SUMMARY OF CHANGES
For Protocol Amendment #8 to:

NCI Protocol #: **9012**
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NCI Version Date: 07/01/2016 (last NCI approved version)
Protocol Date: **07/20/2017 (**current version)

#	Section	Page	Change
1.	TitlePage	1	• Updated protocol amendment number and protocol version date • Replaced COH site PI, Twardowski with Pal.
2.	Protocol history	4	• Updated protocol history section

NCI Protocol #: **9012**
Local Protocol #: **12-0109**

TITLE: A Randomized Gene Fusion Stratified Phase 2 Trial Of Abiraterone With Or Without ABT-888 For Patients With Metastatic Castration-Resistant Prostate Cancer

Coordinating Center: **The University of Chicago Phase II Consortium**

Overall Lead PI:
Maha Hussain, M.D. FACP
Genevieve Teuton Professor of Medicine
Northwestern University (Current address)
Robert H. Lurie Comprehensive Cancer Center
Clinical Center Galter Pavilion
675 North St. Clair, 21st floor
Chicago, IL-60611
Telephone: 312-695-0990
E-mail: maha.hussain@northwestern.edu

Principal Investigator:
David Smith, M.D., F.A.C.P.
University of Michigan
7314 Cancer Center
1500 E. Medical Center Drive
Ann Arbor, MI 48109-5946
Telephone: 734-763-6537
Fax: 734-936-7376
Email: dcsmith@umich.edu

Co-Principal Investigator:
Felix Feng, M.D.
University of Michigan
Department of Radiation Oncology and
Michigan Center for Translational Pathology
University of Michigan Medical Center
1500 E Medical Center Drive
UHB2C490, SPC 5010
Ann Arbor, MI 48109-5010
Telephone: 734-936-4302
Fax: 734-763-7371
Email: ffeng@med.umich.edu

Site Specific and Other Co-Investigators

Emmanuel Antonarakis, M.D Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins Orleans Street, Rm 1M45 The Bunting-Blaustein Cancer Research Building Baltimore, MD 21231 Telephone: 410-502-7528 Email: eantona1@jhmi.edu	David I. Quinn, MBBS (Hons) PhD FRACP Kenneth J. Norris Jr. Comprehensive Cancer Keck School of Medicine University of Southern California 1441 Eastlake Ave Suite 3453 Los Angeles, CA 90033 Telephone: 323-865-3956 Fax: 323-865-0061 Email: diquinn@usc.edu
Glenn Liu, M.D. University of Wisconsin Carbone Cancer Center Wisconsin Institute for Medical Research, Room 7051 1111 Highland Avenue Madison, WI 53075 Telephone: 608-265-8689 Email: gxl@medicine.wisc.edu	Paul Corn, M.D., PhD The University of Texas MD Anderson Cancer Center Pressler Street, CPB7.3481 Houston, Texas 77030 Telephone: 713-792-2830 Email: pcorn@mdanderson.org
Walter Stadler, M.D. University of Chicago 5841 S. Maryland, MC 2115 Telephone: 773-702-4150 Fax: 773-702-3163 Email: wstadler@medicine.bsd.uchicago.edu	Bruce Montgomery, MD University of Washington 1959 NE Pacific St. SP 1301 Box 356158 Seattle, WA 98195 Telephone: 206-598-0860 Fax:.206-598-6986 E-mail: rbmontgo@u.washington.edu
Sumanta Pal, M.D. City of Hope Cancer Center 1500 E. Duarte Road Duarte, CA 91010 Telephone: 626-256-4673 Fax: 626-301-8233 Email: spal@coh.org	Mark Stein, M.D. The Cancer Institute of New Jersey 195 Little Albany Street New Brunswick, NJ 08901 Telephone: 732-235-3336 Email: steinmn@umdnj.edu
Costantine Albany, MD Assistant Professor of Medicine Medical Oncology Indiana University Health Simon Cancer Center 535 Barnhill Drive, RT 473, Indianapolis, IN 46202 Telephone: 317-948-8310 E-mail: calbany@iupui.edu	Young E. Whang, M.D. PhD University of North Carolina at Chapel Hill Lineberger Comprehensive Cancer Center CB #7295, Room 22-016 Chapel Hill, NC 27599 Telephone: 919-843-9983 Fax: 919-966-8212 Email: ywhang@med.unc.edu

Daniel Shevrin, M.D. NorthShore University HealthSystem 2650 Ridge Ave. Evanston, IL 60201 Telephone: 847-570-2515 Email: DShevrin@northshore.org	Stephen Koehler, MD City of Hope Medical Group, Inc. 209 Fair Oaks Avenue South Pasadena, CA 91030 Phone: 626-396-2900 Fax: 626-396-2770 Email: skhoehler@cohmg.com
Biostatistician: Stephanie Daignault-Newton, M.S. N18D11 NIB 300 North Ingalls Ann Arbor, MI, 48109-0473 Telephone: 734-647-3271 Email: sfaruzzi@umich.edu	**Radiology Co-Investigators** Jon A. Jacobson, M.D. University of Michigan Taubman Center, Room 2910L 1500 East Medical Center Dr., SPC 5326 Ann Arbor, MI 48109 Telephone: 734-936-4365 Fax: 734-936-9723 Email: jjacobsn@umich.edu Matthew S. Davenport, M.D. University of Michigan Department of Radiology 1500 E. Medical Center Drive, B1 G505 Ann Arbor, MI 48109-5030 Telephone: 734/615-9016 Email: matdaven@umich.edu
Study Pathologist Lakshmi P. Kunju, M.D. University of Michigan Hospital Department of Pathology 1500 E. Medical Center Drive 2G332 University Hospital, SPC 5054 Ann Arbor, MI 48109-5054 Telephone: 734-647-9125 Email: lkunju@umich.edu	**Study Pathologist** Arul Chinnaiyan, M.D. University of Michigan Hospital Department of Pathology/Urology Surgery 1500 E. Medical Center Drive 5316 Cancer Center, SPC 5940 Ann Arbor, MI 48109-5940 Telephone: 734-615-4062 Email: arul@umich.edu

Administrative Personnel

Responsible Research Nurse: Tamara Huebner, RN, BSN, OCN B1-245 Cancer Center 1500 E. Medical Center Drive Ann Arbor, MI 48109-5913 Telephone: 734-763-4992 Email: thuebner@umich.edu	**Phase II Regulatory Manager:** Supriya Perambakam, CCRP The University of Chicago 5841 S. Maryland Ave., MC 1140, Chicago, IL 60637 Telephone: (773) 702-6793 Email: speramb@.bsd.uchicago.edu

Group Chairman: Walter M. Stadler, M.D. University of Chicago 5841 S. Maryland Ave., MC 2115 Chicago, IL 60637 Telephone: (773) 702-4150 Fax: (773)834-0188 Email:wstadler@medicine.bsd.uchicago.edu	**Responsible Data Manager:** Jaclyn D. Peterson, MBA The University of Chicago Section of Hematology/Oncology 5841 S. Maryland Ave. MC2115 Chicago, IL 60637 Phone: 773-834-1746; Fax: 773-702-4889 Email: jdpeterson@medicine.bsd.uchicago.edu
Technical Director, Michigan Center for Translational Pathology Javed Siddiqui, MS, MT(ASCP, CLsp (MB)) Room 1138 2900 Huron Parkway Suite 100 Ann Arbor, MI 48105 Phone : (734) 232-0829; Fax : (734) 232-0805 Email: siddiqui@med.umich.edu	**University of Michigan Multi-Site Coordinator** Kathleen Granlund, CCRP Cancer Center Clinical Trials Office North Campus Research Complex (NCRC) Building 300 Main Ann Arbor, MI 48109-2800 Phone: 734-936-0563 Fax: 734-232-0744 Email: kemarsh@med.umich.edu

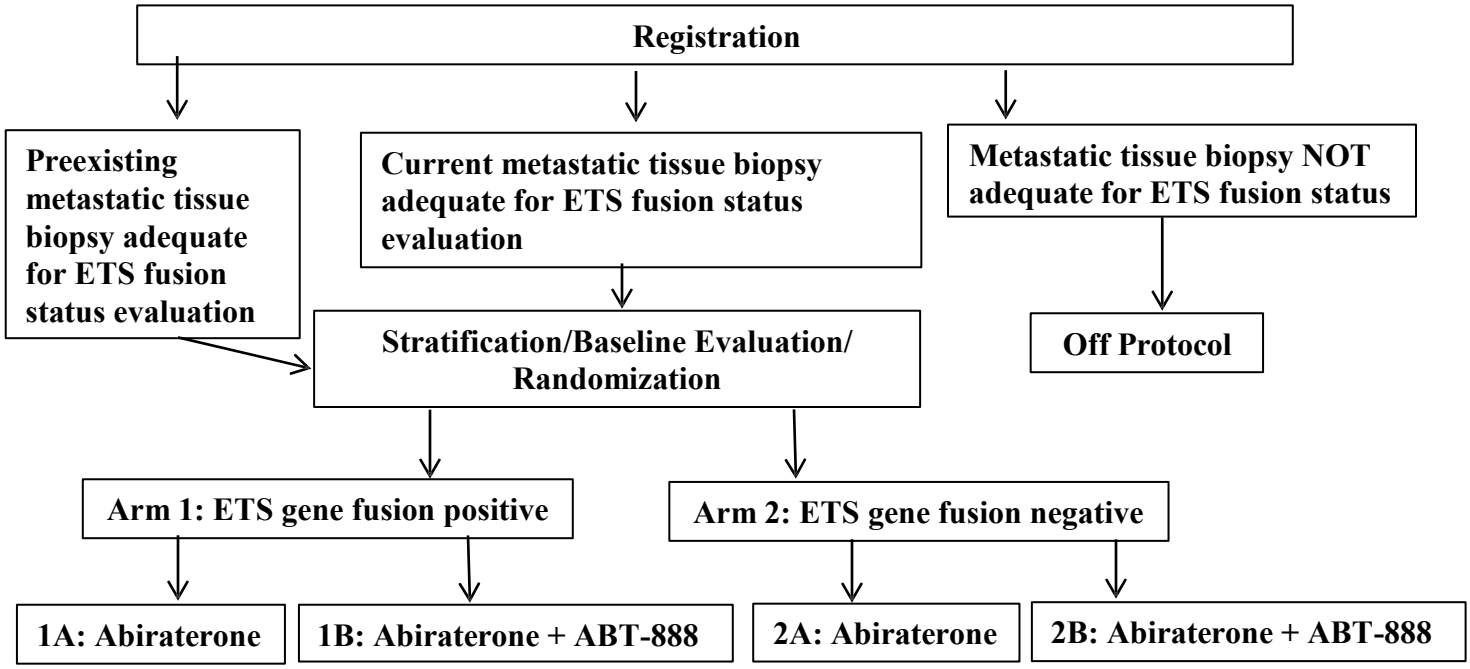
Contract #	
NCI Supplied Agent:	**ABT-888 (NSC # 737664; IND # 77840)**
Commercially Supplied Agent	**Abiraterone (**NSC #748121)
NCI Approval:	**3/30/2012**
UM Protocol Approval:	**4/16/2012**

Protocol History

Version Date	Description of Action
12/23/10	Original Submission to NCI
11/9/11	Consensus Response / Version 2
12/13/11	Follow-up Review/Version 3 and incorporation of ABT-888 CAEPR v. 2.0, 10/28/11 in response to RA received from Dr. Chen on 11.18.11
1/09/12	Follow-up Review /Version 4 and updated Section 5.5 Duration of Follow Up.
6/26/12	Amendment 1: added several participating institutions to the protocol, updated site information/personnel, and corrected/clarified several technical and patient scheduling issues within the protocol.
8/17/12	Amendment 2: being submitted in response to an Action Letter for ABT-888 from Dr. Alice Chen. Removed one participating site and added two patient scheduling windows in

	the study calendar. Updated the exclusion criteria for patients with history of active seizures in Section 3.2.3. Changed FedEx shipping priority in Appendix F from First Overnight to Priority Overnight.
5/29/13	Amendment 3: Added Dr. Stephen Koehler, MD, City of Hope Medical Group, Inc. Updated the University of Chicago regulatory manager. Corrected/clarified several technical and patient scheduling issues within the protocol; increased the number of biopsy samples needed and clarified the SOPs for obtaining these samples (Appendix C and D); updated the eligibility criteria in Section 3.1.8 to indicate that patients with prior exposure to ketoconazole are eligible for this trial. Serum Creatinine level was corrected under Section 3.1.10. Clarified that patients who had been on prednisone prior to registration may continue it during the lead-in ABT-888 therapy in Section 5.1 for Arm 1 and Arm II B. Clarified that the dose of ABT-888 was changed from 300 mg to 200 mg at start and then escalate to 300 mg after the 1st cycle if patient tolerates 200mg in Section 5.2-under agent administration. In section 6.0, dose delays and dose modifications were updated. Dose levels of prednisone and ABT-888 also were changed under sections 6.4 and 6.5, respectively. Added Appendix G: Standard Operating Procedure for Shipment of CTC Clinical Samples to indicate that change to Epic Sciences in the CTC handling and shipping procedures and added that these blood/CTC samples would also be collected at week 12 and at clinical progression. Added the enumeration and molecular characterization of the CTC samples to the correlative analysis plan (section 13.4). Revised the approach from detecting ETV1 fusions from FISH to ISH.
2/10/14	Amendment 4: Updated Indiana University site PI. Updated Adverse Event Expedited Reporting System (AdEERS) with CTEP Adverse Event Reporting System (CTEP-AERS) throughout the protocol.
3/17/16	Amendment 5: Updated version date throughout the protocol. Updated Administrative Personnel. Section 10.0- updated the study calendar.
4/27/16	Amendment 6: Updated version date throughout the protocol. Replaced CAPER for ABT-888 version 2.2, March 22, 2013 with version 2.3, March 4, 2016 per RA letter dated April 19, 2016.
7/1/16	Amendment 7: Administrative amendment to update designation and contact info for DR. Hussain and to include DR. Smith as the new Principal Investigator at Univ. of Michigan..
7/20/17	Amendment 8: Administrative amendment to replace COH site PI, Twardowski with Pal.

STUDY SCHEMA



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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To evaluate the role of ETS gene fusion as a predictive biomarker for response to hormone therapy (abiraterone) alone or hormone therapy plus PARP-1 targeted therapy (ABT-888) in patients with metastatic castration resistant prostate cancer.
1.1.2 To evaluate whether the addition of PARP-1 targeted therapy is superior to hormone therapy alone based on ETS gene fusion status.

1.2 Secondary Objectives

1.2.1 Rate of PSA declines.
1.2.2 Objective response rate.
1.2.3 Progression-free survival.
1.2.4 Evaluate the qualitative and quantitative toxicity of abiraterone acetate with and without ABT-888.

1.3 Correlative Objectives

1.3.1 To determine the concordance in fusion status among prostate cancer samples from the primary site, biopsied metastasis, and CTCs.
1.3.2 To assess if ETS fusion status in the CTCs, at baseline, 12 weeks, and at disease progression (or when off study) is associated with response to therapy.
1.3.3 To evaluate if the number of circulating tumor cells (CTCs), as well as the expression levels of androgen receptor, RAD51, and gamma-H2aX foci in the CTCs at baseline, at 12 weeks, and at disease progression in all patients is associated with response to therapy.
1.3.4 To determine the role of PTEN loss as a predictive biomarker of response to abiraterone, alone or in combination with ABT-888.
1.3.5 To determine the role of PARP1 activity as a predictive biomarker of response to abiraterone, alone or in combination with ABT-888.
1.3.6 To perform next-generation sequencing for discovery of novel gene fusions in prostate cancers negative for ETS fusions.
1.3.7 To perform germline single nucleotide polymorphism (SNP) analysis of genes involved in hormone synthesis, transport, binding, metabolism, and degradation for discovery of novel SNPs predictive of response to abiraterone, alone or in combination with ABT-888.
1.3.8 To determine if ETS fusion RNA levels in blood are predictive of response to abiraterone, alone or in combination with ABT-888.

2. BACKGROUND

2.1 Prostate Cancer and Androgen Signaling

In the United States, metastatic castration-resistant prostate cancer (mCRPC) resulted in 32,050 deaths in 2010.[1] Mounting evidence indicates that mCRPC remains sensitive to androgen signaling through multiple different mechanisms which, in part, is androgen receptor-mediated, including androgen receptor gene amplification and mutations in addition to up-regulation of steroidogenic enzymes (such as CYP17) that lead to increased androgen production. [2-6]

Abiraterone acetate is a selective androgen biosynthesis inhibitor that blocks CYP17 and potently inhibits persistent androgen synthesis from adrenal and potentially intratumoral (autocrine/paracrine) sources. [7-9] In a randomized phase III trial, abiraterone + prednisone significantly improved median overall survival (14.8 months in the abiraterone acetate group and 10.9 months in the placebo group), progression-free survival (5.6 month vs. 3.6 months), and confirmed PSA response rate (29% vs. 6%, P<0.001) in patients with chemotherapy-refractory metastatic CRPC as compared to prednisone and placebo. [10,11] The benefit was consistent irrespective of number of prior chemotherapies (1 vs. 2). This led the FDA to approve abiraterone and confirmed the biologic observation on the continued androgen sensitivity of mCRPC.

While significant survival improvement is seen with abiraterone, the effect is relatively modest (4 months). The latter coupled with a confirmed PSA response rate of 29% suggests that a significant percentage of patients may not be benefiting or have minor benefits from such therapy; hence the need for a potential predictive biomarker to personalize therapy and improve the therapeutic efficacy of abiraterone-based therapy.

