6 months. If there are no clinically significant cardiac findings at the 30-day follow-up visit, a repeat ECHO will be performed within 6 months of the last echocardiogram. Major abnormalities documented by ECHO with Doppler including moderate or severe heart valve function defect including moderate or severe valve stenosis or regurgitation, left ventricular ejection fraction <50%, have septal aneurysm or other heart aneurysm, any aneurysm of the major vessels or any conditions predisposing to aneurysm of the ascending aorta or aortic stress, patent foramen ovale, or bicuspid aortic valve will be excluded from the trial. There has been more data in humans and the investigator's brochure has been updated and does not require frequent testing past baseline.

[11] Approximately 30mL of whole blood to be processed for liquid biopsies are to be taken. In select patients, it may be feasible to interrogate AR mutations using excess cDNA samples. If there is adequate CTC-enriched cDNA remaining after qRT-PCR analysis for AR-V7, cDNA will be subjected to AR mutation analysis, with a focus on known gain-of-function mutations in the ligand-binding domain. See protocol section 9.1.

[12] Approximately 10mL of whole blood to be processed for TGFβ samples are to be taken. See protocol section 9.2.

[13] Approximately 32mL of whole blood to be processed for PBMCs will be drawn. See protocol section 9.3.

[14] Approximately 10mL of whole blood will be collected for serum isolation for immune monitoring will be drawn. Approximately 10mL of whole blood will be collected for plasma isolation will be collected for plasma isolation will be drawn. See protocol section 9.4.

[15] PK sampling will be collected on the first 6 patients at Johns Hopkins Site at the following time points: pretreatment prior to Cycle 1 Day 1, Cycle 1 D14 pretreatment, 30-, 60-minute, and 1.5-, 2-, 3-, 4-, 6-, 8-, and 24-hour. A 7-hour sample may be done instead of the 8-hour sample if necessary. Approximately 45 mL of whole blood will be collected during PK sampling. See protocol section 9.7.

[16] CT scan (chest/abdomen/pelvis or MRI if patient has contrast allergy) and bone scan to be assessed at baseline and every 12 weeks (+/- 1 week), and at End of Treatment/Progression Visit. Scans should include an assessment of the large vessels of the heart.

[17] Designated tests will be performed at C12 and every subsequent cycle, except PSA, TSH, ACTH, and radiologic evaluation which will be performed every 3 cycles.

[18] 30 days after their last dose of study drug or within seven days prior to initiation of a new anti-cancer treatment, whichever comes first.

[19] Laboratory markers for cardiac damage (troponin-I, B-type natriuretic peptide, cystatin C, and high-sensitivity C-reactive protein). **(Post screening timepoints are for Arm 1 only)**.Documented major electrocardiogram (ECG) abnormalities (not responding to medical treatment) are not allowed on study. Mean QT interval corrected for heart rate (QTc) ≥470 msec calculated from one baseline ECG using Bazett's Correction and confirmed with the 2 additional baseline ECGs are not allowed on study.

[20] In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for inperson clinical trial visits or portions of clinical trial visits where determined to be appropriate and where determined by the investigator not to increase the participants risks. Prior to initiating telemedicine for study visits the study team will explain to the participant, what a telemedicine visit entails and confirm that the study participant is in agreement and able to proceed with this method. acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

11. Data Reporting/Regulatory Requirements

Adverse event guidelines and instructions for AE reporting can be found in **Section 7** (Adverse Events: Reporting Requirements).

11.1. Data Management

All information will be collected on study-specific case report forms (CRFs) by study staff. These data will be reviewed for completeness and accuracy by the Principal Investigator at each site.

Protocol Chair

The Protocol Chair is responsible for performing the following tasks:

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
- Assuring that all participating institutions are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.
- Reviewing and ensuring reporting of SAEs.
- Reviewing data from all sites.

Coordinating Center

The Coordinating Center is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
- Managing central patient registration.
- Collecting and compiling data from each site.
- Establishing procedures for documentation, reporting, and submitting of AEs and SAEs to the Protocol Chair, and all applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.

Participating Sites

Participating sites are responsible for performing the following tasks:

- Following the protocol as written, and the guidelines of Good Clinical Practice (GCP).
- Submitting data to the Coordinating Center.
- Registering all patients with the Coordinating Center by submitting the patient registration form and signed informed consent promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.
- Maintaining regulatory binders on site and providing copies of all required documents to the Coordinating Center.
- Collecting and submitting data according to the schedule specified by the protocol.

11.2. Safety Meetings

Scheduled meetings will take place weekly, biweekly or monthly depending on patient enrollement, and will include the protocol principal investigator, study coordinator(s), data manager(s), sub-investigators (as appropriate), collaborators (as appropriate), and biostatisticians (as appropriate) involved with the conduct of the protocol. During these meetings matters related to the following will be discussed: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for objectives.