2.2 The Role of ETS Gene Fusions in Prostate Cancer

Several years ago, our research team discovered the presence of ETS gene fusions in prostate cancers. These gene rearrangements typically involve the fusion of the promoter of an androgen-responsive gene, such as TMPRSS2, to a gene encoding an ETS transcription factor.[12,13] They appear to be present in approximately 50%-60% of all prostate cancers, including metastases, making them the most common genetic translocations associated with any solid malignancy.[12-14] These fusions typically result in the androgen-driven overexpression of ETS transcription factors, such as ERG or ETV1.[14]

The predominant ETS fusion, TMPRSS2:ERG, results in a 20-120 fold overexpression of ERG, with a relatively sharp demarcation in expression levels between prostate cancers containing versus lacking the fusion (Fig. 1). As comparison, EGFR, another commonly investigated biomarker, is overexpressed only 3-10 fold across all prostate cancers, with no clear demarcation between negative versus positive cases. Prostate cancer cells are addicted to ETS fusions in preclinical models, meaning that knockdown of the specific oncogene results in abrogation of the malignant phenotype and overexpression of the oncogene reconstitutes the malignant features[14-

[17]. These alterations are dominant oncogenes, as reflected by their mutual exclusivity. Specifically, ETS fusions are found in approximately 50%-60% (including metastases) of all prostate cancers, respectively with no overlap between subtypes.[12-14] Finally, and possibly most importantly, prostate cancer subtypes determined by ETS fusions are reproducible across multiple cohorts from different institutions. [14,15,18-21]

Once an ETS gene fusion is formed through genomic rearrangement, the subsequent overexpression of an ETS gene fusion protein can contribute to cancer progression through

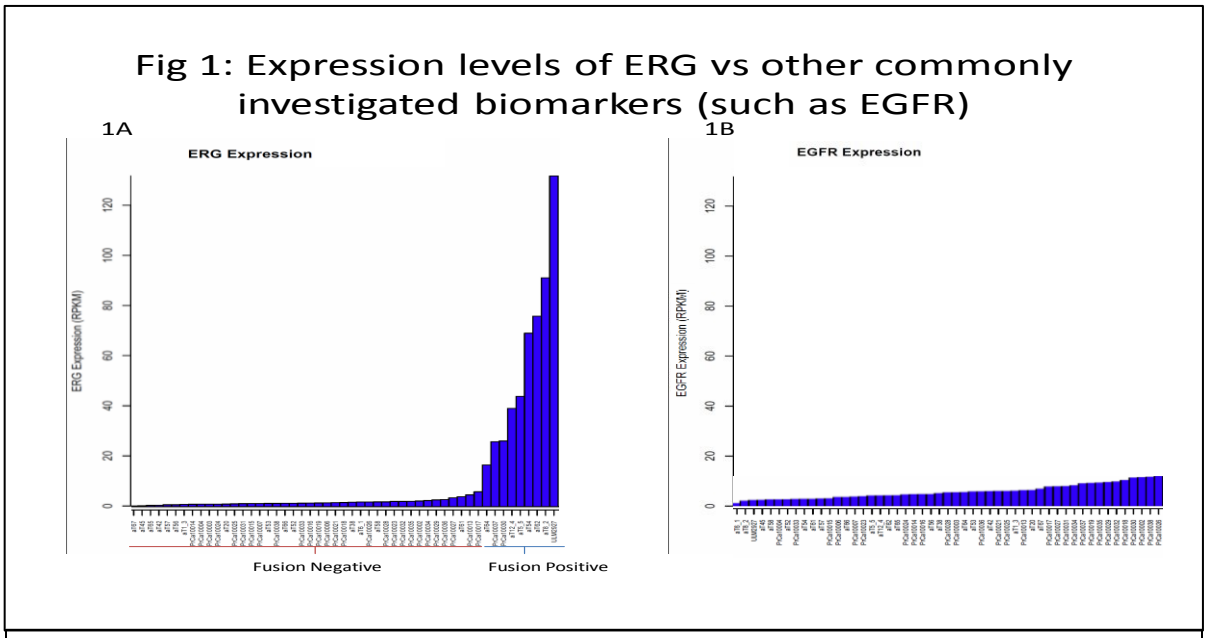

Figure 1: Relative levels of ERG and EGFR expression across a wide range of prostate cancer samples The predominant ETS fusion, TMPRSS2:ERG, results in a 20-120 fold level of ERG overexpression, with a relatively sharp demarcation in expression levels between prostate cancers containing versus lacking the fusion (1A). As comparison, EGFR, another commonly investigated biomarker, is overexpressed only 3-10 fold across all prostate cancers, with no clear demarcation between negative versus positive cases (1B). Note: This cohort was purposely enriched for ETS fusion- negative prostate cancer cases (as a discovery set for non-ETS molecular subtypes). In a population-based cohort of prostate cancer, the frequency of ETS fusions would be expected to be around 50%.

several different mechanisms. For example, we and others have demonstrated that TMPRSS2-ERG gene fusion expression is required for cell growth in cell line models that harbor an endogenous gene fusion both in vitro and in vivo; knockdown of the ETS protein ablates the ability of these cells to form tumors.[15,22-24] Likewise, ETS proteins are active transcription factors that drive cellular invasion through the induction of a transcriptional program highly enriched for invasion-associated genes [15,22,24-26] as reflected by the distinct gene expression signature seen in ETS fusion-positive cancers.[27] Genetically-engineered mice expressing ERG or ETV1 under androgen regulation exhibit prostate intraepithelial neoplasia (PIN)-like lesions.[13,15,26,28-30] Clinically, ERG-positive high-grade PIN is frequently found in close proximity with ERG-rearranged cancers. [31,32] This data suggests that ETS gene fusions are an early driving event in prostate carcinogenesis. Additionally, high levels of ERG and ETV1 gene expression continue to be maintained in advanced or metastatic disease.[14] In fact, one recent study has suggested that metastases may be relatively enriched for ERG positivity; the authors found that in a cohort of prostate cancer patients with multifocal disease containing both ERG-positive and ERG-negative foci, the positive foci have a greater predilection for metastases.[33]

Together, this data suggests that ETS fusions are addicting oncogenes which contribute to both carcinogenesis and progression, and therefore represent a promising target for oncologic therapy in prostate cancer.

2.2.1 Rationale for Targeting ETS Fusion-Positive Prostate Cancers with Abiraterone Based Therapy

The fact that ETS gene fusions are predominantly driven by an androgen-sensitive promoter, [14,16,34,35] coupled with the continued sensitivity of mCRPC to androgen signaling, provides a strong rationale to selectively target mCRPC that harbor such fusions with anti-androgen approaches.

Emerging clinical data provide additional support for this approach. In 150 radical prostatectomy patients, the response to adjuvant androgen deprivation therapy was associated with ERG status, showing more significant treatment effect in ERG(+) patients.[36] In phase I/II studies of mCRPC patients treated with abiraterone, the presence of the predominant ETS fusion, the TMPRSS2:ERG rearrangement, has been demonstrated to correlate with PSA response. Specifically, Attard et al. found that 32 of 77 (41%) of men with CRPC had ERG rearrangements that could be detected in circulating tumor cells (CTCs) prior to treatment; [7,37] 12 of the 32 patients (38%) with ERG-fusion-positive CTCs had a >90% decline in PSA level with abiraterone, compared to 3 of 45 patients (7%) with ERG-fusion-negative CTCs. In this cohort, a decline in PSA level was associated with decreased CTCs and an increased survival rate.

These findings suggest that Abiraterone may have greater therapeutic effect in ETS-fusion positive prostate cancer.

2.2.2 Rationale for Combining Abiraterone and ABT-888

A. *The role of Poly(ADP-ribose)-Polymerase 1 (PARP1) in Prostate Cancer*

- Preliminary observations indicate that castration-resistant tumor cells exhibit increased PARP1 activity. [38]
- PARP1 function is critical to the pro-tumorigenic functions of the androgen receptor (AR): Specifically, new data strongly support the use of ABT-888 as a means to antagonize PARP1-dependent AR function in prostate cancer cells, especially in tumor models with intrinsically high AR activity or which have achieved castration resistance. Preclinical findings in a number of models demonstrate that: i) PARP1 positively regulates AR activity; ii) ABT-888 inhibits expression of AR target genes that are of clinical importance (such as PSA and TMPRSS2); iii) cells with genetic deletion of PARP1 do not support AR function; and iv) ABT-888 markedly represses AR-dependent cellular proliferation in CRPC cells (including those that are ETS fusion negative).[38]
- Since AR-mediated transcription is directly coupled with the induction of DNA damage,[39,40] it is proposed that in metastatic prostate cancer, a combination

approach of hormone-directed therapy with inhibitors of DNA repair (such as PARP inhibitors) "could overwhelm cancer cells with transcription-associated double-strand breaks" and should therefore be used as a therapeutic strategy. [41]

- Additionally, published data from the Knudsen and Feng labs (Fig. 2) confirm that the addition of the PARP inhibitor ABT888 improves the response to hormone therapy in preclinical prostate cancer models.[38]
- Preclinical data from the Chinnaiyan lab (unpublished) has suggested that a short lead-in period of PARP1 inhibition prior to androgen deprivation therapy may modestly improve xenograft control compared to initiation of both therapies at the same time. While the efficacy of abiraterone cannot be well-assessed in preclinical models, we hypothesize that a short lead-in period of PARP1 inhibition, prior to initiation of abiraterone within the relevant treatment arms of this trial, may also confer a benefit. Therefore, the abiraterone + ABT-888 arms of this trial are designed with a short lead-in period of PARP1 inhibition with ABT-888.

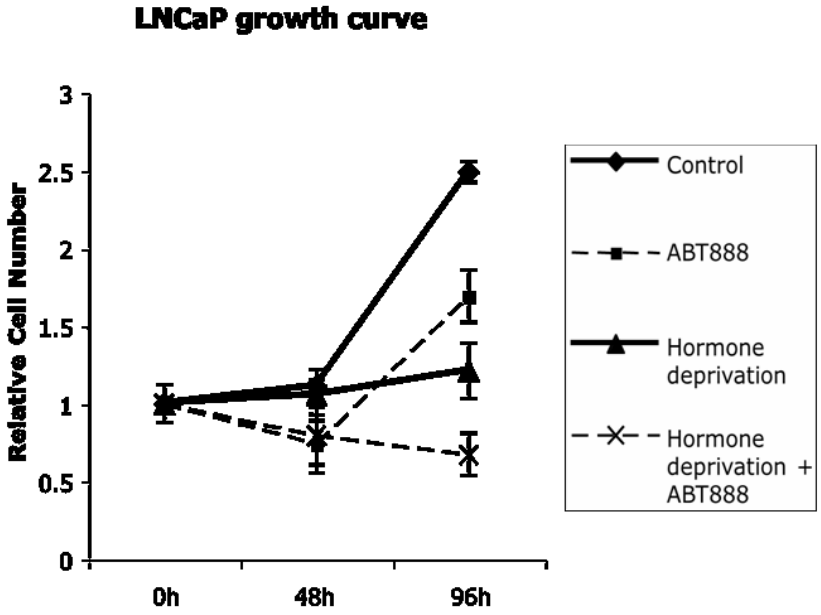

Fig. 2

B. ***The Interaction of ERG and PARP1 in Prostate Cancer***

We recently discovered that, in both preclinical prostate cancer models and clinical prostate cancer specimens, ETS transcription factors, such as ERG, physically interact with poly(ADP-ribose) polymerase-1 (PARP1), a key nuclear enzyme that regulates transcription and facilitates DNA repair through the base excision repair pathway. [42] In addition, we found that PARP1 is required for the malignant phenotype conferred by ETS transcription factors, including ETS-mediated invasion, transcription, and metastasis. [42] Most significantly, we found that PARP1 activity is required to drive the metastatic

approach of hormone-directed therapy with inhibitors of DNA repair (such as PARP inhibitors) "could overwhelm cancer cells with transcription-associated double-strand breaks" and should therefore be used as a therapeutic strategy. [41]

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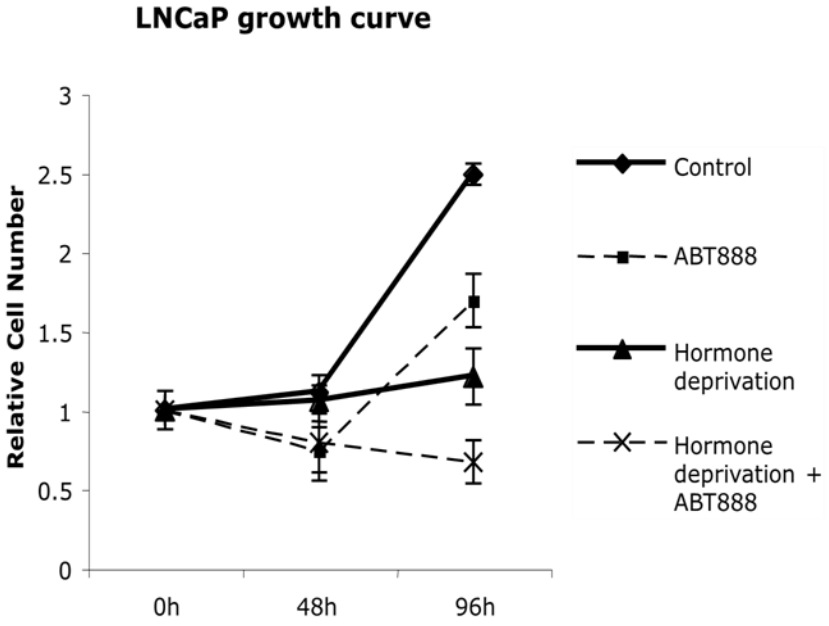

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spread of ETS positive cells. In preclinical models, PARP1 inhibition blocked the formation of liver metastases in an ETS-positive cell line but not an ETS-negative cell line. Moreover, using 8 different prostate cancer xenograft models (4 positive for ETS fusions, 4 negative for ETS fusions), PARP1 inhibition had a much more significant effect on xenograft growth in all of the ETS fusion-positive xenografts compared to the ETS fusion-negative xenografts. (Fig. 3)

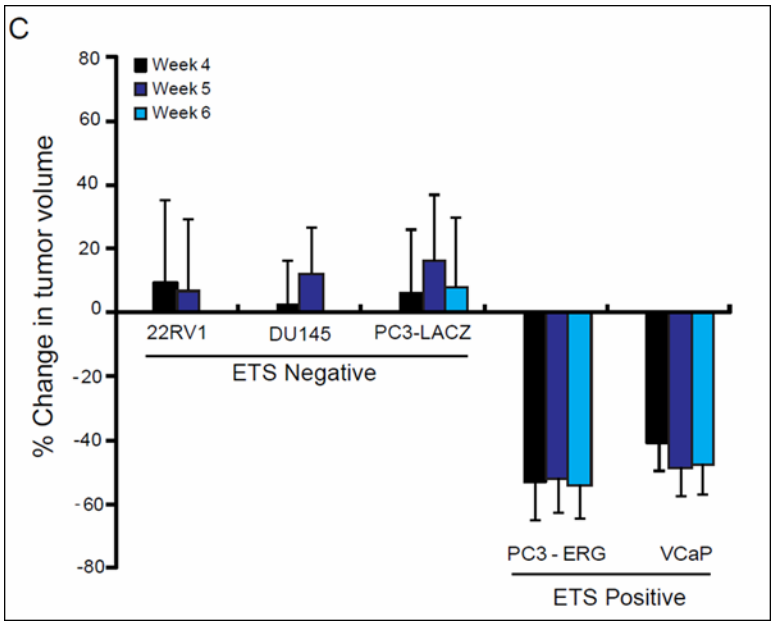

Fig. 3

Taken together, this data suggests that ETS-mediated oncogenic features, such as metastasis and tumor growth, depend on PARP1, and that inhibition of PARP1 can inhibit these phenotypes and specifically target ETS- positive prostate cancers.

2.3 ABT-888 (Veliparib)

ABT-888 is an orally available, small molecule inhibitor of poly(ADP-ribose) polymerase (PARP). PARP is an essential nuclear enzyme that plays a role in recognition of DNA damage and facilitation of DNA repair. Therefore, inhibition of PARP is expected to enhance the effects of DNA damage. Expression of PARP is higher in tumor cells as compared to normal cells. This overexpression has been linked to drug resistance and the ability of tumor cells to withstand genotoxic stress. Hence, it is anticipated that PARP inhibitors will function as sensitizing agents for chemotherapy and radiation therapy that are designed to cause DNA damage.

Mechanism of Action

Poly (ADP-ribosylation) (PAR) occurs after single or double-stranded DNA damage and represents the posttranslational modification of histones and other nuclear proteins by PARP. Based on conserved genetic sequences, encoded for by 18 different genes, 18 nuclear proteins have been classified as members of the PARP superfamily. The superfamily is further subdivided into three branches, the PARP-1 group, the tankyrase group, and other PARP enzymes. The PARP-1 group of NAD^+-dependent enzymes has been extensively studied, and its members PARP-1 and PARP-2 are generally considered as the primary enzymes involved in DNA repair. [43]

PAR has been implicated in many cellular processes including replication, transcription, differentiation, gene regulation, protein degradation, and spindle maintenance. Enhanced PARP-1 expression and/or activity in tumor cells, as compared to normal cells, has been demonstrated in malignant lymphomas ,[44] hepatocellular carcinoma, [45] cervical carcinoma, [46] colorectal carcinoma, [47] non-Hodgkin's lymphoma, [48] leukemic lymphocytes, [49] and colon adenomatous polyps.[50] PARP-1 and PARP-2 are nuclear proteins and are the only members of the PARP family with zinc-finger DNA binding domains. These domains localize PARP-1 and PARP-2 to the site of DNA damage. PARP-1 is highly conserved and has three structural domains (N-terminal DNA-binding domain; automodification domain, and the NAD^+-binding domain). The catalytic domain is located at the C-terminus end of the protein. In knockout mouse models, deletion of PARP-1 is sufficient to impair DNA repair.[51-53] The residual PARP-dependent repair activity (~ 10%) is due to PARP-2. This suggests that only PARP-1 and PARP-2 need to be inhibited to impair DNA repair. [54-56]

The zinc finger domain of PARP binds to both single- and double-stranded DNA breaks, resulting in increased catalytic activity. [54,56,57] Once activated, PARP cleaves NAD^+ and attaches multiple ADP-ribose units to the target nuclear protein. This results in a highly negative charge on the target protein and affects its function. Overactivation of PARP can be induced by DNA damage, leading to the depletion of NAD^+ and energy stores and, thus, cellular demise by necrosis. An alternate mechanism has been identified where PARP overactivation can induce cell death through apoptosis by releasing the Apoptosis Inducing Factor (AIF) from mitochondria.[58] Consequently, multiple mechanisms to prevent overactivation of PARP exist. First, auto-PAR negatively regulates PARP activity. [59] In addition, the cleavage of PARP by caspases yields a peptide fragment that acts as a trans-dominant negative inhibitor for uncleaved PARP. PAR of proteins is a dynamic process with a short half-life ($t_{1/2}$) of <1 min. The enzymes responsible for degrading these polymers are poly(ADP-ribose) glycohydrolase (PARG), which cleaves ribose-ribose bonds, and ADP-ribosyl protein lyase, which removes the protein proximal to the ADP-ribose monomer.

Increased PARP activity is one of the mechanisms by which tumor cells avoid apoptosis caused by DNA damaging agents. PARP activity is essential for the repair of single-stranded DNA breaks through the base excision repair (BER) pathways.[56,60] Therefore, inhibition of PARP sensitizes tumor cells to cytotoxic agents (*e.g.* alkylators [temozolomide, cyclophosphamide, BCNU] and topoisomerase I inhibitors [irinotecan, camptothecin, topotecan]) which induce

DNA damage that would normally be repaired through the BER system. A significant therapeutic window appears to exist between a PARP inhibitor's ability to potentiate therapeutic benefit *versus* potentiation of undesirable side effects.
Ionizing radiation induces both double- and single-stranded DNA breaks. While part of the radiosensitization caused by PARP inhibition is through the inhibition of the single-stranded break repair pathways, it appears likely that repair of double-stranded breaks, which are thought to be more cytotoxic, is also affected. Double-stranded breaks are strong activators of PARP-1, resulting in PARP-1 mediated activation of DNA-PK and Ku80, important components of the non-homologous end-joining (NHEJ) double-stranded break repair pathway. [61,62] Also, small molecule inhibitors of PARP can directly inhibit the repair of double-stranded breaks. [51,63] Thus, it is likely that PARP activity is important for repair of both the single- and double-stranded stranded DNA breaks caused by ionizing radiation and other agents.

Nonclinical Activity

In vitro, ABT-888 inhibited PARP-1 and PARP-2 with K_i values of 3.6 nM and 2.9 nM, respectively. These values were observed in enzyme assays measuring the incorporation of [^{3}H]-NAD^+ into histone H1, an important physiological substrate of PARP. In assays measuring inhibition of H_2O_2-induced poly(ADP-ribosyl)ation in C-41 cervical carcinoma cells, ABT-888 inhibited PARP with an EC_{50} value of 2.4 nM. The extent of DNA damage in cells was indicated by γ-H2AX levels. To determine the effect of ABT-888 in combination with cytotoxic agents on DNA damage, the cellular content of γ-H2AX in C-41 cells was assayed by flow cytometry using an anti-γ-H2AX antibody. Addition of 1 mM of temozolomide alone resulted in increased numbers of γ-H2AX foci, a result which was further potentiated by ABT-888 in a dose-dependent manner. When cell survival was measured by an AlamarBlue assay, ABT-888 potentiated cytotoxicity in the same concentration range as used in the γ-H2AX assay, demonstrating that ABT-888 potentiates cytotoxicity of temozolomide by delaying DNA repair. ABT-888 achieved a maximal potentiation of approximately 15-fold. ABT-888 also potentiates the DNA damage cause by irinotecan.

The combination of PARP inhibitors with different classes of chemotherapeutics was examined. Cisplatin-induced potentiation was observed in a long-term clonogenic assay, but not in the short-term cytotoxicity assay. The potentiation of cisplatin by ABT-888 *in vitro* is consistent with the potent enhancement of the efficacy of platinum agents (cisplatin and carboplatin) observed *in vivo*. PARP inhibition was shown to sensitize cells that are mismatch repair (MMR)-deficient to a greater extent than cells that are MMR competent. [64] Alkylating agents such as temozolomide form methyl adducts in DNA and resistance is frequently encountered in the clinic with either the overexpression of O^6-alkylguanine DNA alkyltransferase (AGT) or functional defects in the MMR system. However, when PARP was inhibited, cells were sensitized to methylpurine formation, regardless of their resistance factors. [65]

There are data to suggest that PARP inhibitors have activity against some BRCA-deficient cells in the absence of any DNA damaging agent. [66,67] These inhibitors did not demonstrate single agent activity in BRCA-competent cells, and restoring functional BRCA to deficient cells abrogated single agent cytotoxicity. It is possible that, in BRCA-deficient cells, PARP inhibition

stops the BER pathway, and thus single-stranded breaks are carried through DNA synthesis, resulting in double-stranded breaks. The increase in double-stranded breaks cannot be repaired by homologous recombination (HR), due to the lack of BRCA1 or 2, resulting in increased cell death. However, since not all BRCA deficient cells are sensitive to the PARP inhibitors, it is unclear why single agent cytotoxicity is observed in some BRCA-deficient cells.

Consistent with PARP-1 being a radiosensitization target, PARP-1 knockout mice showed enhanced sensitivity to γ-radiation. [68,69] There is evidence to suggest that PARP inhibitors sensitize cancer cells to radiation, both *in vitro* and *in vivo.* [70-72] Furthermore, a PARP inhibitor in the same class as ABT-888 potentiated radiation in the HCT116 colon carcinoma model. ABT-888 was tested, in combination with cytotoxic agents, in several tumor models and demonstrated a similar profile of antitumor activity to that seen in the literature (See table below). ABT-888 substantially increased the efficacy of cytotoxic therapies, when measured by either treated/control tumor volumes (%T/C) or by increased time for tumors to grow to a particular size (%ILS).

Table 1: Preclinical data for ABT-888 mediated potentiation of cytotoxic agents

	Breast carcinoma (human MX-1)	**Glioblastoma muliforme (rat 9L)**	**B cell lymphoma (human DOHH2)**	**Melanoma (murine B16F10)**
Carboplatin	Yes			
Cisplatin	Yes		No	
Cyclophosphamide	Yes			
Irinotecan				Yes
Temozolomide		Yes		Yes

ABT-888 potentiated cytotoxic therapy when administered either parenterally or orally (PO). When administered parenterally, significant efficacy was observed at doses as low as 1 mg/kg/day, and maximal efficacy was achieved at approximately 12.5 mg/kg/day. 3.1 mg/kg/day PO (divided, twice daily) provided significant potentiation, with maximal potentiation achieved at approximately 25 mg/kg/day. No increased toxicity was observed at any of these ABT-888 doses, either parenteral or PO. Supratherapeutic doses of ABT-888 (50 mg/kg/day), administered via osmotic minipump (OMP), resulted in skin toxicity at the pump implantation site. The observation that supratherapeutic doses of PARP inhibitors may potentiate toxicity is consistent with preclinical and clinical observations. It is also consistent with the results from a two-week ABT-888/cisplatin combination study. When administered as a continuous infusion, an ABT-888 C_{ss} (plasma concentration at steady-state) of 70 ng/mL was maximally efficacious (area under the curve [AUC]=1.7 µg•hr/mL). Comparable efficacy was seen in oral studies at a 25 mg/kg/day (divided, twice daily) dose that yielded AUCs between 1.6 and 3.0 µg•hr/mL. At this dose, the plasma concentrations were above 70 ng/mL for only 2-4 hours per dose, demonstrating that 24 hour/day coverage above 70 ng/mL was not required for efficacy.

An enzyme-linked immunosorbent assay (ELISA) that can measure PAR formation was used to demonstrate PARP inhibition in murine tumors *in vivo* and human peripheral blood mononuclear cells (PBMCs) *ex vivo* at clinically relevant doses. This ELISA was used as the primary assay for PARP biomarker analysis. The degree of PARP inhibition was assessed in B16F10 syngeneic flank tumors from mice treated *in vivo* using tumor efficacy schedules. In this study, PAR formation was measured in tumors treated with ABT-888 alone. Two hours after administration, ABT-888 inhibited PAR formation in B16F10 tumors in a dose-dependent manner. The same response was reflected in a parallel efficacy experiment, where temozolomide (50 mg/kg/day, PO, daily × 5) was administered with ABT-888. In another study, PAR formation was measured in tumors treated simultaneously with temozolomide and ABT-888. As in the ABT-888 only study, tumor PAR levels in the combination study were also inhibited. Inhibition of PARP activity was significant at 12.5, 5 and 1 mg/kg/day in both the vehicle and temozolomide treated groups. Overall, these results indicate the ability of ABT-888 to inhibit both baseline and cytotoxic-induced PARP activity in tumors treated *in vivo* and provide evidence of the ability of ABT-888 to target PARP *in vivo.*

Nonclinical Pharmacology and Toxicology

The pharmacokinetics (PK) of ABT-888 was evaluated in CD-1 mice, Sprague-Dawley rats, beagle dogs and cynomolgus monkeys. The non-clinical PK profile of ABT-888 was characterized by high plasma clearance (CL) values, ranging from a high of 4.1 L/hr•kg in the mouse to a low of 0.57 L/hr•kg in the dog. ABT-888 exhibits moderate volumes of distribution (V_{ss}) in all species (V_{ss} > 2.0 L/kg), with terminal elimination $t_{1/2}$ in the 1.2-2.7 hr range. In rats and dogs, [^{3}H]ABT-888 was rapidly absorbed and cleared primarily in the urine as intact parent drug. A-925088 (M8), a lactam derivative and the major product of ABT-888 metabolism, was also cleared primarily in the urine. In both rats and dogs, parent drug was the major component in systemic circulation, followed by M8. Elimination of total radioactivity was rapid, with most (>80%) of the dose recovered within 24 hours post-dose, indicating that parent drug and the major metabolites are not likely to accumulate. Bioavailability following an oral dose was high (F>50%) in all species, with values ranging from a low of 56.1% in the monkey to a high of 92.0% in the mouse, and low animal-to-animal variability across all species.

The bioavailability from a non-formulated capsule was only slightly lower than from the solution formulation with values of 59.7% and 65.5% in fasted and non-fasted dogs, respectively. This suggests that there are no major food effects. The compound has high solubility at physiological pH and high permeability. Protein binding values in plasma (assessed *in vitro* as % bound at 5 μM) for ABT-888 were moderate in all species averaging 42% in dog, 41% in monkey, 43% in mouse, 49% in rat and 51% in human. The stability of ABT-888 was evaluated in rat, dog, monkey and human plasma and the drug was found to be very stable, with minimal degradation over the 8-hour incubation interval. *In vitro* metabolism studies indicated that several CYPs (1A1, 1A2, 2C9 and 2C19) have the potential to mediate the formation of M8. However, ABT-888 is not a potent inhibitor of the major human CYPs *in vitro,* indicating a low risk for drug-drug interactions at the anticipated therapeutic concentrations. ABT-888 partitioned slowly into and out of the brain, in both mouse and rat, with high plasma to brain ratios (~3:1) during the

first 3-6 hours after dosing. The plasma to brain ratios approached 1:1 in samples obtained 12 hours after dosing.

PK parameters in humans were estimated by a variety of methods. The oral clearance (CL/F) of ABT-888 was estimated as a function of the projected clearance after IV administration (CL) and the fraction of the dose systemically available after oral administration (F). Clearance predictions were based on allometric scaling. Bioavailability was estimated by simulations with sensitivity analyses using software which took into account human gastrointestinal physiology and the drug's physicochemical characteristics. V_{ss} was estimated either from an average of values observed in animal species, a method averaging the fraction unbound in animal tissues, or by allometric scaling. Terminal phase $t_{1/2}$ values were estimated either by regression relationships between animal and human $t_{1/2}$ values [73], or from the estimates of CL and V_{ss}. The human PK profile is projected to have CL=26 L/hr, with oral bioavailability of ~ 70%. The predicted human $t_{1/2}$ of ABT-888 is ~4 hrs. Simulations of 50 mg twice daily dosing in humans mimic a maximally efficacious dosing regimen in mouse (12.5 mg/kg, twice daily), with concentrations above 71 ng/mL for 8 of 24 hours and an AUC_{24} of 3 µg•hr/mL at steady state. ABT-888 was tested in receptor-binding, CNS/neurobehavioral, cardiovascular, cardiac electrophysiological and gastrointestinal assays. In 74 receptor-binding assays at a concentration of 10 µM (2.4 µg/mL), ABT-888 displaced control-specific binding at the human H_1 (61%), the human 5-HT_{1A} (91%), and the human 5-HT_7 (84%) sites only, with IC_{50} values of 1.2-5.3 µM.

ABT-888 did not display clear adverse CNS effects in the rat and mouse between 3-30 mg/kg PO. At 100 mg/kg PO, mild sedation-like effects were observed, followed in time by mild excitation. At 300 mg/kg PO, more moderate to marked CNS effects were observed, including abnormal gait and sedation. Further, at 100 mg/kg, PO, there was an increased incidence of death after electrically-induced tonic convulsions in mice. Death was also noted in a second convulsant model (audiogenic seizures in mice). In a repeated dosing mini-Irwin observational test, in which rats were dosed with ABT-888 at 30, 100, and 300 mg/kg intraperitoneally (IP) every day for 5 days, tonic-clonic seizures/death were observed in approximately 50% of the animals treated at the highest dose on day 1. A similar incidence of seizures was observed after dosing the remaining animals at the same dose on each of the subsequent days. In an acute follow-up study with rats dosed with ABT-888 300 mg/kg IP, protection against seizures was not provided by pretreatment with either valproic acid (300 mg/kg IP, 15 min prior to ABT-888) or diphenylhydantoin (75 mg/kg IP, 100 min prior to ABT-888). In a 2-week toxicology study, seizures were also noted in dogs treated with ABT-888 at either 60 mg/kg/day, 30 mg/kg twice daily, or 30 mg/kg every day. Plasma concentrations in dogs with seizures were in excess of 5.4 µg/mL (26-fold the predicted clinical C_{max} of 0.21 µg/mL).
In the anesthetized dog, ABT-888 produced no physiologically relevant changes in mean arterial pressure, heart rate, dP/dt_{max}, pulmonary arterial pressure, or systemic or pulmonary vascular resistance compared to vehicle controls at mean plasma concentrations as high as 4.45 ± 0.13 µg/mL (21-fold the predicted clinical C_{max} of 0.21 µg/mL). As mean plasma concentrations increased to 12.96 ± 0.92 µg/mL (62-fold), ABT-888 produced a modest reduction in mean arterial pressure (–16 ± 5% below baseline) and systemic vascular resistance (–10 ± 7% below baseline).

ABT-888 blocked hERG current with an IC_{50} value of 57.6 ± 1.7 µg/mL (236 ± 7 µM), a value 278-fold higher than the predicted clinical C_{max}. The M8 metabolite of ABT-888 (A-925088) minimally affected hERG at the highest concentration tested (81.5 µg/mL). While no effect on repolarization (*in vitro* action potential duration measures) was noted at the lowest measured concentration of ABT-888 (0.42 µg/mL, 2-fold higher than the predicted clinical C_{max}), ABT-888 prolonged the action potential duration at the intermediate and highest measured concentrations (4.8% and 18.6% prolongation at 4.22 ± 0.02 and 39.49 ± 0.70 µg/mL respectively), suggesting delayed repolarization risk between 20- and 190-times the C_{max}. There was a trend (7%) towards delayed repolarization in the anesthetized dog model (QTc intervals) at plasma concentrations 21-fold higher than the predicted clinical C_{max}; greater concentrations elicited prolongation (15 ± 3% above baseline [QTcV] at 12.96 ± 0.92 µg/mL). In humans, QTc prolongation is predicted to be less than 3 msec at the anticipated dose of 50 mg twice daily. These cardiac effects need to be monitored during clinical trials.

Gavage administration of ABT-888 up to 10 mg/kg was generally well tolerated in the ferret emesis model. No emesis was noted at this dose (resulting in mean plasma concentrations of 3.80 ± 0.11 µg/mL, a value 18-fold greater than the predicted C_{max}), with significant emesis noted in response to the 20 mg/kg dose (resulting in mean plasma concentrations of 6.61 ± 0.26 µg/mL, a value 31-fold greater than predicted C_{max}). Parenteral (subcutaneous) dosing of ABT-888 at doses and plasma concentrations similar to those used in the gavage study revealed a similar emetic dose-response relationship, suggesting a centrally-mediated emetic response. ABT-888 had no significant effect on gastrointestinal transit up to 100 mg/kg (resulting in a mean plasma concentration of 1.63 ± 0.14 µg/mL, a value 7-fold greater than the predicted clinical C_{max}).

ABT-888 dihydrochloride was evaluated in repeated dose toxicity studies in rats and dogs. When administered as a sole agent to rats, the compound did not result in adverse effects at C_{max} values that were greater than 19-fold the estimated therapeutic peak plasma drug concentration (highest dose tested). When rats were administered ABT-888 dihydrochloride in conjunction with a cytotoxic agent (cisplatin), no clinically meaningful exacerbations of cisplatin-associated toxicity were apparent at C_{max} values that were up to 8-fold greater for ABT-888 than the estimated therapeutic value. Exacerbation of cisplatin-associated toxicity was limited to rats that received ABT-888 dihydrochloride in conjunction with cisplatin at the highest dose that yielded C_{max} values 22-fold greater than the estimated therapeutic peak plasma drug concentration. In dogs, emesis, body weight losses related to anorexia, and convulsions were observed at doses of 30 mg base/kg/day with C_{max} values 26-fold greater than the estimated therapeutic peak plasma concentration. ABT-888 dihydrochloride was found to be negative *in vitro* for both mutagenicity and clastogenicity.

The non-toxic dose observed in the most sensitive mammalian species (beagle dogs) was 300 mg/m^2. Emesis and QT prolongation were observed in animal models, at 31-fold and 21-fold higher concentrations than the predicted clinical C_{max} (0.21 µg/mL), respectively. Based on different sensitivities to seizures between rodents and dogs, the plasma concentration that would be associated clinically with pro-convulsant activity will be difficult to define.

Clinical Investigations

A single-dose pharmacokinetic and pharmacodynamic endpoint study in cancer patients was initiated under an exploratory IND by the National Cancer Institute as the initial study in their phase 0 program (Kummar *et al.*, 2009). In this study, participants had baseline assessments of PAR in peripheral blood mononuclear cells (PBMCs) and at higher dose levels, in tumor from needle biopsies, assessed by a validated immunoassay. Participants received a single dose of ABT-888 at 10, 25, or 50 mg. PBMCs were collected over a 24 hour period at all dose levels, and tumor biopsies were obtained at the 25 mg dose level, approximately 3 to 6 hours after administration of ABT-888. A total of 6 patients have been studied so far, 3 each for the 10 mg and 25 mg cohorts. No treatment related adverse events have been observed. The target plasma C_{max} of 210 nM was exceeded in 2 of 3 patients at the 10 mg dose level, and in all three patients for at least 4 hours at the 25 mg dose level. Levels of PAR were reduced 80-99% from baseline levels after administration of ABT-888 in both the PBMCs and tumor samples at the 25 mg dose level. Thus, there is reason to believe that target inhibition is seen at least at the 25 mg dose level, and may be occurring at doses lower than 25 mg.

Currently, several combination phase I trials are underway. Also, a single agent trial had been initiated in the BRCA-deficient population.

2.4 Abiraterone Acetate

Abiraterone acetate and its metabolite abiraterone are potent and selective inhibitors of CYP17 α-hydrxylase and $C_{17,20}$–lyase activites, both essential steps in androgen biosynthesis.[74] In the two Phase III studies, COU-AA-301 and COU-AA-302, subjects have been exposed to abiraterone acetate and placebo in a 2:1 ratio, and 1:1 ratio, respectively. [10,11,75] Overall, abiraterone acetate has been very well tolerated with no dose limiting toxicity observed in several trials treating patients with up to 2,000 mg daily. Some evidence of secondary mineralocorticoid excess (hypertension, hypokalemia and edema) has been observed in the initial studies; it is now recommended that abiraterone acetate be given with a low dose of glucocorticoid such as prednisone 5 mg PO twice daily to abrogate these symptoms, while others have advocated the use of a mineralocorticoid receptor antagonist. [74,76] Based on the PK profile and the overall toxicity pattern, the recommended Phase II testing dose of abiraterone acetate was 1,000 mg daily and this regimen in combination with prednisone of 5 mg twice a day was tested in the Phase III trials.