11.3. Monitoring

This is a DSMP Level "High Risk" study under the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center (SKCCC) Data Safety Monitoring Plan (DSMP, 02/21/2019). Eligibility for all sites will be monitored by the JHU SKCCC CRO. Data monitoring of this protocol will occur on a regular basis with the frequency dependent on the rate of subject accrual and the progress of the study. Additional data and safety monitoring oversight will also be performed by the SKCCC Safety Monitoring Committee (SMC - as defined in the DSMP). The protocol will be monitored internally by the Principal Investigator at each site. External monitoring will occur according to the following guidelines:

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring. Participating site(s): Data required by the study will be collected in Case Report Forms provided by the Coordinating Center at SKCCC. The participating site will be required to submit a paper Eligibility Checklist along with supporting source documents at the time of patient registration. All other data will be collected on the electronic case report forms (eCRFs) in CRMS, provided by SKCCC. Site staff access to CRMS will be initiated at the time of the site activation.

Case Report Form Completion
The eCRFs will be completed within 2 weeks of the patient coming to the clinic and all relevant supporting documentation such as scans, progress notes, blood work, pathology reports, etc., will be submitted to the Study Manager, Serina King, at sking18@jhmi.edu. All patient names or other identifying information will be removed prior to being sent to the Coordinating Center (SKCCC) or non- redacted source documents can be sent via a password -protected/ secured document transfer based on each institution's guidelines.

-

Authorized representatives of the Coordinating Center may visit the satellite sites to perform audits or inspections, including source data verification. The purpose of these audits or inspections is to systematically and independently examine all trial-related activities and documents to determine whether these activities were conducted and data were recorded,

analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and any applicable regulatory requirements.

Dr. Channing Paller will be holding the IND for this study. She will comply with all regulated reporting requirements to the FDA.

12. Statistical Considerations

Study design: The proposed study is a randomized, Phase II study of radiologic progression free survival (rPFS) comparing combination TGF-β receptor I kinase inhibitor (LY2157299) plus enzalutamide to single agent enzalutamide in patients with mCRPC with elevated CRP (>1.0 mg/dL). While LY2157299 has been combined with other drugs in previous studies, this is the first time that it will be combined with enzalutamide. The first part of this study will therefore be a run-in phase where patients will be observed for grade 3-4 adverse events through cycle 1. In the second portion of the study, patients will be randomized 3:2 to LY2157299 + enzalutamide (Arm 1) or enzalutamide alone (Arm 2). Information from the randomized control group in this study will be augmented with information obtained in a recent study of similar patients treated with enzalutamide alone from the cancer center at Johns Hopkins.[7] We will use a method originally proposed by Pocock[60] to incorporate the historical information in the analysis of the current study according to how well it matches the contemporaneous controls.

Primary Objective

To compare radiographic progression-free survival (rPFS) in chemotherapy-naïve, post-abiraterone-treated metastatic castration resistant prostate cancer patients treated with enzalutamide and LY2157299 versus enzalutamide alone.

Secondary Objectives

1. To compare the PSA response rate and duration of benefit of chemotherapy-naïve, post-abiraterone metastatic castration resistant prostate cancer patients treated with a combination of enzalutamide and LY2157299 vs. enzalutamide alone.

2. To summarize and compare overall survival in chemotherapy-naïve, post-abiraterone metastatic castration resistant prostate cancer patients treated with a combination of enzalutamide and LY2157299 vs. enzalutamide alone.

3. To assess safety and tolerability the combination of enzalutamide and LY2157299 as measured by CTCAE v 4.03 criteria.

4. To assess the pharmacokinetics of LY2157299 in the presence of enzalutamide during the safety run-in phase.

Exploratory Objectives: Correlates

1. To measure correlates that may, individually or as a group, be predictive of response or lack of response to the combination of enzalutamide and LY2157299: AR-V7 splice variant status, C-reactive protein (CRP), TGF-β, and SMAD2 in peripheral blood.

2. To quantify and compare the function of the peripheral $CD8^+$ and $CD4^+$ cells from patients in each of the two treatment groups.

3. To evaluate and compare the serum cytokine profile from patients in each of the two treatment groups (including but not limited to: IL-1,IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, TNF-α, and IFNγ).
4. To evaluate and compare the generation of novel antibody specificities to prostate-associated antigens in the serum of patients in each group, after the receipt of protocol therapy.
5. To evaluate markers of epithelial to mesenchymal transition in tumor biopsies pre and post therapy: E-cadherin (CDH1), N-cadherin (CDH2), TGF-β1, vimentin (VIM), ZEB1, and SMAD2.

Measurement of effect definitions
Radiological progression-free survival is defined per PCWG2 as the time from start of treatment until progression (based on local assessment) or death from any cause. Progression is defined as the occurrence of either tumor progression in soft tissue according to RECIST 1 .1 or progression in bone.

For bone scans, progression at the first assessment (week 13 $\pm$1) is defined as at least 2 new lesions compared to baseline, confirmed by a subsequent scan 6 or more weeks later showing at least 2 additional new bone lesions as compared to the first assessment scan as per PCWG2. For subsequent evaluations, according to PCWG2, bone progression is defined as at least 2 new lesions as compared to the previous scan, should be confirmed by a subsequent scan 6 or more weeks later. Progression on CT scan at first assessment are confirmed by a subsequent scan 6 or more weeks later as per PCWG2. Progression on CT scans (soft tissues) and progression on bone scans are analyzed separately.

PSA response is defined as a greater than or equal to a 30% decrease in PSA from the baseline without clinical or radiologic evidence of progression according to the Response Evaluation Criteria in Solid Tumors (RECIST).

Overall survival is defined as time from randomization to death, due to any cause.

Safety run-in: LY2157299 150 mg PO BID plus enzalutamide 160 mg PO will be tested in 6 patients in the safety run-in portion of the study. Previous experience with LY2157299 combined with gemcitabine or lomustine demonstrated that the incidence of grade 3-4 toxicities is acceptable, (4-11% with lomustine and 1-36% with gemcitabine). The primary DLTs of concern for this safety monitoring will be grade 3-4 of the types listed in **Section 5.7**. They will be monitored continuously for six patients in the run-in phase through cycle 1 of treatment. In the safety run-in, no more than one patient will be accrued in any seven day period.

We will use a probability-based decision rule to decide if the combination therapy is safe enough to continue to the randomized study. The prior for this decision rule is Beta(0.5, 0.5) to reflect the lack of experience with this strategy. We will declare the combination safe for the randomized portion of the study if, after treating six patients, the upper bound of a 95% credible interval for safety is below 50%. The run-in will be considered unacceptable if the lower bound of the 95% credible interval is greater than 50%. DLTs are defined in **Section 5.7.** If three or more of the first six patients experience a DLT with the combination therapy

during the run-in, the study will be discontinued. Overall, the study will continue without pause to the randomized phase if none or one of the first 6 patients experience DLT in the run-in. If two patients experience a DLT, the study will be paused for a review and may or may not continue. If the protocol is resumed after review, the dose of LY2157299 will be lowered to 80 mg PO BID. Subjects not completing the run-in safety phase for reasons other than dose-limiting toxicity will be replaced.

The following table shows the 95% credible intervals for the underlying probability of toxicity, based on different numbers of toxicities out of six patients, using a Beta(0.5, 0.5) prior. As an example, if 1 out of 6 patients experience toxicity, we will be confident that toxicity is below 50%.

Toxicity	95% Credible Interval	Safe
If 0 out of 6 pts:	(0.0, 26.4)	Yes
If 1 out of 6 pts:	(0.1, 49.5)	Yes
If 2 out of 6 pts:	(5.4, 68.0)	Uncertain
If 3 out of 6 pts:	(16.7, 83.3)	Uncertain
If 4 out of 6 pts:	(32.0, 94.6)	Uncertain
If 5 out of 6 pts:	(50.5, 99.9)	No
If 6 out of 6 pts:	(73.6, 100.0)	No

Analysis plans:
The primary analysis will compare mean rPFS based on the method by Pocock.[60] The method assumes survival times will be exponentially distributed, a reasonable assumption for studies in advanced cancer. Mean survival is estimated as the sum of the exposure times divided by the number of observed events. Assuming exponential survival, the ratio of the means equals the reciprocal of the hazard ratio. The logarithm of the ratio of the two treatment arms' means can be considered as normally distributed and normal theory applied. The design applies Bayesian methodology, first to combine the historical and randomized control estimators of mean survival, and then to compare this posterior distribution for the controls to the posterior for the treated group. The primary analysis will use a z test of the log of the ratio of these posterior means with a two-sided alpha of 0.05. We will also compute the posterior probability interval for the ratio of the means and the posterior probability that the ratio of mean survival ($\bar{x}_t / \bar{x}_c$) is greater than 1.0 (i.e. that the hazard ratio, λ_t / λ_c, is less than 1.0).

Randomization:
We evaluated the effect of bias on the optimal number of patients that should be randomized to the control group (enzalutamide alone). The optimal number is determined such that the variance in the difference of the posterior means between the treatment group (LY2157299 + enzalutamide) and the combined control information in the final analysis would be minimized.[53] Here, bias is in terms of the difference between the randomized control patients (enzalutamide alone) and the historical information.[7] Given a total of 60 patients in the historical control group and assuming the current number of observed events 18 in this cohort, if bias is 5% (approximately a difference of two weeks), then we should randomize 22 patients to control. If the bias is 10%, we should randomize 23 to control; and if the bias is 15%, then

we should randomize 24 to controls. We have therefore chosen to randomize 24 patients to the control treatment and 36 patients to the arm with LY2157299 (i.e., 3:2 randomization).