Several trials involving mCRPC have been recently reported.[77] COU-301 randomized mCRPC patients with prior docetaxel therapy in a 2:1 ratio to abiraterone + prednisone vs placebo + prednisone. 1,195 men were enrolled and treated. There was a statistically significant improvement in median overall survival (14.8 versus 10.9 months) favoring the abiraterone arm ($p<0.001$). There was also improvement in the time to PSA progression, progression-free survival and PSA response rate in the abiraterone acetate treatment arm. [11] Abiraterone with prednisone was approved by the FDA in April 2011 for use in mCRPC following docetaxel. The most common adverse events, which were associated with increased mineralocorticoid levels, included hypokalemia, fluid retention, and hypertension; these events were largely abrogated by coadministering low-dose glucocorticoids.[11]

Recently this agent also received FDA approval for use in patients with mCRPC and no prior chemotherapy based on improvement in progression-free survival. [78]

2.5 Rationale for Combination Abiraterone and ABT-888 Therapy Irrespective of ETS Gene Fusions Status

This biomarker stratified and randomized phase II trial will evaluate the role of ETS gene fusion as a predictive biomarker for response to hormone therapy alone or hormone therapy plus PARP-1 targeted therapy in patients with mCRPC. The study will also evaluate whether the addition of PARP-1 targeted therapy is superior to hormone therapy based on gene fusion status. The scientific rational for this study is supported by:

1. Abiraterone is FDA approved based on prolonging survival in patients with mCRPC post docetaxel and progression free survival in patients with no prior docetaxel therapy; however the effect is modest and not all patients benefit.
2. ETS gene fusions are predominantly driven by an androgen-sensitive promoter. Data from radical prostatectomy series suggest that ETS fusion status predicts for response to adjuvant androgen deprivation therapy[36] and preliminary data from phase I/II studies of

mCRPC patients suggest that abiraterone may have greater therapeutic effect in ETS-fusion positive prostate cancer patients. [7,37]

3. There is interaction of PARP1 with the androgen signaling cascade, regardless of ETS fusion status and with ETS fusions; our data indicate that ERG-positive xenografts are preferentially sensitive to PARP-1 inhibitors.
4. ABT-888 has been demonstrated to have efficacy across a wide range of tumor types in preclinical studies.[79] ABT-888 has been demonstrated to inhibit PARP1 in a clinical phase 0 study, and is currently being assessed as a component of combination therapy across a range of tumor types clinically, including breast, liver, and ovarian cancer, as well as an unselected metastatic prostate cancer population. We have conducted in collaboration with Abbott a clinical trial with ABT-888: M11-070 Protocol A Pilot Study Combining ABT-888 (an Oral PARP Inhibitor) + Temozolomide in Patients with Metastatic Castration Resistant Prostate Cancer Who Have Failed Up to Two Non-hormonal Systemic Therapies. The interim data suggests its feasible to administer ABT-888 in combination and that there is a signal of clinical activity.

2.6 Correlative Studies Background

2.6.1 The Role of ETS Gene Fusions (in Metastases) as a Predictive Biomarker for Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic Castration-Resistant Prostate Cancer (CRPC) (Primary Objective #1)

2.6.2 The Role of ETS Gene Fusions (in Metastases) as a Predictive Biomarker for Treatment Benefit for the Addition of ABT-888 to Abiraterone (Primary Objective #2)

Correlative Studies 2.6.1 and 2.6.2 represent the primary objectives of this biomarker-based translational clinical trial--to determine whether ETS fusion status predicts for outcome following abiraterone alone and abiraterone in combination with ABT-888, and to determine whether ETS fusion status predicts which patients will specifically benefit from the addition of ABT-888 to abiraterone. The background and rationale for these two correlative studies have been described in section 2.2.

2.6.3 Concordance in ETS Fusion Status among Primary Tumor, Metastases, and Circulating Tumor Cells in Patients

The concordance of ETS fusion status among primary tumors, metastases and circulating tumor cells (CTCs) is not well known at this time. In one limited study conducted at the time of autopsy, there was complete concordance among samples from metastases and primary tumor from 10 patients in which both these samples were available at the time of autopsy (5 ETS-positive, 5 ETS-negative)[80] and from 11 similar patients on a second study (5 ETS-positive, 6 ETS-negative).[37,81] However, the concordance between metastases and primary tumor is unknown in the context of multifocal primary disease in the prostate. As the correct assignment of ETS fusion

status in the metastases is critical in this study, as it will dictate assignment to treatment arms, ETS fusion status will be assessed in metastatic samples, unless an interim analysis planned after accrual of 38 patients to each ETS strata (see Section 13.1, Interim Analysis Plan) demonstrates sufficient concordance between the primary and metastatic samples.

Of note, in the previously mentioned autopsy study, of the 20 patients who had multiple metastases from which ETS fusion status could be determined, it was noted that all metastases had the same ETS status, and when an ETS rearrangement was present in one metastases within a patient, the exact same rearrangement was present in all assessable metastases within the same patient, supporting a clonal origin to the metastases.[80] This finding supports the rationale for biopsying only one metastatic site (and not more) in this trial.

The concordance of ETS status between primary tumor and CTCs has also not been extensively studied. However, a recent study demonstrated that the concordance of ETS status between primary tumor and CTCs was only 65% (concordant in 15 out of 23 cases).[81] The concordance of ETS status between CTCs and metastases has also been studied in limited patients, with 100% concordance (7 ETS positive, 4 ETS negative).[37] Therefore, a secondary correlative objective of this study is to establish the concordance among ETS fusion status, in a significantly larger study than any previously conducted, among primary tumors, metastases, and CTCs. If the concordance between primary tumor and metastases, or CTCs and metastases, is confirmed to be high, then ETS status in the primary tumor or CTCs may serve as a surrogate for ETS status in the metastases, on future studies of ETS fusion-directed therapy.

To assess ETS fusion status in the circulating tumor cell study, we will utilize a platform developed by Epic Sciences for CTC isolation and identification, which has previously been described.[82-86] This platform allows for analysis of specific biomarkers, such as analysis of the status of ERG, in CTCs. The remainder of the CTC studies described below and in this protocol will utilize these same assays.

2.6.4 The Role of ETS Gene Fusions (in Circulating Tumor Cells) as a Predictive Biomarker of Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic CRPC

2.6.5 Circulating Tumor Cell Count and Biomarker Status as a Predictive Biomarkers of Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic CRPC

Based on the results of a small study,[37] ETS fusion status in CTCs may predict for PSA response in mCRPC patients treated with abiraterone based on the results of a 89 patient phase I/II study, though this association was not found to be statistically

significant on a smaller 41 patient study.[81] We hypothesize that ETS fusion status in CTCs, as well as CTC count, may predict for longer-term PSA responses, and will explore this in the larger cohort proposed for this study. In addition, given the previously demonstrated relationships (in preclinical models) between ETS fusions and the androgen receptor,[14,87] as well as ETS fusions and DNA damage,[88] we hypothesize that treatment sensitivity to abiraterone +/- a PARP1 inhibitor may also correlate to the status of the androgen receptor and markers of DNA damage/repair (such as gamma H2aX and RAD51 foci).

2.6.6 The Role of PTEN Loss as a Predictive Biomarker for Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic Castration-Resistant Prostate Cancer (CRPC)

In addition to ETS fusion status, PTEN loss is a promising predictive biomarker of response to abiraterone alone or in combination with PARP1 inhibition. Specifically, preclinical work from Sawyers laboratory in Memorial Sloan Kettering Cancer Center demonstrated that, in genetically engineered mouse models of prostate cancer, conditional loss of PTEN reduced expression of androgen receptor target genes.[89] Using these models, they further demonstrated that PTEN-loss renders prostate cancers less androgen-dependent, and unresponsive to combined androgen blockade.[89] Additionally, using transcriptomic analyses of 218 primary prostate tumors, this group confirmed that prostate cancers with PTEN loss demonstrated decreased androgen-receptor transcriptional output.[89] Therefore, PTEN loss is a promising predictor of resistance to next-generation anti-androgens, such as abiraterone.

Additionally, PTEN deletion has been demonstrated to confer a defect in homologous recombination, which can result in synthetic lethality with PARP1 inhibition, analogous to BRCA mutations.[90,91] Moreover, PTEN deletion has been demonstrated to confer sensitivity to PARP1 inhibition in multiple preclinical cancer models.[90,92] Thus, PTEN loss may also predict response to PARP1 inhibition. Based on a recent abstract demonstrating extremely high concordance between PTEN status in the CTCs and the metastases from a limited number of patients, we will assess for PTEN loss, by FISH, in both CTCs and metastases. [93]

2.6.7 The Role of PARP1 Activity (in Metastases) as a Predictive Biomarker for Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic Castration-Resistant Prostate Cancer (CRPC)

Similar to PTEN deletion, increased PARP1 activity may predict resistance to abiraterone and sensitivity to ABT-888. Unpublished data from Karen Knudsen at Thomas Jefferson University show a correlation between PARP1 activity (as detected by PAR levels) and castration resistance in cell line models of prostate cancer. Specifically, the androgen-sensitive cell lines VCaP, LAPC4, and LnCAP

demonstrated no or low PAR levels, but the androgen-resistant cell lines C42 and LnCAP-abl demonstrated increased PAR levels. This increased PAR levels was coincident with heightened, differential AR activity, consistent with castration-resistance. Therefore, PARP1 activity, as reflected in PAR levels, may be a biomarker of resistance to abiraterone. Conversely, increased PARP1 activity may suggest a dependence on the PARP1 pathway for a malignant phenotype, and therefore may predict for sensitivity to PARP1 inhibition.

2.6.8 Discovery of Novel Gene Fusions Through Paired-End Transcriptome Sequencing of Metastases

While ETS gene fusions are the most common gene fusions found in prostate cancer, there are other gene fusions, such as RAF fusions[17] that can also be found. Additionally, there are molecular alterations, such as massive overexpression of SPINK1, for which the underlying genetic cause has not yet been discovered.[94] The ETS fusions, RAF fusions, and SPINK1 overexpression are unique molecular events, in the sense that, in hundreds of patients analyzed by our group to date, there is no overlap between these alterations--they are mutually exclusive.[17,94] Thus, there is rationale to suggest that they may be unique molecular subtypes of prostate cancer, and potentially targetable defining alterations. The goal of this discovery-based correlative aim is to perform next-generation sequencing approaches to identify novel targetable gene fusions in prostate cancer management.

2.6.9 Discovery of novel SNPs Predictive of Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic Castration-Resistant Prostate Cancer (CRPC)

Single nucleotide polymorphisms (SNPs) are single nucleotide base pair changes in DNA sequences which occur in at least 1% of the population.[95] SNPs can affect gene function through multiple mechanisms including: missense mutations causing conformational changes in protein structure, nonsense mutations creating aberrant stop codons and premature truncation of RNA transcription, and mutations in non-coding gene regulatory regions causing abnormal gene splicing and transcription. Due their high frequency and the availability of high-throughput multiplex SNP array platforms, SNPs have been a frequent target of cancer biomarker investigations.

In metastatic prostate cancer patients, SNPs in genes critical to hormone synthesis, transport, binding, metabolism, and degradation have been analyzed to identify subsets of patients with favorable therapy outcomes. Specifically, investigators from the Dana Farber Cancer Institute have reported an improved time to progression in CRPC patients treated with ketoconazole (an older, less potent CYP17-lyase inhibitor than abiraterone) in association with a germline SNP in the SLCO1B3 gene, a gene involved in steroid transport.[96] Similarly, investigators at the Indiana University Simon Cancer Center have recently reported an improved overall survival in CRPC patients treated with ketoconazole in association with additional germline SNPs

involved in hormone pathway genes including: AKR1C3, CYP19A1, CYP11B1, and HSD17B4.[97] Taken together, these studies provide preliminary evidence that common variations in genes critical to hormone synthesis, signaling, and degradation may impact clinical efficacy of additional hormonal therapies in CRPC patients. No investigations of SNPs associated with favorable clinical outcomes to abiraterone therapy in CRPC patients have been reported. The goal of this correlative aim will be to identify candidate germline SNPs associated with improved clinical outcomes to abiraterone therapy in CRPC patients.

2.6.10 The Role of ETS fusion RNA levels (in blood) as Predictive Biomarkers for Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic Castration-Resistant Prostate Cancer (CRPC)

As described in Section 2.2, ETS gene fusions result in androgen-dependent over-expression of ETS transcription factors. Messenger RNA (mRNA) levels of the predominant ETS fusion, TMPRSS2:ERG (found in 50% of all prostate cancers) can be measured in blood from patients with CRPC.[98] The goals of this study will be to determine the correlation between TMPRSS2:ERG mRNA levels in the blood and TMPRSS2:ERG fusion status in metastatic and primary tumors, and to determine of TMPRSS2:ERG levels may predict for therapeutic response to abiraterone +/- ABT-888 in patients with TMPRSS2:ERG-positive tumors.

3. PATIENT SELECTION

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday 4 weeks later would be considered Day 28. This allows for efficient patient scheduling without exceeding the guidelines. If Day 28, 42, or 56 falls on a weekend or holiday, the limit may be extended to the next working day.

3.1 Eligibility Criteria

At registration all patients must:

3.1.1 Have a histologic or cytologic diagnosis of prostate cancer.

3.1.2 Have progressive metastatic castration resistant prostate cancer, on androgen deprivation therapy, based on **at least one** of the following criteria:

a) PSA progression defined as 25% increase over baseline value with an increase in the absolute value of at least 2 ng/mL that is confirmed by another PSA level with a minimum of a 1 week interval with a minimum PSA of 2 ng/ml.

b) Progression of bidimensionally measurable soft tissue (nodal metastasis) assessed within one month prior to registration by a CT scan or MRI of the abdomen and pelvis *(please refer to section 11.1.2 for definitions of measurable disease).*

c) Progression of bone disease (evaluable disease) (new bone lesion(s)) by bone scan. *(please refer to section 11.1.2 for definitions of evaluable disease)*.

3.1.3 Agree to undergo a biopsy of at least one metastatic site for gene fusion status analysis (*Please refer to Appendices C and D for specific procedures*). Adequate metastatic tissue from prior biopsy/resection can be used if available in lieu of a biopsy. Patients will only be eligible for protocol therapy if the biopsy has tumor and the tissue is evaluable for ETS fusion status.

3.1.4 ECOG performance status of 0-2.

3.1.5 Age ≥ 18 years.

3.1.6 Have testosterone < 50 ng/dL. Patients must continue primary androgen deprivation with an LHRH analogue if they have not undergone orchiectomy.

3.1.7 Patients must discontinue antiandrogen therapy for at least 4 weeks (e.g. flutamide, bicalutamide, nilutamide) prior to registration with no evidence of a falling PSA after

washout. Patients on steroids are eligible as long as they will be switched to prednisone as outlined in the Treatment Plan, Section 5.

3.1.8 Have no prior exposure to CYP-17 (other than ketoconazole) or PARP inhibitors for prostate cancer. Patients with prior exposure to ketoconazole are eligible.

3.1.9 Patients with up to 2 prior chemotherapy regimens are eligible.

3.1.10 Patients must have normal organ and marrow function as defined below obtained within 14 days prior to registration:

WBC	≥ 3,000/µl
ANC	≥ 1,500/µl
Platelet count	≥ 100,000/µl
Serum Creatinine	≤ 1.5 x the institutional upper limits of normal or corrected creatinine clearance of ≥ 50 mg/ml/hr/1.73 m^2 BSA
Potassium	≥ 3.5 mmol/L
Bilirubin	within the institutional limits of normal
SGOT (AST) SGPT (ALT)	≤ 2 x upper limits of normal ≤ 2 x upper limits of normal

3.1.13 The effects of ABT888 and Abiraterone on the developing human fetus at the recommended therapeutic dose are unknown, and may cause fetal harm or loss of pregnancy. Men must agree to use effective contraception during treatment and for at least 1 week after the last administration of therapy.

3.1.14 Patients must be able to take oral medication without crushing, dissolving or chewing tablets.

3.1.15 Patients may have received prior radiation therapy or surgery. However, at least 21 days must have elapsed since completion of radiation therapy or surgery and patient must have recovered from all side effects at the time of registration.

3.1.16 Ability to understand and the willingness to sign a written informed consent document that is approved by the local institutional review board.

3.2 Exclusion Criteria

3.2.1 Patients may not be receiving any other investigational agents. Any prior investigational products must be stopped at least 14 days (2 week washout) prior to registration.

3.2.2 Patients who have had chemotherapy, radiotherapy or oral antifungal agents (Ketoconazole, itraconazole, fluconazole) within 3 weeks prior to entering the study

or those who have not recovered (e.g. back to baseline or grade 1) from adverse events due to agents administered more than 3 weeks earlier.

There is a potential drug interaction when abiraterone is concomitantly used with a CYP2D6 substrate narrow therapeutic index (e.g., thioridazine, dextromethorphan), or strong CYP3A4 inhibitors (e.g., atazanavir, erythromycin, indinavir, itraconazole, Ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, and voriconazole) or strong inducers (e.g., carbamazepine, Phenobarbital, phenytoin, rifabutin, rifampin, rifapentine).
Caution should be used when patients are on one of these drugs.

3.2.3 Patients with history of active seizures are not eligible.

3.2.4 Patients with a history of pituitary or adrenal dysfunction, active or symptomatic viral hepatitis or chronic liver disease are not eligible.

3.2.5 Patients with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

3.2.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to ABT-888 or Abiraterone.

3.2.7 Patients may continue on a daily Multi-Vitamin, calcium and Vitamin D, but all other herbal, alternative and food supplements (i.e. PC-Spes, Saw Palmetto, St John's Wort, etc.) must be discontinued before registration. Patients must not be planning to receive any concurrent cytotoxic chemotherapy, surgery, or radiation therapy during protocol treatment. Hormonal-acting agents (including diethylstilbestrol/DES, aldosterone, and spironolactone) are forbidden during the trial and must be stopped prior to registration. No washout period will be required for any of these agents. Patients on megesterol acetate for hot flashes are allowed to continue therapy.

3.2.8 Patients on stable doses of bisphosphonates or denosumab which have been started prior to registration may continue on this medication, patients who are not on bisphosphonates or denosumab are eligible as long as they initiate therapy prior to registration.

3.2.9 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure (New York Heart Association Class III and IV heart failure), unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements or concurrent medications that alter cardiac conduction.

3.2.10 Patients with a "currently active" second malignancy other than non-melanoma skin cancers are not eligible. Patients are not considered to have a "currently active" malignancy if they have completed all therapy and are now considered without evidence of disease for 1 year.

3.2.11 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with ABT-888. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.

3.3 Inclusion of Women and Minorities

This study applies only to men. All races and ethnic groups are eligible for this trial. The anticipated accrual in the ethnicity/race categories is shown in the table below.