Sample size:
Table 1 shows the power of the study based on 1,000 simulations with a total sample size of 60 patients and the inclusion of the historical control information from this institution, Antonarakis.[52] The expected mean rPFS for enzalutamide alone, calculated from the Antonarakis[7] study, was 35.7 weeks corresponding to an event rate, λ_h, of 0.028 per week. We assume that the accrual rate to the study will be about 30 patients per year, with an extra 12 months of follow-up after the last patient enrolls. Simulations in Table 1 are shown for a range of hazard ratios and levels of bias in the historical mean survival times. The simulations show that by including the historical information, along with the concurrent randomized controls, the power is 81% (with bias = 15%) to detect a doubling of mean rPFS (35.7 weeks to 71.4 weeks). We have included power for the Log-rank test in Table 1 for comparison. The power for the Log-rank test will be increased to 75% for a hazard ratio of 0.05.

Table 1. Power based on 1,000 simulations with 60 patients (3:2 randomization), and the Pocock method for inclusion of Antonarakis[7] data. Two-sided alpha level of 0.10.

Simulated mean rPFS Control vs Trt (weeks)	Hazard Ratio λ_t/λ_c	Historical mean (weeks) rPFS	Prob H0 rejected (bias 10%)	Prob H0 rejected (bias 15%)	Prob H0 rejected (bias 34%)	Power Log-rank
35.7 vs 89.3	0.40	35.7	98.8%	98.2%	96.3%	91.3%
35.7 vs 85.7	0.42	35.7	98.3%	97.6%	96.3%	92.0%
35.7 vs 82.2	0.43	35.7	96.8%	96.1%	94.0%	88.0%
35.7 vs 78.6	0.45	35.7	94.8%	94.1%	90.8%	84.7%
35.7 vs 75.0	0.48	35.7	92.1%	92.3%	89.1%	82.4%
35.7 vs 71.4	0.50	35.7	89.6%	90.0%	81.9%	75.2%
35.7 vs 64.3	0.56	35.7	78.9%	79.4%	75.4%	67.4%
35.7 vs 53.6	0.67	35.7	51.5%	48.1%	47.8%	44.0%
35.7 vs 46.5	0.77	35.7	27.6%	27.1%	25.3%	24.7%
35.7 vs 35.7 (null scenario)	1.0	35.7	7.6%	7.2%	9.7%	12.2%

Analysis plans for correlative studies:
The randomized design will allow us to determine if there is a difference in plasma TGF-β, CRP, and PSA levels between patients treated with the combination of enzalutamide+LY2157299 and the control arm treated with enzalutamide alone. Several analyses will be performed to assess the hypothesis that treatment with enzalutamide+LY2157299 results in TGF-β pathway inhibition and that on-target effects are associated with improved rPFS or increased probability of PSA response (>30% decline). Initially, raw data values and log fold changes of markers in each study arm will be visualized using boxplots with data points superimposed. The ratios of week 12 to baseline measures will be the basis for this comparison. We expect that fold changes in the combination arm

TGF-β will be down modulated, and the other three markers may have varying levels of down regulation.

Two group comparisons of the log fold change of tumor markers will be based on t-tests of log transformed data. We assume that the coefficient of variation in the fold change in each treatment group is at most 40%. If we are not able to get paired biopsies on all men, the fold difference we can detect with 80% power get larger. With a sample size of 48 men (10% dropout), power will be around 84% to detect a 1.4 fold difference in means. With 36 men providing paired samples, we will have 80% power to detect 1.45 fold difference. With 32 and 28 paired samples, we will have 80% power to detect 1.49 and 1.53 fold differences, respectively. The sample sizes have assumed roughly equal numbers of men providing paired samples in each treatment group, which should be pretty close to what we find, even though the randomization is 3:2.

We will test the significance of the association between baseline TGF-β (>4.9 ng/mL) and rPFS with a Cox regression model. The one month change in TGF-β (from blood) will be correlated with rPFS using a landmark Cox proportional hazards model. Joint modelling of the longitudinal TGF-β data and the time to event data may also be used.[61] We will adjust for the AR-V7 splice variant and possibly assess if the effect of TGF-β is different depending on the presence or absence of AR-V7. If there is evidence that the effect of TGF-β is different depending on AR-V7, a stratified Cox regression model will be used to obtain estimates specific for AR-V7 variant status by including stratum-covariate interaction effects.[62,63] We expect around 40% of patients will exhibit the AR-V7 splice variant at baseline.

We will also examine the fold change in $CD4^+$ and $CD8^+$ T cell counts, IFNγ, and various cytokine levels and their associations with treatment. The cytokines will include (but are not limited to) IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, TNF-α, IFNγ, and CRP. A within-patient median normalization will be applied before analysis. For the cytokines, we will apply the multivariate O'Brien OLS statistic[64] to compare fold change (on the log scale) between treatment groups. The primary analyses will evaluate the ratios to baseline of levels measured taken day 1 of each of the first four cycles and every third cycle thereafter in both arms of the study. We will also examine the ratio of levels at disease progression, relative to baseline, as more of a hypothesis generating analysis. We expect that treating men with mCRPC with LY2175299 will lead to decreasing levels of the pro-inflammatory cytokines. As long as the coefficient of variation is 40% or less, we will have at least 80% power to detect a fold change of 25% or greater. Based on the information in Zabransky et al.,[48] we expect the coefficients of variation for the cytokines to range from 5 to 27%. Similar considerations will apply to measurement of pSMAD (as a fraction of total SMAD, tSMAD) and levels of antigen-specific IgG that are measured from peripheral blood as documented in the study calendar.

The pharmacokinetics variables for LY2157299 will be tabulated and descriptive statistics (e.g., geometric means and coefficients of variation) calculated for each dose level. This will be compared to historical controls.[65]

13. References

1. Fitzsimmons D, Johnson CD, George S, et al. Development of a disease specific quality of life (QoL) questionnaire module to supplement the EORTC core cancer QoL questionnaire, the QLQ-C30 in patients with pancreatic cancer. EORTC Study Group on Quality of Life. European journal of cancer 1999;35:939-41.
2. Wang X, Yin L, Rao P, et al. Targeted treatment of prostate cancer. Journal of cellular biochemistry 2007;102:571-9.
3. Beer TM, Armstrong AJ, Sternberg CN, et al. Enzalutamide in men with chemotherapy-naive metastatic prostate cancer (mCRPC): Results of phase III PREVAIL study. J Clin Oncol 32, 2014 (suppl 4; abstr LBA1).
4. Cheng HH, Nadal R, Gulati R, et al. The effect of prior abiraterone use on the activity of enzalutamide in men with metastatic castration resistant prostate cancer. J Clin Oncol 32, 2014 (suppl 4; abstr 18).
5. Bianchini D, Lorente D, Rodriguez-Vida A, et al. Antitumour activity of enzalutamide (MDV3100) in patients with metastatic castration-resistant prostate cancer (CRPC) pre-treated with docetaxel and abiraterone. European journal of cancer 2014;50:78-84.
6. Schrader AJ, Boegemann M, Ohlmann CH, et al. Enzalutamide in castration-resistant prostate cancer patients progressing after docetaxel and abiraterone. European urology 2014;65:30-6.
7. Suzman DL, Luber B, Schweizer MT, Nadal R, Antonarakis ES. Clinical activity of enzalutamide versus docetaxel in men with castration-resistant prostate cancer progressing after abiraterone. The Prostate 2014;74:1278-85.
8. Li P, Yang R, Gao WQ. Contributions of epithelial-mesenchymal transition and cancer stem cells to the development of castration resistance of prostate cancer. Molecular cancer 2014;13:55.
9. Derynck R, Akhurst RJ. Differentiation plasticity regulated by TGF-beta family proteins in development and disease. Nature cell biology 2007;9:1000-4.
10. Wahl SM, Hunt DA, Wong HL, et al. Transforming growth factor-beta is a potent immunosuppressive agent that inhibits IL-1-dependent lymphocyte proliferation. Journal of immunology 1988;140:3026-32.
11. Kirkbride KC, Blobe GC. Inhibiting the TGF-beta signalling pathway as a means of cancer immunotherapy. Expert opinion on biological therapy 2003;3:251-61.
12. de Bono JS, Logothetis CJ, Molina A, et al. Abiraterone and increased survival in metastatic prostate cancer. The New England journal of medicine 2011;364:1995-2005.
13. Ryan CJ, Smith MR, de Bono JS, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. The New England journal of medicine 2013;368:138-48.
14. Scher HI, Fizazi K, Saad F, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. The New England journal of medicine 2012;367:1187-97.
15. Schweizer MT, Antonarakis ES. Abiraterone and other novel androgen-directed strategies for the treatment of prostate cancer: a new era of hormonal therapies is born. Therapeutic advances in urology 2012;4:167-78.
16. Thadani-Mulero M, Portella L, Sun S, et al. Androgen receptor splice variants determine taxane sensitivity in prostate cancer. Cancer research 2014;74:2270-82.