Ethnic Category	**Males**	**Total**
Hispanic or Latino	16	16
Not Hispanic or Latino	132	132
Total Ethnic	148	148
Racial Category		
American Indian or Alaskan Native	1	1
Asian	4	4
Black or African American	27	27
Native Hawaiian or other Pacific Islander	0	0
White	116	116
Racial Category: Total of all Subjects	148	148

4.0 REGISTRATION PROCEDURES

4.1 Initial Registration

All patients must be registered with the University of Chicago (UC) Registrar Jeffrey Bozeman at 773-834- 3095 or jbozema1@.bsd.uchicago.edu at least 2 business days prior to any planned biopsy. Because a Multi-Site Study Coordinator of the Clinical Trials Office at the University of Michigan will work collaboratively with the UC registrar, to review the submitted documents and process the registration, participating sites should inform the UC registrar Mon-Fri, 8:15am-4:00pm CST of a potential registration. Same day registrations cannot be guaranteed.

Confirm all selection criteria listed in Section 3.0, and then call the UC Registrar with the following information:

Copy of required laboratory tests
Signed patient consent form

Completed Eligibility checklist
Provider of information
Study # and Institution
Treating Physician
Patient name and hospital ID number
Patient's zip code of residence Date
of signed informed consent Race,
gender, date of birth of patient
Diagnosis and date of initial diagnosis

As noted, the UC Registrar will work collaboratively with Dr. Hussain and her research team at the University of Michigan to verify each patient's eligibility and then issue a confirmation of registration. To complete the registration process, the UC registrar will:

- Assign a patient study number
- Register the patient on the study
- Fax or e-mail the patient study number to the participating site and to the Michigan Center for Translational Pathology (see section 9)
- Call the research nurse or data manager at the participating site and verbally confirm registration.
- Fax a copy of the completed Section Two of the Eligibility Worksheet signed and dated by the UC registrar to the requesting site.
- Patients found to be ineligible for participation after being consented will be considered screen failures, and documented as such in the Screening and Enrollment Log. These patients will not have study identification number assigned to them, and will not undergo a biopsy and not receive study treatment.

4.2 Biopsy and randomization

Following registration, patient biopsies must be performed within 10 calendar days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient is eligible for treatment, therapy must start within three weeks from the biopsy dates.

Biopsy tissue should be processed and shipped to the Michigan Center for Translational Pathology as specified in section 9. The coordinator for the Michigan Center for Translational Pathology will inform University of Chicago (UC) Registrar Jeffrey Bozeman at 773-834- 3095 or jbozema1@.bsd.uchicago.edu as well as the participating site whether the patient has adequate tissue for fusion analysis.

If the patient does not have adequate tissue for fusion analysis, he will be removed from protocol unless the patient is willing to undergo another biopsy. Repeat biopsy will need to be done within 10 calendar days as long as patient still fulfills other eligibility criteria. No study labs are needed but safety labs can be done as deemed necessary by the managing oncologist. Patients will be randomized if on repeat biopsy tissue is adequate for ETS fusion status evaluation.

If the patient has adequate tissue for fusion analysis, the coordinator for the Michigan Center for Translational Pathology will also inform the statistician, Stephanie Daignault-Newton at 734-647-3271 or sfaruzzi@umich.edu of the fusion status so appropriate randomization stratified by ETS gene fusion status can be performed. (See Section 5, Treatment Plan). Randomization will be conducted using pre-printed cards and sealed envelopes.

Randomized patients should begin protocol treatment within 7 working days from randomization. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. University of Chicago (UC) Registrar Jeffrey Bozeman should be notified of cancellations as soon as possible.

4.3 Drug ordering

Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov).

4.4 Registration Process & Data Submission for all Participating Sites

Registration
Submit the **Clinical Trial Patient Registration Form** and all source documentation for the protocol required eligibility criteria and pre-study procedures.

All required forms and source documentation should be faxed or mailed to:

UC Phase II Clinical Research Associates
5841 S. Maryland Avenue MC 2115
Chicago, IL 60637
Fax: (773) 702-4889

Weekly:
Submit the **Clinical Trial Patient Weekly Treatment Summary Form** with supporting source documentation by noon on Friday of each week, for review at the weekly Phase II Conference. All other source documentation for protocol required procedures should be submitted within a week after it is created or modified.

Evaluations:
At each response evaluation as specified in the protocol, submit supporting source documentation for the response.

Off-study:
Submit the **Clinical Trial Patient Off Treatment Form** with the appropriate source documentation

Follow-up:
Submit the **Phase II Consortium Affiliate Clinical Trial Patient Follow-Up Form** with appropriate source documentation**.**

5. TREATMENT PLAN

5.1 Stratification and Randomization

All eligible patients with adequate tissue for fusion analysis will be stratified by their prior ketoconazole therapy (Yes/No) and ETS gene fusion status (Positive or Negative) and then randomized to receive Abiraterone alone or Abiraterone +ABT-888 as follows. As noted above, treatment must start within 7 working days of randomization, and patients must continue to meet the noted eligibility criteria specified in section 3:

Arm 1: ETS Fusion-Positive Prostate Cancer
Patients will be randomized to one of the following arms:

Arm IA: Abiraterone + Prednisone
Abiraterone 1000 mg orally once daily and prednisone 5 mg orally twice daily, days 1-28 in 28 day cycles.

Arm IB: Abiraterone + Prednisone + ABT-888
Abiraterone 1000 mg orally once daily and Prednisone 5 mg orally twice daily. **Only for cycle 1, Abiraterone and Prednisone* will start on day 8 to allow lead-in with ABT-888 and will continue through day 28.** All subsequent cycles Abiraterone/prednisone will start on day 1 and continue daily in 28 days cycle.
ABT-888 200 mg will be given BID orally on days 1-28 in 28 day cycles. If after the first cycle of therapy the patient tolerates therapy well then the ABT-888 dose can be escalated to 300 mg BID for cycle 2 and thereafter.
** patients who have been on prednisone prior to registration may continue it during the lead in ABT-888 therapy.*

Arm II: ETS Fusion-Negative Prostate Cancer
Patients will be randomized to one of the following arms:

Arm IIA: Abiraterone + Prednisone
Abiraterone 1000 mg orally once daily and prednisone 5 mg orally twice daily, days 1-28 in 28 day cycles.

Arm IIB: Abiraterone + Prednisone + ABT-888
Abiraterone 1000 mg orally once daily and Prednisone 5 mg orally twice daily. **Only for cycle 1, Abiraterone and Prednisone will start on day 8 to allow lead-in with ABT-888 and will continue through day 28.** All subsequent cycles Abiraterone/prednisone will start on day 1 and continue daily in 28 days cycle. ABT-888 200 mg will be given BID orally on days 1-28 in 28 day cycles. If after the first

cycle of therapy the patient tolerates therapy well then the ABT-888 dose can be escalated to 300 mg BID for cycle 2 and thereafter.
** patients who have been on prednisone prior to registration may continue it during the lead in ABT-888 therapy.*

5.2 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

REGIMEN DESCRIPTION				
Agent	**Dose**	**Route**	**Schedule**	**Cycle Length**
Abiraterone*#	1000mg	Oral	Once daily, Days1-28 #	28 days (4 weeks)
Prednisone**#	5 mg	Oral	Twice daily. Days 1-28 #	
ABT-888***	Start at 200 mg escalate to 300 mg after the 1st cycle if patient tolerates 200 mg	Oral	BID, Days1-28	

***Abiraterone acetate**: Four (250 mg) tablets (total dose /day 1,000 mg) orally on an empty stomach, 1 hour before or 2 hours after a meal. The tablets are to be swallowed whole with water. Tablets cannot be broken, crushed, or chewed. Abiraterone acetate therapy must be accompanied by prednisone.
** **Prednisone**: Predisone 5 mg to be taken with food twice daily.
#: For patients randomized to Arms IB and IIB: **For Cycle 1 only** Abiraterone and Prednisone will start on day 8 daily to allow lead in ABT888 therapy. For all subsequent cycles Abiraterone/prednisone will start on day 1 and continue daily in 28 days cycle.
***ABT888:** Take the capsules to be taken orally twice daily with or without meals. Tablets are not to be crush, broken, or chewed

- Patients will continue treatment until disease progression or other reason for discontinuation of protocol treatment.

Intake Calendar: Abiraterone acetate, prednisone and ABT- 888 compliance will be recorded on the Intake Calendar (see Appendix F and G). Institutional staff will review and ascertain patient adherence with protocol treatment. If a dose is missed, patients are to take the normal dose on the following day. If more than one daily dose is missed, the study doctor or study team must be informed5.3 General Concomitant Medication and Supportive Care Guidelines

5.3.1 Several medications are specifically disallowed. Antiandrogens (e.g. flutamide, bicalutamide, and nilutamide) may not be used. Five-alpha reductase inhibitor (e.g. finasteride and dutasteride) are not permitted. Other excluded therapies include **ketoconazole,** diethylstilbestrol/DES, aldosterone, spironolactone, and PC-SPES. Other excluded treatments include chemotherapy, immunotherapy, and radiopharmaceuticals or any other therapy intended to treat prostate cancer. **Oral** antifungal medication such as **ketoconazole**, fluconazole and itraconazole (or related antifungal medication) are not allowed.

5.3.2 In case participants develop nausea/vomiting/diarrhea or myelosuppression, supportive medications will be prescribed as per Clinical Center and ASCO guidelines. Seizures were seen in some animal toxicology studies, although at doses much higher than those anticipated for this study. Seizures in animals were successfully treated with lorazepam. The use of supportive care medications is allowed according to institutional standards.

5.3.3 Because there is a potential for interaction of Abiraterone 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.

Potential Drug Interaction with Abiraterone Acetate

Effects of Abiraterone on Drug Metabolizing Enzymes

Abiraterone is an inhibitor of the hepatic drug-metabolizing enzyme CYP2D6. In a CYP2D6 drug-drug interaction trial, the Cmax and AUC of dextromethorphan (CYP2D6 substrate) were increased 2.8- and 2.9-fold, respectively, when dextromethorphan was given with abiraterone acetate 1,000 mg daily and prednisone 5 mg twice daily.

Avoid co-administration of abiraterone acetate with substrates of CYP2D6 with a narrow therapeutic index (e.g., thioridazine). If alternative treatments cannot be used, exercise caution and consider a dose reduction of the concomitant CYP2D6 substrate drug

Drugs that Inhibit or Induce CYP3A4 Enzymes

Based on in vitro data, Abiraterone is a substrate of CYP3A4. The effects of strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, indinavir, nelfinavir, voriconazole) or inducers (e.g., phenytoin, carbamazepine, rifampin, rifabutin, rifapentine, phenobarbital) on the pharmacokinetics of abiraterone have not been evaluated, in vivo. Avoid or use with caution, strong inhibitors and inducers of CYP3A4 during Abiraterone treatment

While abiraterone acetate exhibited inhibition of P450 CYPs 2C19, 2D6, and 1A2 in the in vitro enzyme interaction studies, these inhibitory effects were an order of magnitude weaker than those for classic inhibitors of 1A2 and 2D6, and 5 fold weaker than for classic inhibitors of 2C19.

There have been no reports of clinically significant drug-drug interactions involving abiraterone. Table below identifies medications that could potentially be affected in subjects receiving abiraterone acetate.

Drug Name	**P450 Mechanism**	**Possible Effect**
Clopidogrel	2C19 needed for prodrug activation	Decreased efficacy
Ticlopidine	2C19 needed for prodrug activation	Decreased efficacy
Metoprolol	Metabolized by 2D6	Increased drug levels
Propranolol	Metabolized by 2C19	Increased drug levels
Flecainide	Metabolized by 2D6	Increased drug levels
Propafenone	Metabolized by 2D6	Increased drug levels
Haloperidol	Metabolized by 2D6	Increased drug levels
Fluoxetine	Metabolized by 2C19	Increased drug levels
Paroxetine	Metabolized by 2C19	Increased drug levels
Sertraline	Metabolized by 2D6	Increased drug levels
Amoxapine	Metabolized by 2C19	Increased drug levels
Clomipramine	Metabolized by 2D6	Increased drug levels
Desipramine	Metabolized by 2D6	Increased drug levels
Doxepin	Metabolized by 2D6	Increased drug levels
Imipramine	Metabolized by 2D6	Increased drug levels
Nortriptyline	Metabolized by 2D6	Increased drug levels
Protriptyline	Metabolized by 2D6	Increased drug levels
Oxycodone	Metabolized by 2D6	Increased drug levels
Codeine	Prodrug activated by 2D6 to form Morphine	Decreased efficacy
Tamoxifen	Prodrug activated by 2D6	Decreased efficacy
Theophylline	Metabolized by 1A2	Increased drug levels
Cimetidine	Metabolized by 2C19	Increased drug levels

5.3.4 ABT-888 is not known to be a potent inhibitor of the major human CYPs in vitro, indicating a low risk for drug-drug interactions at the proposed dosing concentrations.
Specifically, ABT-888 (veliparib) is not a potent inhibitor of CYP1A2, CYp2A6, CYP2C9, CYP2Ci19, CYP2D6, CYP2E1, CYP2B6, CYP2C8, or CYP3A4 in human liver microsomes, (IC50 > 30 μM), suggesting that ABT-888 is not likely to inhibit the metabolism of drugs that are substrates of the aforementioned CYPs at the projected therapeutic concentration (≈1 μM).

ABT-888 did not significantly induce the activities of CYP1A2, 2B6, 2C9, or 3A4 in human hepatocytes from 4 donors at the drug concentrations up to 10 μM.

5.3.5 Several medications are specifically allowed. LHRH agonist or antagonist must be continued throughout the study and are considered a standard of care treatment for this patient population. Conventional multi-vitamins and additional glucocorticoid use, beyond prednisone 5 mg twice daily, is allowed as deemed medically necessary.

5.3.6 Transfusions and hematologic growth factors are allowed in accordance with institutional guidelines.

5.4 Duration of Therapy

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

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s) (see section 6),
- Therapy delay for more than 4 weeks
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.5 Duration of Follow Up

Patients removed from study for progression will be followed for 4 weeks or resolution or stabilization of any therapy related adverse events.

Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. Once off study for any reason, follow-up visits to assess progression will be completed every 6 months, until objective progression or 2 years, whichever occurs first.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 CTCAE

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 4.0 for toxicity and Serious Adverse Event reporting. A copy of the CTCAE Version 4.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE Version 4.0.

6.1.1 Expedited Reporting of Toxicities

Toxicities (including suspected reactions) that meet the expedited reporting criteria as outlined in section 7.0 of the protocol must be reported to the Coordinating Center, Study Coordinator and NCI via CTEP-AERS, and to the IRB per local IRB requirements.

6.2 Guidance Regarding Dose Adjustments for Adverse Event Based on Attribution

For laboratory or clinical Adverse events (AEs) that warrant a dose reduction, the dose-reduction of the drug will be based on the attribution of the AE. If the event is related to one drug the dose-reduction will be made to only that drug, if AE is related to both then both drugs should be dose reduced.

6.3 PI contact number

For treatment or dose modification related questions, please contact Dr. Maha Hussain at 734/936-8906.

6.4 Abiraterone and Prednisone dose levels are defined below:

Dose Level	Abiraterone acetate	Prednisone
0	1,000 mg daily	5 mg twice daily
-1	750 mg daily	5 mg twice daily
-2	500 mg daily	5 mg twice daily

- A dose reduction will be pursued for any serious adverse event, defined as any Grade 4 toxicity and also for any Grade 3 toxicity that is believed to potentially impact the safety of the participant. The dose of abiraterone will not be re-escalated once a dose reduction has occurred. Abiraterone will be held and the subjects followed until resolution of any Grade 3 or 4 adverse event to ≤ Grade 1.

- Asymptomatic laboratory abnormalities would not be considered a serious adverse event unless the investigator believes it may potentially impact the participant's safety or unless it is specifically addressed separately in the protocol (e.g. hypokalemia and liver test abnormalities).

- Due to the known side effect of hypokalemia, oral replacement of potassium is to be started with grade 1 hypokalemia. With Grade 3 or 4 hypokalemia, abiraterone acetate is to be held until it resolves to ≤ Grade 1 and IV potassium replacement is to be given with appropriate monitoring.

- If Grade 2 or higher liver function test abnormalities (AST, ALT or bilirubin) develop, the liver function tests should be followed at least weekly until they resolve to ≤ Grade 1. If Grade 3 liver test abnormities (AST, ALT or bilirubin) develop, abiraterone acetate and any other hepatotoxic drugs should be held until they resolve to ≤ Grade 1. In the specific case of Grade 3 AST, ALT or bilirubin abnormalities, a mandatory dose reduction of abiraterone acetate to 500 mg daily must be undertaken, with every 2 week assessments of the liver function tests for at least 3 months. If Grade 4 liver test abnormalities (AST, ALT or bilirubin) occur, the drug should be permanently discontinued. The Multi-site study coordinator should also be notified with any Grade 3 or 4 liver function test abnormality.

- If abiraterone acetate is delayed more than 4 weeks for any reason, or if a dose reduction of abiraterone acetate below 500 mg daily is required, patients will be removed from protocol treatment.

- Prednisone is included in this regimen primarily as a safety medication to reduce the potential incidence of mineralocorticoid excess from abiraterone acetate. For this reason, the prednisone cannot be dose reduced or held without holding the abiraterone acetate. If Grade 3 or 4 toxicity develops which is known to be related to prednisone (e.g. hyperglycemia) and this is believed to potentially impact the safety of participation and if the toxicity cannot be managed with medical therapy while maintaining the dose of the prednisone, then the prednisone dose may be reduced to 75% of the starting dose (7.5 mg/day). If Prednisone cannot be tolerated by the patient and therapy is deemed unsafe by the treating physician then patient will be removed from protocol.

6.5 ABT-888 dose levels are defined below

Dose Level	ABT888
+1	300 mg BID
0 starting dose level	200 mg BID
-1	150 mg BID
-2	100 mg BID

Patients randomized to ABT-888 will be started at 200 mg bid. If after the first cycle of therapy the patient tolerates therapy well then the dose can be escalated to 300 mg BID for cycle 2 and thereafter.

Note: *All treatment modifications must be expressed as a specific dose or amount rather than as a percentage of the starting or previous dose. ABT-888 will be available in 10 and 50 mg increment capsules.*

6.5.1 Dose reductions based on hematologic nadirs: Dosage adjustments to be implemented at the beginning of each treatment cycle will be based upon the nadir counts as follows:

Granulocytes		Platelets	ABT888DoseAdjustment
ANC ≥ 500	and	≥20,000	Full dose
ANC < 500	and/or	<20,000	Decrease ABT888 by 1 dose level

- **If the ANC nadir is <500 and/or the platelet nadir is <20,000 after a 1 dose level reduction of ABT888, then the next cycle will be started at -2 dose level.**
- Patients requiring more than two dose reductions should discontinue ABT888

6.5.2 General Non-Hematological Toxicities:

Patients requiring more than two dose reductions (3 if they were treated at the +1 dose level) should discontinue ABT888.