17. Li Y, Chan SC, Brand LJ, Hwang TH, Silverstein KA, Dehm SM. Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. Cancer research 2013;73:483-9.
18. Pu H, Collazo J, Jones E, et al. Dysfunctional transforming growth factor-beta receptor II accelerates prostate tumorigenesis in the TRAMP mouse model. Cancer research 2009;69:7366-74.
19. Liu GL, Yang HJ, Liu T, Lin YZ. Expression and significance of E-cadherin, N-cadherin, transforming growth factor-beta1 and Twist in prostate cancer. Asian Pacific journal of tropical medicine 2014;7:76-82.
20. Sporn MB, Roberts AB. Autocrine growth factors and cancer. Nature 1985;313:745-7.
21. Jakowlew SB. Transforming growth factor-beta in cancer and metastasis. Cancer metastasis reviews 2006;25:435-57.
22. Yingling JM, Blanchard KL, Sawyer JS. Development of TGF-beta signalling inhibitors for cancer therapy. Nature reviews Drug discovery 2004;3:1011-22.
23. Eastham JA, Truong LD, Rogers E, et al. Transforming growth factor-beta 1: comparative immunohistochemical localization in human primary and metastatic prostate cancer. Laboratory investigation; a journal of technical methods and pathology 1995;73:628-35.
24. Steiner MS, Barrack ER. Transforming growth factor-beta 1 overproduction in prostate cancer: effects on growth in vivo and in vitro. Molecular endocrinology 1992;6:15-25.
25. Kyprianou N, Isaacs JT. Expression of transforming growth factor-beta in the rat ventral prostate during castration-induced programmed cell death. Molecular endocrinology 1989;3:1515-22.
26. Armstrong AJ, Marengo MS, Oltean S, et al. Circulating tumor cells from patients with advanced prostate and breast cancer display both epithelial and mesenchymal markers. Molecular cancer research : MCR 2011;9:997-1007.
27. Yoon G, Kim JY, Choi YK, Won YS, Lim IK. Direct activation of TGF-beta1 transcription by androgen and androgen receptor complex in Huh7 human hepatoma cells and its tumor in nude mice. Journal of cellular biochemistry 2006;97:393-411.
28. Hu R, Dunn TA, Wei S, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer research 2009;69:16-22.
29. Giannelli G, Villa E, Lahn M. Transforming Growth Factor-beta as a Therapeutic Target in Hepatocellular Carcinoma. Cancer research 2014;74:1890-4.
30. Calvo-Aller E, Baselga J, Glatt S, et al. First human dose escalation study in patients with metastatic malignancies to determine safety and pharmacokinetics of LY2157299, a small molecule inhibitor of the transforming growth factor-beta receptor I kinase. J Clin Oncol (Meeting Abstracts) May 2008 vol. 26 no. 15_suppl 14554.
31. Rodon J, Carducci M, Sepulveda-Sanchez J, et al. Integrated data review of the first-in-human dose study evaluating safety, phramacokinetics, and pharmacodynamics of the oral transforming growth factor -beta receptor I kinase inhibitor, LY2157299 monohydrate. J Clin Oncol 31, 2013 (suppl; abstr 2016).
32. Ahnert J, Baselga J, Calvo E, and Carducci M, Sepulveda-Sanchez J, et al. First human dose (FHD) study of the oral transforming growth factor -beta receptor I kinase

inhibitor, LY2157299 in patients with treatment-refractory malignant glioma. J Clin Oncol 29, 2011 (suppl; abstr 3011).
33. Carpentier A, Brandes A, Kesari S, et al. Safety interim data from a 3-arm phase 2 study evaluating safety and pharmacokinetics of the oral transforming growth factor-beta receptor I kinase inhibitor LY2157299 monohydrate in patients with glioblastoma at first progression. J Clin Oncol 31, 2013 (suppl; abstr 2061).
34. Faivre S, Santoro A, Kelley R, et al. Growth Factor-beta (TGF-β) Receptor I Kinase Inhibitor, LY2157299 Monohydrate, in Patients With Advanced Hepatocellular Carcinoma (HCC). 2014 Gastrointestinal Cancers Symposium, Jan 16-18, 2014; San Francisco.
35. Liu FT, Patterson RJ, Wang JL. Intracellular functions of galectins. Biochimica et biophysica acta 2002;1572:263-73.
36. Beer TM, Schellhammer PF, Corman JM, et al. Quality of life after sipuleucel-T therapy: results from a randomized, double-blind study in patients with androgen-dependent prostate cancer. Urology 2013;82:410-5.
37. Hu R, Lu C, Mostaghel EA, et al. Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. Cancer research 2012;72:3457-62.
38. Hu R, Denmeade SR, Luo J. Molecular processes leading to aberrant androgen receptor signaling and castration resistance in prostate cancer. Expert review of endocrinology & metabolism 2010;5:753-64.
39. Antonarakis ES, Lu C, Wang H, et al. AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer. The New England Journal of Medicine, Sep 3, 2014 [Epub ahead of print].
40. Prins RC, Rademacher BL, Mongoue-Tchokote S, et al. C-reactive protein as an adverse prognostic marker for men with castration-resistant prostate cancer (CRPC): confirmatory results. Urologic oncology 2012;30:33-7.
41. Baselga J, Rothenberg ML, Tabernero J, et al. TGF-beta signalling-related markers in cancer patients with bone metastasis. Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals 2008;13:217-36.
42. Ito M, Saito K, Yasuda Y, et al. Prognostic impact of C-reactive protein for determining overall survival of patients with castration-resistant prostate cancer treated with docetaxel. Urology 2011;78:1131-5.
43. Zeng M, Smith AJ, Wietgrefe SW, et al. Cumulative mechanisms of lymphoid tissue fibrosis and T cell depletion in HIV-1 and SIV infections. The Journal of clinical investigation 2011;121:998-1008.
44. Sheikh NA, Petrylak D, Kantoff PW, et al. Sipuleucel-T immune parameters correlate with survival: an analysis of the randomized phase 3 clinical trials in men with castration-resistant prostate cancer. Cancer immunology, immunotherapy : CII 2013;62:137-47.
45. De Marzo AM, Platz EA, Sutcliffe S, et al. Inflammation in prostate carcinogenesis. Nature reviews Cancer 2007;7:256-69.
46. Dluzniewski PJ, Wang MH, Zheng SL, et al. Variation in IL10 and other genes involved in the immune response and in oxidation and prostate cancer recurrence. Cancer epidemiology, biomarkers & prevention : a publication of the American

Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2012;21:1774-82.
47. Kattan MW, Shariat SF, Andrews B, et al. The addition of interleukin-6 soluble receptor and transforming growth factor beta1 improves a preoperative nomogram for predicting biochemical progression in patients with clinically localized prostate cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2003;21:3573-9.
48. Zabransky DJ, Smith HA, Thoburn CJ, et al. Lenalidomide modulates IL-8 and anti-prostate antibody levels in men with biochemically recurrent prostate cancer. The Prostate 2012;72:487-98.
49. Maricque BB, Eickhoff JC, McNeel DG. Antibody responses to prostate-associated antigens in patients with prostatitis and prostate cancer. The Prostate 2011;71:134-46.
50. Sreekumar A, Laxman B, Rhodes DR, et al. Humoral immune response to alpha-methylacyl-CoA racemase and prostate cancer. Journal of the National Cancer Institute 2004;96:834-43.
51. Wang X, Yu J, Sreekumar A, et al. Autoantibody signatures in prostate cancer. The New England journal of medicine 2005;353:1224-35.
52. Basch EM, Reeve BB, Mitchell SA, et al. Electronic toxicity monitoring and patient-reported outcomes. Cancer journal 2011;17:231-4.
53. Highlights of Prescribing Information for XTANDI, http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/203415lbl.pdf, accessed 7/27/2014.
54. XTANDI Package Insert. https://www.astellas.us/docs/us/12A005-ENZ-WPI.pdf Accessed Feb 23.
55. Transforming Growth Factor-Beta Receptor I Kinase Inhibitor LY2157299 Monohydrate Investigator's Brochure, Lilly, October 15, 2013.
56. Horsted F, West J, Grainge MJ. Risk of venous thromboembolism in patients with cancer: a systematic review and meta-analysis. PLoS medicine 2012;9:e1001275.
57. Scher HI, Halabi S, Tannock I, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2008;26:1148-59.
58. Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression and cancer progression. Nature genetics 2001;29:117-29.
59. McCarthy, EF. CT-guided needle biposies of bone and soft tissue tumors: a pathologist's perspective. *Skeletal Radiol.* 2007:36:181-2.
60. Pocock SJ. The combination of randomized and historical controls in clinical trials. Journal of chronic diseases 1976;29:175-88.
61. Song X, Davidian M, Tsiatis AA. A semiparametric likelihood approach to joint modeling of longitudinal and time-to-event data. Biometrics 2002;58:742-53.
62. Eide GE, Omenaas E, Gulsvik A. The semi-proportional hazards model revisited: practical reparametrizations. Statistics in medicine 1996;15:1771-7.
63. Thall PF, Lachin JM. Assessment of stratum-covariate interactions in Cox's proportional hazards regression model. Statistics in medicine 1986;5:73-83.

64. O'Brien PC. Procedures for comparing samples with multiple endpoints. Biometrics 1984;40:1079-87.
65. Rodon J, Carducci M, Sepulveda-Sanchez JM, et al. Pharmacokinetic, pharmacodynamic and biomarker evaluation of transforming growth factor-beta receptor I kinase inhibitor, galunisertib, in phase 1 study in patients with advanced cancer. Investigational new drugs 2015;33:357-70.

Appendices

Appendix A: Performance Status Criteria

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

Appendix B: Concomitant Medications to Use with Caution

Per Enzalutamide package insert:

1. Avoid strong CYP2C8 inhibitors (see examples below), as they can increase the plasma exposure to Enzalutamide: (If co-administration of a strong CYP2C8 inhibitor is necessary, reduce the dose enzalutamide as described in Section 6.2.)

   gemfibrozil

2. Avoid strong or moderate CYP3A4 or CYP2C8 inducers as they can alter the plasma exposure to enzalutamide (see examples below):

barbiturates	etravirine	oxcarbazepine	rifampin
bosentan	efavirenz	phenobarbital	rifapentine
carbamazepine	modafinil	phenytoin	St. John's Wort
clarithromycin	nafcillin	primidone	ritonavir
dexamethasone	nevirapine	rifabutin	troglitazone

3. Avoid CYP3A4, CYP2C9 and CYP2C19 substrates with narrow therapeutic indices, as enzalutamide may decrease the plasma exposures of these drugs (examples listed below). If enzalutamide is co-administered with warfarin (CYP2C9 substrate), conduct additional INR monitoring.

alfentanil	ergotamine	pimozide	tacrolimus
cyclosporine	fentanyl	sirolimus	warfarin
dihydroergotamine	phenytoin	s-mephenytoin	

Appendix C: Medication Diaries

PATIENT DIARY FOR ENZALUTAMIDE

Name: _____History #_____ Cycle #: _____

- Take enzalutamide tablet once a day (with or without food) and swallowed with water. Do not chew, dissolve, or open the capsules.
- Enzalutamide should be taken at the same time on each day. If you forget a dose, take it as soon as you remember. If it is within 12 hours of your next dose, omit the missed dose. Do not take more than 1 dose in a 24 hour period. If you vomit within 30 minutes of taking your daily dose and you can see the pill, you may repeat the dose.
- The use of any natural/herbal products or other "folk remedies" is discouraged.
- Bring your diary, pill supply, and all pill bottles with you to each clinic visit**.**

Day	Date	Time Taken	Day	Date	Time Taken	Comments
1		AM/PM	**15**		AM/PM	
2		AM/PM	**16**		AM/PM	
3		AM/PM	**17**		AM/PM	
4		AM/PM	**18**		AM/PM	
5		AM/PM	**19**		AM/PM	
6		AM/PM	**20**		AM/PM	
7		AM/PM	**21**		AM/PM	
8		AM/PM	**22**		AM/PM	
9		AM/PM	**23**		AM/PM	
10		AM/PM	**24**		AM/PM	
11		AM/PM	**25**		AM/PM	
12		AM/PM	**26**		AM/PM	
13		AM/PM	**27**		AM/PM	
14		AM/PM	**28**		AM/PM	

Completed By _____ Date: _____

RN Review _____ Date: _____

PATIENT DIARY FOR LY2157299

Name: _____History #_____ Cycle #: _____

- Take LY2157299 tablet twice each day and swallowed with water. The tablets are to be swallowed whole.
- LY2157299 should be taken each morning and night at the same time on designated days. If you forget a dose, take it as soon as you remember and then resume the next dose at the scheduled time. If it is within 8 hours of your next dose, omit the missed dose. Do not take more than 2 doses in a 24 hour period. If you vomit within 30 minutes of taking your daily dose and you can see the pill, you may repeat the dose.
- The use of any natural/herbal products or other "folk remedies" is discouraged
- Bring your diary, pill supply, and pill bottles with you to each clinic visit**.**

Day	Date	Time Taken	Number of Pills	Time Taken	Number of Pills	Comments
1		AM		PM		
2		AM		PM		
3		AM		PM		
4		AM		PM		
5		AM		PM		
6		AM		PM		
7		AM		PM		
8		AM		PM		
9		AM		PM		
10		AM		PM		
11		AM		PM		
12		AM		PM		
13		AM		PM		
14		AM		PM		

Completed By _____ Date: _____

RN Review _____ Date: _____

Appendix D: Serious Adverse Event Reporting Form

Please notify IND Sponsor, Channing Paller, MD within 24 hours via Lead Study Coordinator, Serina King (Fax: 410-614-7287, Email: sking18@jhmi.edu and cpaller1@jhmi.edu)
Lilly within 24 hours (Fax:)

<table>
<tr><td>Protocol Title:</td><td colspan="3">Overcoming drug resistance in metastatic castration resistant prostate cancer (mCRPC) with first-in-human novel combination of TGF-β inhibition and enzalutamide: a randomized multi-site phase II study</td></tr>
<tr><td>Protocol Number:</td><td>Signature of PI:</td><td>Principal Investigator:</td><td>Date:</td></tr>
<tr><td>Report Type:
☐ Initial
☐ Follow-up
☐ Final Follow-up
☐ Death
☐ Addendum to:</td><td>Serious Criteria (check all that apply):
☐ Death
☐ Life-threatening
☐ Hospitalization or Elongation of Existing Hospitalization
☐ Persistent or Significant Disability
☐ Congenital Anomaly
☐ Other Important Medical Event
☐ Cancer
☐ Overdose</td><td>Hospital Admission Date:

Hospital Discharge Date:</td><td>Date Event Discovered:</td></tr>
<tr><td colspan="4">Section A: Subject Information</td></tr>
<tr><td colspan="2">Subject ID:</td><td>Subject Initial:</td><td>Subject Gender:
☐ Male
☐ Female</td></tr>
<tr><td colspan="4">Section B: Event Information</td></tr>
<tr><td rowspan="3">Event diagnosis or symptoms:</td><td>Date of First Dose:</td><td colspan="2" rowspan="3">Action taken with the study drug:

☐ None
☐ Interrupted
☐ Discontinued
☐ Delayed</td></tr>
<tr><td>Date of Last Dose prior to Event:</td></tr>
<tr><td>Number of Total Doses:</td></tr>
</table>

Event Onset Date:	Event End Date:

Relationship to:	LY2157299	Underlying Disease
Unrelated	☐	☐
Probably Unrelated	☐	☐
Possible Related	☐	☐
Probably Related	☐	☐
Definitely Related	☐	☐

Section C: Brief Description of the Event:

Section D: Relevant Medical History

Section E: Concomitant Drug (Not related to Serious Adverse Event)

Name of the Drug	Start Date	Stop Date	Route	Dose	Frequency

Section F: Comments

Additional Documents: ☐ Please specify