General Non-Hematological Toxicities including Nausea, Vomiting, and Diarrhea:

- For Grade 1 and 2 toxicities there will be no routine requirement for a dose reduction and appropriate medical therapy is to be given accordingly.
- If patients develop intolerable G1 or G2 toxicities (per patient) then they can undergo a dose reduction.
- > Grade 3 toxicities despite optimal medical therapy: Hold ABT-888 until ≤ Grade 1. Resume at one dose level reduction.

General Non-Hematological Toxicities excluding nausea, vomiting, and diarrhea:
Grade ≥ 3: delay until recovery to grade ≤ 1 and reduce one dose level.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

7.1 Comprehensive Adverse Events and Potential Risks list (CAEPR) For Veliparib (ABT-888, NSC 737664)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 2310 patients.* Below is the CAEPR for ABT-888 (Veliparib).

NOTE: Report AEs on the SPEER **ONLYIF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.3, March 4, 2016[1]

Adverse Events with Possible Relationship to ABT-888 (Veliparib) (CTCAE 4.0 Term) [n= 2310]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 3)***
	Febrile neutropenia		***Febrile neutropenia (Gr 3)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Constipation		***Constipation (Gr 2)***
	Diarrhea		***Diarrhea (Gr 3)***
Nausea			***Nausea (Gr 3)***
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 3)***
INVESTIGATIONS			
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
	Neutrophil count decreased		***Neutrophil count decreased (Gr 4)***
Platelet count decreased			***Platelet count decreased (Gr 4)***
	Weight loss		***Weight loss (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 4)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
	Dehydration		***Dehydration (Gr 3)***
	Hypophosphatemia		***Hypophosphatemia (Gr 3)***
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Dysgeusia		***Dysgeusia (Gr 2)***
	Headache		***Headache (Gr 3)***

Adverse Events with Possible Relationship to ABT-888 (Veliparib) (CTCAE 4.0 Term) [n= 2310]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Seizure	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Rash maculo-papular		
VASCULAR DISORDERS			
		Thromboembolic event[2]	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Thromboembolic events, including deep vein thrombosis and pulmonary embolism, have been observed at a higher frequency compared to control arm when administered in combination with temozolomide.

Adverse events reported on ABT-888 (Veliparib) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that ABT-888 (Veliparib) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (bone marrow failure); Blood and lymphatic system disorders - Other (pancytopenia)
CARDIAC DISORDERS - Cardiac disorders - Other (Takotsubo cardiomyopathy); Heart failure; Left ventricular systolic dysfunction; Palpitations; Sinus bradycardia; Sinus tachycardia
EAR AND LABYRINTH DISORDERS - Vertigo
EYE DISORDERS - Blurred vision
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Colitis; Colonic obstruction; Dental caries; Dry mouth; Duodenal ulcer; Dyspepsia; Dysphagia; Enterocolitis; Esophagitis; Flatulence; Gastritis; Gastroesophageal reflux disease; Lower gastrointestinal hemorrhage; Mucositis oral; Obstruction gastric; Rectal hemorrhage; Rectal pain; Small intestinal obstruction
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema limbs; Fever; Flu like symptoms; Malaise; Non-cardiac chest pain; Pain
HEPATOBILIARY DISORDERS - Hepatic failure; Hepatobiliary disorders - Other (cirrhosis)
INFECTIONS AND INFESTATIONS - Appendicitis; Catheter related infection; Infections and infestations - Other (peritonsillar abscess); Infections and infestations - Other (shingles); Lung infection; Lymph gland infection; Mucosal infection; Sepsis; Skin infection; Upper respiratory infection; Urinary tract infection
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising; Dermatitis radiation; Injury, poisoning and procedural complications - Other (radiation proctitis)
INVESTIGATIONS - Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Cardiac troponin I increased; Creatinine increased; Electrocardiogram QT corrected interval prolonged; Lipase increased
METABOLISM AND NUTRITION DISORDERS - Hyperglycemia; Hypernatremia; Hypoalbuminemia; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Arthritis; Back pain; Bone pain; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (muscle spasms); Myalgia; Neck pain; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Myelodysplastic syndrome; Treatment related secondary malignancy; Tumor pain
NERVOUS SYSTEM DISORDERS – Ataxia; Cognitive disturbance; Depressed level of consciousness;

Dysarthria; Extrapyramidal disorder; Intracranial hemorrhage; Lethargy; Memory impairment; Movements involuntary; Paresthesia; Peripheral motor neuropathy; Peripheral sensory neuropathy; Presyncope; Reversible posterior leukoencephalopathy syndrome; Stroke; Syncope; Tremor
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression; Insomnia; Psychiatric disorders - Other (emotional instability); Psychosis; Restlessness
RENAL AND URINARY DISORDERS - Hematuria; Proteinuria; Renal and urinary disorders - Other (dysuria)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Dyspnea; Epistaxis; Hypoxia; Nasal congestion; Pharyngolaryngeal pain; Pleural effusion; Pneumonitis; Respiratory failure
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Purpura; Rash acneiform; Skin and subcutaneous tissue disorders - Other (nail bed changes)
VASCULAR DISORDERS - Flushing; Hot flashes; Hypertension; Hypotension; Vascular disorders - Other (brainstem infarction)

Note: ABT-888 (Veliparib) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 Adverse Event List(s) for Abiraterone

Please refer to Section 8.3 for detailed information regarding reported toxicities for Abiraterone and ***the Physician Desk Reference and package insert for complete information.***

7.3 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section 7.1 above) for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR (Section 7.1.1).

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

7.4 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTTEP-AERS (Adverse Event Expedited Reporting System), accessed via the CTEP Web site (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov). These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 Since this is a multicenter trial CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients.

7.3.3 ExpeditedReportingGuidelines

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention[1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days			24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required		10 Calendar Days	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR

ExpeditedAEreportingtimelinesaredefinedas:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.5 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must also be reported in routine study data submissions.**

7.6 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6.1 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified.

8. PHARMACEUTICAL INFORMATION

8.1 CTEP IND Agent:

8.1.1 Veliparib (ABT-888) (NSC 737664)

Chemical Name: 1*H*-Benzimidazole-7-carboxamide, 2-[(2*R*)-2-methyl-2-pyrrolidinyl]-

Other Names: Veliparib, A-861695.0

Classification: Poly (ADP-ribosome) polymerase (PARP) Inhibitor

CAS Registry Number: 912444-00-9

Molecular Formula: $C_{13}H_{16}N_4O$ **M.W.:** 244.29

Approximate Solubility: A-861695.0 is freely soluble at pH < 6.9, soluble at pH 6.9 to 7.1, and slightly soluble at pH > 7.1.

Mode of Action: ABT-888 inhibits the formation of poly (ADP-ribose) (PAR) polymers in vitro and in vivo. It inhibits the repair of DNA when the DNA is damaged by cytotoxic agents. ABT-888 increases antitumor efficacy when added to DNA-damaging therapies such as temozolomide, cisplatin, carboplatin, cyclophosphamide, irinotecan, or radiation therapy.

How Supplied:

Abbott Laboratories supplies and DCTD distributes ABT-888. ABT-888 capsules are available in 10 mg, **20 mg, 40 mg**, 50 mg, and **100 mg** immediate release capsules. The inactive ingredients are microcrystaline cellulose, colloidal silicon dioxide, magnesium stearate, gelatin, sodium lauryl sulfate, FD&C yellow #5, and titanium dioxide. The capsules are packaged in HDPE bottles, and each HDPE bottle contains 16 capsules or 64 capsules.

Note:

ABT-888 capsules may be repackaged from the supplied HDPE bottles into amber (or other low-actinic) child resistant pharmacy dispensing bottles. Expiration will be 30 days from the repackaging date (or the original retest date, whichever is earlier) when stored at 15°C to 25°C (59°F to 77°F).

Storage: Store at 20^0C to 25^0C (68^0F to 77^0 F).

Stability: Excursions permitted to 15^0 C to 30^0 C (59^0F to 86^0 F).

Route(s) of Administration: Oral

Method of Administration: Administer ABT-888 orally without regards to meals.
Potential Drug Interactions: Clinical studies evaluating the metabolism of ABT-888 have not been conducted. However, results from the in vitro analysis reveal that this agent is metabolized by multiple isoenzymes – CYP1A1, 2D6, 2C19 and 3A4. ABT-888 is neither a potent inhibitor nor a potent inducer of the CYP-450 isoenzymes. Use caution when concomitantly administer with drugs that are substrate, inhibitor, inducer of CYP1A1, 2D6, 2C19 and 3A4.

ABT-888 clears primarily in the urine as intact parent drug along with metabolites suggesting that renal function plays an important role in the drug clearance and its metabolites. Use caution when concomitantly administer with oxalipaltin, carboplatin, cisplatin, and topotecan in patients with pre-existing renal impairment.

Special Handling: Handle ABT-888 according to your institutional guideline for hazardous drugs.

Patient Care Implications: Patients may feel fatigue or tiredness. Loss of appetite and losing weight are common. Provide appropriate supportive care for diarrhea. Avoid long-sun exposure as it might exacerbate skin rash.

i. Availability

ABT-888 is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

ABT-888 is provided to the NCI under a Collaborative Agreement between Abbott Laboratories and the DCTD, NCI (see Section 12.3).

8.1.2 Agent Ordering and Agent Accountability

8.1.2.1 NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and

Financial Disclosure Form (FDF).

If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password. Alternatively, site personnel can fax completed Clinical Drug Requests (NIH-986) to the Pharmaceutical Management Branch at (240) 276 7893. For questions about drug orders, transfers, returns, or accountability, call (240) 276 6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.2.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.2 Commercial Agents

8.2.1 Abiraterone Acetate (CB7630) (NSC #748121) (IND-111552)

a. DESCRIPTION

General: Abiraterone acetate is a pro-drug of abiraterone. Abiraterone inhibits the conversion of pregnenolone or progesterone into DHEA or androstenedione, which are 2 precursors of testosterone in the adrenal glands and testes. Testosterone is further converted to the more potent androgen dihydrotestosterone (DHT). Both testosterone and DHT stimulate prostatic growth. Current therapies (orchiectomy and GNRH) ablate androgen production by the testes but do not affect androgen production by the adrenals. Since the steroid hormones upstream of aromatase and downstream of CYP17 could be important in the activation of ER-α and possibly other nuclear steroid hormone receptors in breast cancer growth, abiraterone may impact breast cancer cell proliferation and survival by virtue of its estrogen suppressing activity.

Molecular Formula: C26H33NO2

Molecular Weight: 391 Daltons

PHARMACOLOGY:

Mechanism of Action: Abiraterone is a selective irreversible steroidal inhibitor of 17α hydroxylase/C17, 20-lyase (cytochrome P450c17 [CYP17]). Inhibition of CYP17 inhibits the conversion of pregnenolone or progesterone into DHEA or androstenedione, respectively, which are two precursors of testosterone in the adrenal glands and testes.

Pharmacokinetics

Data indicate that the pharmacokinetics of abiraterone acetate administered orally in either tablet or capsule formulations were very similar. Abiraterone acetate is rapidly converted to abiraterone after oral administration. Time to reach maximum plasma concentration is approximately 2 to 4 hours. The terminal half-life is estimated to be between 5 to 16 hours. Following a single oral tablet dose of abiraterone acetate, up to 25 – fold variation was observed, while up to 9 – fold variation was observed following administration with a capsule formulation. Although there is a variation in plasma levels between the formulations, there is no significant difference between the Cmax observed in each group. Food increases bioavailability of abiraterone acetate, however, to minimize the variability in drug exposure as a result of dietary variations, abiraterone is to be administered under the fasting condition.

TOXICOLOGY

Human:
Reported Adverse Events and Potential Risks:
Hypertriglyceridemia (62.5%)
Aspartate aminotransferase increased (30.6%)
Arthritis (29.5%)
Hypokalemia (28.3%)
Edema limbs (26.7%)
Arthralgia (26.2%)
Investigations – Other, specify (low phosphorus) (23.8%)
Flushing (19.0%)
Diarrhea (17.6%)
Urinary tract infection (11.5%)
Alanine aminotransferase increased (11.1%)
Cough (10.6%)
Hypertension (8.5%)
Ventricular arrhythmia (7.2%)
Urinary frequency (7.2%)
Blood bilirubin increased (6.6%)
Renal and urinary disorders –Other, specify (nocturia) (6.2%)
Dyspepsia (6.1%)

Upper respiratory infection (5.4%)
Chest pain - cardiac (3.8%)
Heart failure (2.3%)
Adrenal insufficiency (<1%)

Pregnancy and lactation: Abiraterone acetate has not been tested in pregnant or lactating women. As abiraterone acetate affects the levels of androgens and estrogens, fetal abnormalities or miscarriage may occur if the drug is administered during pregnancy.

Drug interactions: Please refer to Physician Desk Reference and package insert for complete information.

ADMINISTRATION (DOSING):

See Section 5.0 Treatment Plan.

STORAGE/STABILITY

Bottles of abiraterone acetate tablets should be stored at room temperature (15-30o C; 59-86o F) in the original container/closure with the cap sealed tightly. Do not refrigerate.

HOW SUPPLIED

Abiraterone acetate is standard agent for this study and is commercially available as 250 mg strength tablet and should be purchased by a third party. The tablets are oval, and white to off-white.

Please refer to the Physician Desk Reference and package insert for complete information

8.3 Prednisone (NSC-10023)

DESCRIPTION

Prednisone is a glucocorticoid which is rapidly absorbed from the GI tract.

TOXICOLOGY

Human Toxicology: Possible adverse effects associated with the use of prednisone are: fluid and electrolyte disturbances, congestive heart failure in susceptible persons, hypertension, euphoria, personality changes, insomnia, mood swings, depression, exacerbation of infection (e.g., tuberculosis), exacerbation or symptoms of diabetes, psychosis, muscle weakness, osteoporosis, vertebral compression fractures, pancreatitis, esophagitis, peptic ulcer disease,

dermatologic disturbances, convulsions, vertigo and headache, endocrine abnormalities, ophthalmic changes, and metabolic changes. Some patients have experienced itching and other allergic, anaphylactic or other hypersensitivity reactions. Withdrawal from prolonged therapy may result in symptoms of adrenal insufficiency including fever, myalgia and arthralgia. Phenytoin, phenobarbitol, and ephedrine enhance metabolic clearance of corticosteroids.

Corticosteroids should be used cautiously in patients with hypothyroidism, cirrhosis, ocular herpes simplex, existing emotional instability or psychotic tendencies, nonspecific ulcerative colitis, diverticulitis, fresh intestinal anastomoses, peptic ulcer disease, renal insufficiency, hypertension, osteoporosis and myasthenia gravis. Immunization procedures (especially smallpox vaccination) should not be undertaken in patients on corticosteroids.

PHARMACOLOGY

Pharmacokinetics: Natural and synthetic glucocorticoids are readily and completely absorbed from the GI tract. Prednisone is slightly soluble in water. Glucocorticoids have salt-retaining properties. The anti-inflammatory property of this drug is due to a down-regulation of the body's immune system and as a consequence it can suppress the body's response to viral as well as bacterial infections.
Formulation: Prednisone is available in 2.5 mg, 5 mg, 10 mg, 20 mg and 50 mg tablets.

Storage and Stability: Prednisone should be stored at room temperature.

Administration: Prednisone is administered orally.
Supplier: Prednisone is commercially available and should be purchased by third party. Prednisone will not be supplied by the NCI.

Please refer to the Physician Desk Reference and package insert for complete information.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Laboratory Correlative Studies

9.1.1 Collection and Handling of Specimens

From each patient, specimens will be collected from three sources:

a) A metastatic site of disease (via biopsy) or adequate metastatic tissue from prior biopsy or resection.
b) The primary tumor site (via retrieval of previously archived specimens)
c) Blood/circulating tumor cells (pre-treatment, week 12, and at clinical progression or when off study, whichever is first)
d) Blood/pharmacogenomic SNP analysis (pre-treatment)
e) Blood/TMPRSS2:ERG mRNA(pre-treatment and at weeks 5 and 9)

The rationale, and a brief overview, for collection and handling of these specimens is as described below. Further technical details regarding biopsies of metastastic sites and handling of these biopsy specimens can be found in Appendices C and D, respectively.

9.1.1a: Biopsy of metastatic site: Rationale and Procedure (Appendices C and D)

a). The primary objectives of this study, and the subsequent assignment of patients to treatment arms, depend on determination of ETS fusion status in each patient's metastases. The concordance of ETS fusion between primary tumor and metastases has been assessed on only limited samples[80] and is not well known at this point in time. Thus, all patients registered on this study, will undergo a biopsy from a metastatic site unless they have adequate available metastatic tissue from prior biopsy or resection. If the ETS fusion status of the metastatic tissue is known from previous testing, the ETS fusion status of this tissue will be reconfirmed by immunohistochemistry (IHC) for ERG testing and ISH-based assays detailed in Section 9.1.5a and 9.1.5b. **Biopsies must be scheduled for Monday to Thursday to allow for overnight shipping to be received by Friday.**

b). Patients will be scheduled for a CT-guided biopsy of a bone or soft tissue abnormality consistent with prostate cancer metastasis. The site of biopsy will be determined by the radiologist after review of available radiographs, CT, MRI, and/or bone scan, and will be selected based whatever site is expected to result in the best access and best yield.

- An ultrasound (US) can be used for lymph node or soft tissue biopsies provided that the target lymph node or soft tissue mass is visible with the US and can be properly accessed.

Verbal and written informed consent will be obtained after discussion of risks (bleeding, infection, soft tissue and bone injury, inconclusive biopsy results) of the procedure.

Standard laboratory tests to assess coagulation and bleeding will also be completed prior to the procedure. **A standard operating procedure for bone biopsy is provided in Appendix C**. **A standard operating procedure for soft tissue biopsy is provided in Appendix D**.

In order to ensure adequate tumor sample as long as it is deemed safe and based on accessibility, target lesion size, specimen integrity, specimen appearance, intra-procedural bleeding, tumor biopsy should attempt to obtain:
1. Bone biopsy: 2-8 bone biopsy samples. In addition, at the time of biopsy, it will be determined if a bone marrow aspirate is feasible, and if performed, the bone marrow aspirate will be sent to University of Michigan for analysis.

2. Lymph node or soft tissue: If obtaining a 1-cm-long 18-gauge core specimen, attempts should be made to obtain at least 6 cores. If obtaining a 2-cm-long 18-gauge core specimen, attempts should be made to obtain at least 4 cores.

c). The biopsy of the metastases, and any accompanying aspirate, will be obtained exclusively for research purposes. The biopsy specimens and the aspirate will be collected and will be immediately frozen in dry-ice ethanol bath in Tissue-Tek O.C.T Compound (Sakura Finetek, Torrance, CA) to ensure the integrity of tissue and proteins for both immunoblotting and immunohistological analysis of samples. Upon enrollment of each patient on study, a sample kit containing necessary reagents for tissue collection (Tissue-Tek standard-size cryomolds, Tissue-Tek OCT, blue pads, markers, and an insulated bucket) will be shipped to the enrolling site coordinator. It is expected that dry ice will be provided by the enrolling site. In addition, written SOPs, with specific details regarding tissue collection with this kit, along with a contact sheet with mailing addresses for sample shipment, will be mailed to each site coordinator.

d). Each Tissue-Tek cryomold will be labeled with the clinical protocol number, the patient's study registration number, and the tissue type and the biopsy time and date. In addition, a separate information sheet accompanying each specimen will be filled out. This information sheet will include various details necessary for quality assurance of the specimen, including:

1) Date of collection
2) Specimen ID
3) Site from which biopsy obtained (bone versus soft tissue, and then location)
4) Type of biopsy device
5) Biopsy diameter: gauge, and length: cm
6) Time biopsy needle introduced
7) Time biopsy snap-frozen on dry ice
8) Time aspirate performed (if applicable)
9) Time aspirate snap-frozen on dry ice

A standard operating procedure for handling of the biopsy and aspirate specimens has been attached to this protocol as Appendix D.

Following biopsy, samples from metastatic sites need to be immediately frozen prior to shipment within 24 hours to the University of Michigan, per shipping directions described in 9.1.2.

9.1.1b: Archived samples from the primary tumor site: Rationale and Procurement Procedure

Archived primary tumor samples, where available, will be requested from the enrolling institution. These primary tumor samples should consist of either radical prostatectomy

specimens or biopsies from the prostate. Archived primary tumor samples can be maintained at room temperature. **Either a block from the primary tissue sample, OR 1 H&E stained slide and 8 unstained charged slides (4 micron thickness) (4 of the unstained slides to be used for ISH should be sent without baking the slides) should be shipped at room temperature to the University of Michigan, per shipping directions described in 9.1.2.**

9.1.1c: Circulating Tumor Cells: Rationale and Sample Collection Procedure

Blood will be collected pre-treatment, week 12, and at disease progression or when off study. Blood samples will be drawn into three 10-mL evacuated blood draw tubes (Cell-Free DNA; Streck) (one at each time point). These blood samples can be maintained at room temperature, **but must be shipped to Epic Sciences within 48 hours after collection**, per shipping directions described in 9.1.2.

9.1.1d: Blood/PharmacogenomicSNPAnalysis:RationaleandSampleCollection Procedure

Blood for pharmacogenomic SNP analysis will be collected at pre-treatment. Blood samples will be drawn into a single 10-mL EDTA lavender top tube. These blood samples need no processing, can be maintained at room temperature, but need **to be shipped to the University of Michigan within 24 hours after collection**, per shipping directions described in 9.1.2.

9.1.1e: Blood/TMPRSS2:ERGmRNAAnalysis:RationaleandSampleCollection Procedure

Blood will be collected at pre-treatment, and at 5 and 9 weeks post-treatment. At each time point, 2.5 mL blood samples will be drawn into two 10-mL PAXgene Blood RNA Tubes (Becton Dickinson, Cat# 762165) containing 7.5 mL of solution. These blood samples can be maintained at room temperature, **but need to be shipped to the University of Michigan within 24 hours after collection**, per shipping directions described in 9.1.2.

9.1.2: Shipping of Specimens after Collection

All samples, except the blood samples for CTC analysis, must be shipped to the University of Michigan, at the following address:

Javed Siddiqui, MS, MT (ASCP, CLsp (MB))
Technical Director
Michigan Center for Translational Pathology
Room 1138
2900 Huron Parkway Suite 100
Ann Arbor, MI 48105
Phone: (734) 232-0829
Fax: (734) 232-0805
siddiqui@med.umich.edu

Shipping must occur only Monday-Thursday, and an e-mail or phone call to the contact listed on the laboratory contact sheet, alerting her to the shipment, is required.

9.1.2a Biopsiesandaspiratesfrommetastaticsites: If biopsies are performed on metastatic sites on Monday through Thursday, the biopsy and aspirate samples should be shipped overnight, on dry ice, to the UniversityofMichigan.
9.1.2b Primarytumorsamples: The primary tumor samples can be shipped via standard mail, at room temperature, to the UniversityofMichigan. It is requested that efforts be made to ship the primary tumor samples within a month of patient enrollment onto the trial.
9.1.2c BloodsamplesNOTforCTCanalysis: The blood samples should be shipped overnight, at room temperature, to the UniversityofMichigan.

9.1.2d BloodsamplesforCTCanalysis: The blood samples should be shipped overnight, at room temperature, toEpicSciences,atthefollowingaddress:

EpicSciences
10975NTorreyPinesRd
LaJolla,CA92037
858-356-6610

Shipping must occur only Monday-Thursday, and an e-mail should be sent to partners@epicsciences.com, alerting to the shipment, the inclusion of the sample ID, number of samples, tracking number, and time of blood

draw, is required. Please note that the Epic Sciences shipping address is to be used ONLY FOR THE CTC SAMPLES, and that the remainder of the samples (metastases, primary tumor, other blood samples) should be sent to the University of Michigan address listed above section 9.1.2a.

For further details regarding shipping, please refer to Appendix E, F & G which are the standard operating procedures for the shipment of samples.

9.1.3 Sites Performing Correlative Studies

Aside from the circulating tumor cell studies, all correlative studies will be performed at the University of Michigan, within the Michigan Center for Translational Pathology (MCTP). Dr. Felix Feng, co-Principal Investigator, and Dr. Arul Chinnaiyan, head of the MCTP, will oversee these correlative studies. The circulating tumor cell studies will be performed by Epic Sciences.

9.1.4 Processing of Specimens after Receipt at the University of Michigan

a. With the receipt of each biopsy specimen, Javed Siddiqui at the University of Michigan (or a pre-specified designee) will place the samples in the -80˚C tissue repository described below and will record two additional items for further quality assurance:

1) The date/time of receipt of the biopsy specimen at the University of Michigan
2) The date/time that the biopsy specimen is placed at -80°C

b. Biopsies from metastatic sites: The frozen tissue samples from either biopsy or from preexisting metastatic tissue, obtained for research from patients taking part in this protocol will be held in the University of Michigan Prostate Oncology Program Tissue Bank. The Tissue Bank is located in Room 1143, 2900 Huron Parkway, Suite 100, University of Michigan, Ann Arbor, Michigan 48105. This laboratory has multiple -80˚C freezers, one of which will be devoted to the biopsies from this trial. The freezers have an alarm system and self-dialing emergency system. It is anticipated that at least two samples will be obtained from each metastatic biopsy. From bone biopsies, these metastases may consist of two biopsies or a biopsy and an aspirate. One sample from each metastatic biopsy (the biopsy sample in cases where there is a biopsy sample and an aspirate) will be fixed in formalin shortly after receipt at the University of Michigan. Six 5 um section slides will be cut from this formalin fixed sample -- one for H&E staining to confirm the presence of tumor, two for determination of ETS fusion status, one for analysis of PTEN status, one for analysis of PAR (indicative of PARP1 activity), and one for analysis of PARP1 levels. The determination of ETS status for the primary objectives will be prioritized above all others, both in schedule and in cases of limited tissue

availability, as these studies are necessary to complete the primary objectives of this clinical trial. In cases where two samples are available from a metastatic biopsy, the second sample will be preserved at -80˚C for subsequent transcriptome sequencing, as described below. For samples in which the biopsies contain insufficient tumor samples for analysis, the collected aspirates (if available) will be used for analysis.

c. Primarytumorsamples: Retrieved primary tumor samples will be stored at room temperature in the University of Michigan Prostate Oncology Program Tissue Bank

d. Bloodsamplesforcirculatingtumorcellstudies:
The Epic Sciences platform will be utilized for circulating tumor cell analyses. [82-86] Peripheral blood samples will be drawn at accruing sites following informed consent and processed following standard operating procedures. Using a standardized and ISO-certified shipping process, samples are transferred to the Epic Sciences central laboratory using Federal Express as a carrier. Samples are then processed after 48 hours of blood draw. The red blood cells are lysed and the remaining nucleated cells are plated on custom glass slides and frozen for storage. As needed, blood sample slides are thawed and processed to bind the appropriate cell markers with fluorescently labeled antibodies. Depending on peripheral blood counts, up to 16 slides are generated from each subject. Two to four processed slides, representing one patient test, the identification of CTCs consists of three markers (DAPI-nucleus, cytokeratin – epithelial marker, and CD-45 – white blood cell marker) and are imaged on custom-built automated slide imagers. The resulting images are then automatically processed to produce data for technical analysis which consists of removing imaging artifacts and selecting potential CTC candidates which are then presented in a web-based report to a pathologist for final review. Remaining slides are stored for subsequent analysis. These subsequent analyses will include immunofluorescence or FISH assays for both ERG and ETV1 fusions, androgen receptor, androgen receptor splice variants, RAD51, γH2AX and PTEN biomarkers.

e. Blood samples for SNP and mRNA analysis: Germline DNA from patient peripheral blood mononuclear cells will be extracted from blood collected in EDTA lavender top tubes using Qiagen DNAEasy assay (Qiagen, Valencia, CA) according to manufacturer methods. TMPRSS2:ERG mRNA levels in PAXgene tubes will be quantified using a transcription-mediated amplification assay.[99] The target RNA is captured onto magnetic microparticles and amplified. Amplification products are then detected using chemiluminescent DNA probes, and the raw signal is converted to RNA copies using calibrators of known TMPRSS2:ERG copy levels. The assay is specific for the TMPRSS2(Exon1)-ERG(Exon4) RNA isoform. To verify the presence and integrity of prostate RNA in the blood samples, PCA3 (prostate cancer gene 3) and PSA (prostate specific antigen) mRNAs will also be quantified using the same methodology.[100]

9.1.5 Description of Correlative Studies

9.1.5a. The Role of ETS Gene Fusions (in Metastases) as a Predictive Biomarker for Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic Castration-Resistant Prostate Cancer (CRPC) (Primary Objective#1)

and

9.1.5b. TheRoleofETSGeneFusions(inMetastases)asaPredictiveBiomarkerfor TreatmentBenefitfortheAdditionofABT-888toAbiraterone(PrimaryObjective#2)

To determine gene fusion status in metastatic biopsies or metastatic prexisting tissue samples, our trial will utilize the following assays:

- immunohistochemistry for ERG (the TMPRSS2:ERG gene fusion is the predominant ETS gene fusion, and comprises close to 90% of all ETS fusions)
- ISH-based assays for ETV1 fusions (the second most common ETS fusions)

Together, the ERG and ETV1 fusions comprise approximately 97% of all ETS fusions. Until recently, fluorescence in situ hybridization (FISH) approaches have been primarily used for the detection of gene fusions involving ERG. However, recently, a rabbit anti-ERG antibody has been demonstrated to exhibit extremely high concordance with FISH approaches for the detection of ERG fusions, with a sensitivity of approximately 96% and a specificity of approximately 97%.[101] The diagnostic performance of this ERG antibody was independently evaluated in two large patient cohorts from two different institutions.[101] Given the increased ease and decreased cost of performing immunohistochemistry compared to FISH, evaluation of ERG with this antibody approach is now offered in our CLIA lab at the University of Michigan, starting in March 2011, and is the assay of choice, for detection of ERG fusions, in this gene-fusion guided clinical trial.

While a robust antibody approach has been successfully been developed for detection of ERG, FISH approaches were, until recently, the gold standard for the detection of ETV1 fusions. However, recently, using 329 samples of both metastatic and localized prostate cancer, our team has demonstrated the RNA-ISH (in-situ hybridization) is a good approach for detecting ETV1 rearrangements, with benefits in detection compared to the DNA-based FISH (fluorescence-in-situ-hybridization approaches). The primary benefit of ETV1 RNA-ISH over ETV1 DNA-FISH is in the ability to detect rearrangements using a smaller amount of tissue, as is often the case with bone biopsies.[102] Of the first 25 patients enrolled on this trial, 13 have had assessment for ETV1 fusions by both ETV1 FISH and ETV1 ISH. Two cases were positive by both FISH and ISH assays; 10 were negative by both assays. The thirteenth case was inconclusive for ETV1 fusions by FISH, but positive for ISH, confirming that even within this initial sample set, ETV1 ISH was superior to ETV1 FISH in performance. Thus, moving forward, ETV1 ISH will be used as the gold standard for ETV1 fusion detection.

As noted, our research team has significant experience and competence with the ERG IHC and ETV1 ISH assays. Our study pathologist, Dr. Arul Chinnaiyan and his team, discovered both ERG and ETV1 gene fusions several years ago[14,15] and has helped develop the ISH assays used to detect ETS fusions.[14,15,102] In addition, Dr. Chinnaiyan's group has helped perform the preliminary studies validating the performance of the ERG antibody.[101] Based on our experience, we expect a 7-9 days from receipt of tissue to reporting of read-out of ETS fusion assays.
The impact of ETS fusion status upon treatment outcome following abiraterone vs abiraterone + ABT888 will be determined, as described in section 13.1. In addition, the role of ETS fusion in predicting benefit of the addition of ABT888 to abiraterone will also be determined, per section 13.1.

9.1.5c Concordance in ETS Fusion Status among Primary Tumor, Metastases, and Circulating Tumor Cells in Patients

ETS fusion status will be evaluated in metastases, primary tumor, and CTCs for assessment of concordance.

- To determine gene fusion status in formalin-fixed metastatic and primary tumor biopsies, our trial will utilize the following assays:
  a) immunohistochemistry for ERG (the TMPRSS2:ERG gene fusion is the predominant ETS gene fusion, and comprises close to 90% of all ETS fusions)
  b) ISH-based assays for ETV1 fusions (the second most common ETS fusions)
- To determine gene fusion status in isolated CTCs, immunofluorescence-based assays for ERG fusions will be performed.
- Statistical assessment for concordance among metastases, primary tumor, and CTCs is described in Section 13.4.

9.1.5d The Role of ETS Gene Fusions (in Circulating Tumor Cells) as a Predictive Biomarker of Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic CRPC

9.1.5e Circulating Tumor Cell Count, and biomarker status of androgen receptor, PTEN, RAD51, and gammaH2aX, at baseline, 12 weeks post-therapy, and at progression, as a Predictive Biomarkers of Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic CRPC

To determine gene fusion status in isolated CTCs, immunofluoresence-based assays for ERG will be performed utilizing the Epic Sciences System. The CTCs will be enumerated using the Epic Sciences System utilizing automated fluorescence- based microscopy systems that permit computer-generated reconstruction of cellular images. Associations between outcome, and CTC count and percent CTCs harboring ETS fusions, will be performed as per Section 13.4. In addition, immunofluoresence-based approaches will be used to assess levels of the androgen receptor, RAD51, and gammaH2aX in thte CTCs. All CTC assays will be performed at baseline, at 12 weeks, and at time of progression.

9.1.5f The Role of PTEN Loss (in Metastases) as a Predictive Biomarker for Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic Castration-Resistant Prostate Cancer (CRPC)

We will assess for PTEN loss in metastatic samples using immunohistochemistry for a PTEN antibody (138G6–Cell Signaling Technology–Rabbit Monoclonal Ab) which has been validated in a CLIA certified lab with excellent performance characteristics[103] and is also offered in our CLIA-certified lab. We will also assess for PTEN loss in CTCs using a FISH-based assay.[93] CRPC samples from metastases and CTCs will be stratified by PTEN status, and the impact of PTEN loss on PSA response following treatment with abiraterone +/- ABT-888 will be assessed statistically, as described in Section 13.4.

9.1.5g The Role of PARP1 Activity (in Metastases) as a Predictive Biomarker for Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic Castration-Resistant Prostate Cancer (CRPC)

We will use immunohistochemical approaches to quantitate PAR and PARP1 levels, as previously published, in metastases and determine the association between PAR levels with PSA response to abiraterone, alone and in combination with PARP1 inhibition.

9.1.5h Discovery of Novel Gene Fusions Through Paired-End Transcriptome Sequencing of Metastases

In frozen biopsy samples from the metastatic lesion, we propose to perform next-generation sequencing, for discovery of novel targetable gene fusions distinct from ETS fusions. The cancer genome is often highly aneuploid (aberrant chromosome numbers) or polyploid (with aberrant sets of chromosomes), and almost always highly rearranged, with several areas of gains and losses. To adequately analyze these complex sequences, requires extra deep coverage of the genome that is not yet routinely feasible (or economical) over large sample sizes. To circumvent these formidable sequencing needs we have considered a complementary approach of focusing on the 'expressed' component of the genome, namely the transcriptome. Sequencing the transcriptome not only provides an in depth coverage of entire

genomic coding sequences, but also serves as a direct readout of gene expression, alternatively spliced isoforms, gene fusions (including RNA chimeras) and SNPs/mutations, thus enriching the data for 'functional' aberrations with considerably reduced sequencing resources and time as compared to genomic sequencing. We have recently applied transcriptome sequencing to discover multiple novel gene fusions and RNA chimeras in prostate cancer, including the discovery of a recurrent chimera, *SLC45A3-ELK4* in a subset of prostate cancer tissues[11]. Subsequently, in a proof of concept study we have improved our technique by developing the method of 'paired end transcriptome sequencing' to systematically identify gene fusions and chimeric transcripts in cancers.[17,104,105]

We will now focus on applying transcriptome sequencing to characterize recurrent gene fusions and other transcript aberrations in prostate cancer biospecimens collected by this protocol. Two types of next-generation sequencing libraries will be constructed and sequenced from the sample RNA. Specifically, paired-end transcriptome libraries from polyadenylated RNA, as well as from ribosomal RNA depleted samples (Ribominus), will be sequenced. The aim will be to discover novel cancer-specific, recurrent gene fusions and other signature genetic aberrations in these prostate cancer samples. The specific aims are to 1. Generate high throughput transcriptome sequencing data from these prostate cancer samples 2. Bioinformatically identify cancer-specific, recurrent gene fusions, SNPs and alternatively spliced (AS) transcript isoforms in prostate cancer. 3. Experimentally validate candidate transcript aberrations and screen big sample cohorts to determine recurrence and to 4. Functionally characterize novel, recurrent aberrations with clinical implications. These sequencing approaches, and the required bio-informatic pipelines necessary to analyze the sequencing data, have previously been established by our research team, led by Dr. Arul Chinnaiyan.[14,15,104,105]

9.1.5i Discovery of novel SNPs Predictive of Response to Abiraterone, Alone or in Combination with the PARP1 Inhibitor ABT-888, in Patients with Metastatic Castration-ResistantProstateCancer(CRPC)

Germline DNA will be extracted from patient peripheral blood mononuclear cells using Qiagen DNAEasy assay (Qiagen, Valencia, CA) according to manufacturer methods. The extracted DNA will be genotyped for 128 SNPs utilizing a TaqMan OpenArray GT Kit (Applied Biosystems, Carlsbad, CA) according to manufacturer specifications. SNPs have been chosen to include previously implicated SNPs of interest (i.e. *SLCO1B3* (rs4149117)) as well as SNPs in genes with critical roles in the metabolism, transport, elimination, and mechanism of action of hormonally targeted therapies. Details regarding the 128 SNPs of interest have been previously reported.[106]

9.1.5j The Role of blood TMPRSS2:ERG RNA as a Predictive Biomarker of Response to Abiraterone,AloneorinCombinationwiththePARP1InhibitorABT-888,inPatients with MetastaticCRPC

Blood TMPRSS2:ERG mRNA levels will quantified at baseline and after initiation of treatment. PCA3 and PSA mRNA levels will also be measured as controls, and may be used to normalize TMPRSS2:ERG RNA levels for the amount of prostate-specific RNA in blood. Associations between outcome, and baseline TMPRSS2:ERG mRNA levels (or changes in TMPRSS2:ERG mRNA levels) will be performed per Section 13.4.

10. STUDY CALENDAR

Pre-Study evaluations are to be conducted within 3 weeks prior to registration. Biopsy must be done within 10 calendar days post registration and results will be reported within 10 calendar days. Patients who are eligible and randomized must have Cycle 1 Day 1 evaluations and procedures completed and begin therapy within 7 working days from randomization. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. **Please refer to # below for Abiraterone + Prednisone Cycle 1 schedule for patients randomized to Arms IB and II B (Abiraterone + ABT888).**

Return visits after initiation of therapy can be done within ± 4 days from actual due date and lab draws (CBC w/diff, plts and Serum chemistry) on day 8 and 15 can be done ± one day. Correlative blood samples to be collected post-randomization but before starting treatment on day 1.

		Cycle 1				Cycle 2				Cycle 3					
Required Treatment or Studies	Pre-Study	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22	Subsequent Courses	Off Study[c]
ABT-888		A	A	A	A	A	A	A	A	A	A	A	A	A	
Abiraterone+ Prednisone #		# B	B	B	B	B	B	B	B	B	B	B	B	B	
Informed consent	X														
History & Physical	X	X				X				X				X&	X
Weight & PS	X	X				X				X				X&	X
Concurrent meds	X	X----------X												X	
Metastatic disease biopsy*	X														
Serum PSA	X	X[e]				X				X				X	X
CBC w/diff, plts	X	X[d]		X		X		X		X[d]				X	X
Serum chemistry[a]	X	X[e]	X	X	X	X				X				X	X
EKG (as indicated)	X														
Testosterone	X														
PTT/ PT	X														
Adverse event evaluation		X----------X													X
Tumor measurements	X	Tumor measurements are repeated every 12 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.													
Radiologic evaluation[b]	X	Radiologic measurements should be performed every 12 weeks+/-1 week												&	

Blood samples for ETS fusion mRNA studies		X				X				X					
Blood samples for CTC studies [f]		X											X	X	X
Blood samples for SNP studies		X													
	A: ABT-888: Dose as assigned; *administration schedule* B: Abiraterone / Prednisone: Dose as assigned*; administration schedule* # For patients randomized to Arms IB and IIB: For Cycle 1 only Abiraterone and Prednisone will start on day 8 to allow lead in ABT888 therapy. For all subsequent cycles Abiraterone/prednisone will start on day 1 and continue daily in 28 days cycle. * Biopsy must be done after patient has consented and registered to the study (unless prexisiting metastatic tissue is available). & After the first 3 months if patients are tolerating therapy well and clinically stable clinic visit for H&P can be done every three months. Chemistry and PSA will be done on D1 of each cycle as specified. For patients coming every 3 months a 3 month supply of ABT-888 can be provided per institutional guidelines. Imaging will be done every 3 months until disease progression or removal from study for patients on study for < 1 year. For patients who have completed ≥ 1 year of therapy, imaging can be done every 4 months . For patients who have completed ≥ 2 years of therapy, imaging can be done every 6 months.. Irrespective of duration on therapy imaging can be done sooner than the specified intervals as clinically indicated. a:Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium. b. Include bone scan, CT/MRI abdomen and pelvis, CT chest or C XR as appropriate. All disease sites must be reassessed. c: 30 day off study follow-up for adverse event assessment.. d. CBC w/diff, plts will be monitored on Day 1 only of each cycle after Cycle 1 and 2 if no hematologic toxicity is observe on Day 15 of cycle 1 and 2. CBCw/diff after cycle 2 can be done as otherwise clinically indicated. e. Cycle 1 Day 1 labs will always be reviewed prior to dosing. f.. Pretreatment, end of C#3 and disease progression or removal from study														

11. MEASUREMENT OF EFFECT

- For the purposes of this study, patients should be reevaluated for objective (radiologic) response every 3 cycles (ideally, every 12 weeks +/- 1 week).
- Patients demonstrating evidence of objective progression by bone scan but no objective measurable disease progression or PSA progression at the first tumor assessment after 3 cycles should continue on treatment and repeat scans should be done in 6 weeks:
    - If no additional objective progression is noted (SD), then patients will continue on therapy.
    - If additional objective progression is demonstrated then patients are removed from study. The date of the imaging at 3 cycles (12 weeks) will be recorded as the date of progressive disease.

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

- Pain is a criteria for progression. Please refer to section 11.1.5.5 for specific definitions.

11.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with Abiraterone +/-ABT-888.

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

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

11.1.2 Disease Parameters

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

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. *If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol*.

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

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

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts

should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

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

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

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

11.1.3 MethodsforEvaluationofMeasurableDisease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 6 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

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

lesion, is recommended.

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

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

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

11.1.4 Methods of Evaluation of Bone disease:

Bone disease will be evaluated using Radionuclide bone scan.

11.1.5 ResponseCriteria

11.1.5.1 Evaluation of Target Lesions

CompleteResponse(CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
PartialResponse(PR):	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

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

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

11.1.5.2 Evaluation of Non-Target Lesions

CompleteResponse(CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).
Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

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

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

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

11.1.5.3 Outcomes based on Radionuclide bone scans:

The subjectivity in interpreting serial changes in a radionuclide bone scan is well recognized. Thus, the primary outcome will be whether the scan is stable or improved, vs. worse or progression. Changes in intensity will not be used as an outcome measure.

Stable or Improved: A stable or improved classification requires that no new lesions appear at the 3rd cycle (12 weeks) ± 1 week assessments, or that new pain has not developed in an area that was previously visualized.

Progression (Non-Response): Appearance of **two or more** new skeletal lesions. *An increase in the size or intensity of known skeletal lesions will not be considered progression*.

11.1.5.4 Outcomes based on post-therapy PSA changes:

These definitions are intended to characterize the PSA changes on study for the purpose of reporting of results.

Complete Response (CR): Undetectable PSA (≤0.2 ng/ml) that is confirmed by another PSA level at no less than 4 weeks interval.

Partial Response (PR): Decrease in PSA value by ≥ 50% that is confirmed by another PSA level at no less than 4 weeks interval.

Stabilization (SD): Patients who do not meet the criteria for PR or PD for at least 90 days on study (2 cycles of treatment) will be considered stable

Progression (PD): 25% increase over baseline or nadir whichever is lower and an increase in the absolute value of PSA level by 2 ng/ml that is confirmed by another PSA level at no less than 4 weeks interval.

11.1.5.5 Definition of Progression Based on Pain:

Progression by pain criteria is based on pain due to prostate cancer requiring one or more of the following palliative interventions:

- OpioidTherapy: Intravenous, intramuscular or subcutaneous opioid therapy administered as a single dose; oral or transdermal opioid analgesic use administered for 10 out of 14 consecutive days, and/or requiring Radionuclideor Radiationtherapy.

- Evidence of disease at the site of pain is required. Pain requiring only non-opioid analgesics will not be considered disease progression.

11.1.6 Progression-Free Survival

Progression-free survival (PFS) is (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

11.2 Evaluation of Best Overall Response

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

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	

SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

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

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

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

11.3 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis, either by FTP burst of data or via the CDS web application. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov/reporting/cdus.html).

Note: If your study has been assigned to CDUS-Complete reporting, **all** adverse events (both routine and expedited) that have occurred on the study and meet the mandatory CDUS reporting guidelines must be reported via the monitoring method identified above. If your study has been assigned to CDUS-Abbreviated reporting, no adverse event reporting (routine or expedited) is required to be reported via CDUS.

12.1.2 Responsibility for Data Submission

Study participants are responsible for submitting CDUS data and/or data forms to the Coordinating Center quarterly by January 31, April 30, July 31, and October 31 to allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP (see Section 12.1.1). For trials monitored by CTMS, the monthly data submission to CTEP from Theradex should be copied to the Coordinating Center.

The Coordinating Center is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.2 CTEP Multicenter Guidelines

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the

Coordinating Center (Study Coordinator) and the procedures for auditing are presented in Appendix B.

- *The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.*
- *Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) except for Group studies.*

12. 3 Data and Safety Monitoring Procedures

Data Safety and Monitoring will occur as specified by the University of Chicago Phase 2 Consortium Procedure Manual. Adverse event and serious adverse event reporting is discussed in Section 7. As per the University of Chicago Comprehensive Cancer Center NCI approved DSM Plan, routine data and safety monitoring will occur at the weekly University of Chicago Phase II Consortium meetings, which are led by Phase 2 Consortium Chairman, Dr. Stadler or a senior level medical oncologist designee. The meeting is held by tele- and web conferencing for participation by the protocol PI or her designate. At each meeting, all active Phase II Consortium studies are reviewed for safety and progress toward completion. Toxicities and adverse events are reviewed as well and a Data Safety and Monitoring form is completed for each protocol and signed by either the principal investigator, the Chairman of the Phase II Consortium or by his designate if neither is available.

In addition, a formal review of all collected data will be conducted by the PI after the initial 3 subjects have completed 2 months of therapy and quarterly thereafter. Formal accrual reports will be generated for PI review on a quarterly basis as well.

12. 4 Quality Assurance and Audits

The Cancer Therapy Evaluation Program (CTEP), as the sponsor of clinical trials of investigational agents on behalf of the Division of Cancer Treatment and Diagnosis (DCTD), requires that all institutions conducting clinical trials involving NCI-sponsored investigational agents be site visited at least once every three years. The Clinical Trials Monitoring Branch (CTMB), in CTEP, is responsible for monitoring and overseeing the site visit program. Theradex, a Clinical Trials Monitoring Service (CTMS) contractor, administers the site visit audit program on behalf of CTMB. Theradex is a pharmaceutical consulting and development company based in Princeton, New Jersey. The audit teams who conduct the audit are composed of experienced clinical research associates, pharmacists and outside physicians, as necessary, who have specialized experience and expertise relevant to the types of protocols being reviewed.

All institutions (Lead site and Affiliates) that accrue patients to this study during a three year

period will be eligible for an audit at least every thirty-six months but may be selected for audit at any time. The objectives of the audit are to bring to the attention of the individual investigators, the Food and Drug Administration regulatory requirements which affect various aspects of the conduct of clinical studies. The items reviewed will include:

1. Full and non-contingent Institutional Review Board approval.
2. Copies of the Institutional Review Board approval to be available, including approvals for al amendments.
3. Copies of the annual reports on the progress of the study and copies of Institutional Review Board re-approval.
4. An IRB approved informed consent for all patients entered on study.
5. Adherence to the protocol.
6. Classification of adverse experiences and the reporting to the NCI drug monitor of unusual or unexpected events.
7. Follow up on drop outs with due diligence.
8. The regulatory requirements as they affect record keeping and record retention.
9. Test article or investigational drug accountability. This involves a discussion with pharmacy the methods of record keeping which best satisfies the federal regulations and NCI guidelines and requirements.

12.5 Collaborative Agreements Language

ABT-888 supplied by CTEP, DCTD, NCI in this protocol is provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between Abbott (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. ABT-888 may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must

be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

The goal of this phase II study is to assess the role of the ETS fusion biomarker on efficacy of Abiraterone + ABT-888 in comparison to Abiraterone alone in mCRPC patients. The primary endpoint will be the confirmed PSA response rate. Overall comparison of treatments and comparison of treatments within strata will describe the role of the ETS fusion biomarker on the endpoint by treatment.

The biomarker-stratified design[107] will be used to determine if there is a difference in PSA response between the ETS gene fusion-positive and fusion-negative strata in PSA response to Abiraterone compared to Abiraterone + ABT-888. An overall improvement of 20% in PSA response, from 30% expected with Abiraterone versus 50% with Abiraterone + ABT-888, is detectable with 80% power at a one-sided 5% significance level assuming 148 total patient are randomized 1:1 to Abiraterone vs Abiraterone + ABT-888. Thus, there will be 74 patients per treatment. Enrollment will continue until 148 patients have been randomized and are response evaluable. Response evaluable patients are defined as patients receiving at least 2 cycles of therapy or patients removed from study due to toxicity before completing 2 cycles. It is estimated that 55% of patients enrolled will be ETS fusion-positive and 45% ETS fusion-negative which results in 81 fusion-positive in the ETS fusion-positive stratum and 67 patients in the ETS fusion-negative stratum. In addition, 81 fusion-positive patients randomized provide 87% power to detect a 30% difference in PSA response rates between Abiraterone versus Abiraterone + ABT-888 at a 5% one-sided significance level in this stratum; 67 fusion-negative patients randomized provides 80% power to detect a 30% difference in response between treatments at a 5% one-sided significance level in this stratum.

Primary Endpoint Analysis Plan: Confirmed PSA response rates and the corresponding binomial confidence intervals will be reported for each arm. A logistic model including treatment group, fusion status, and prior ketoconazole use will be used to determine the interaction between the rates of PSA response between Abiraterone and Abiraterone + ABT-888 and ETS fusion status. The interaction will be tested with a significance threshold of 0.15. If the interaction is not significant, the overall PSA response rates will be compared overall. Four pair-wise comparisons will be made between treatment arms. These comparisons will be:

1) ETS fusion-positive Abiraterone vs ETS fusion-positive Abiraterone + ABT-888.
2) ETS fusion-negative Abiraterone vs ETS fusion-negative Abiraterone + ABT-888
3) ETS fusion-positive Abiraterone vs ETS fusion-negative Abiraterone
4) ETS fusion-positive Abiraterone + ABT-888 vs ETS fusion-negative Abiraterone + ABT-888

- Using α_{int}=0.15, the interaction test has 85% power when there is a 40% increase in PSA response between treatments in ETS fusion-positive patients and no treatment effect

in ETS fusion-negative patients and 67% power when there is a 30% increase in PSA response in ETS fusion-positive patients and no treatment effect in ETS fusion-negative patients.

- Probability of Significant Treatment Effect in ETS Fusion-Positive Patients :

PSA Response Difference in ETS+	Probability of Significant Treatment Effect in ETS Fusion-Positive Stratum
0.2	0.58
0.25	0.74
0.3	0.87
0.35	0.95
0.40	0.98

Interim Analysis Plan and Early Stopping:

After accrual of 34 response evaluable patients in each ETS stratum, accrual will be halted and an interim analysis will be completed. The results of the interim futility analysis will be discussed with CTEP to determine the arms that will be re-opened for enrollment. A comparison within each stratum will be made. If the observed PSA response is the same or worse in the Abiraterone + ABT-888 treatment arm compared to Abiraterone alone then the Abiraterone + ABT-888 treatment arm in that stratum will be stopped. Under the null, the early stopping probability is 57% and under the alternative, has probability of 15.7%.

In addition, each Abiraterone alone arm will be tested for efficacy. If we have evidence in either stratum that Abiraterone alone has a response rate < 5% then we would want to stop treating patients in that arm. Using a binomial comparison with the null hypothesis of PSA response of 30% compared to a 5% PSA response rate this treatment arm will close with less than 2 PSA responses out of the 17 patients. If the fusion positive cohort crosses a futility boundary and the fusion negative cohort has not crossed the futility boundary, the data will be jointly reviewed by CTEP and the investigators to determine whether accrual to the study will continue. If both combination arms are closed for futility the trial will close regardless of futility in the single agent arms.

Concordance in ETS fusion status between the primary and metastatic biopsies will be examined during the interim analysis if there is more than half of the patients have both tissue types available for analysis. The binomial confidence interval will be used to assess concordance with a requirement that the lower confidence limit be greater than 80% to consider allowing the requirement of metastatic biopsies to be relaxed.

Safety Assessment:

Review of the dose of the combination treatment of Abiraterone + ABT-888 will be conducted after the first 10 and again after the first 20 patients who receive the combination treatment. If toxicity, defined as Grade 4 or greater that is at least possibly related to the study drug(s), in the first cycle is greater than 33% at either assessment, then an amendment for dose adjustments will be recommended. The dose recommended will be based upon final phase I data and dose reduction data from this trial. The table below outlines the probability of such adjustments based upon a range of true toxicity probabilities.

	P(Tox in 4 or more out of 10)	P(Tox in 4 or more out of 20)
True toxicity rate = 10%	0.013	0.002
True toxicity rate = 20%	0.121	0.087
True toxicity rate = 30%	0.350	0.392
True toxicity rate = 40%	0.618	0.750
True toxicity rate = 50%	0.828	0.942

13.2 Sample Size/Accrual Rate

Proposed Sample Size: 148 gene fusion assessable and response evaluable patients will be stratified based on gene fusions and randomized. Assuming that 55% of patients harbor ETS fusion-positive prostate cancers, 81 patients will be randomized amongst two proposed treatments for ETS fusion-positive cancer. The 67 remaining patients (ETS fusion negative) will be randomized between the same two treatments as the ETS fusion positive patients. We plan to accrue 8 – 10 patients per month. Thus this trial will take approximately 15-19 months to accrue. It is expected that the trial will be halted to accrual for interim efficacy analysis for 4 months. Final follow-up for the primary endpoint will be approximately 6 months for the last enrolled patients. Final trial time will be approximately 2 to 3 years.

This timeline will change if arms close at the interim due to efficacy. If one arm is closed after the interim analysis, the final sample size will be at most 132 patients; two arms closed will have a maximum of 116 patients.

13.3 Stratification Factors

Patients will be stratified by ETS gene fusion status into ETS positive and ETS negative groups and by use of ketoconazole (yes/no) prior to enrollment.

13.4 Analysis of Secondary Endpoints

Secondary endpoints: Secondary aims will include rates of PSA decline, objective response rates, progression free survival, and toxicity of Abiraterone and Abiraterone + ABT-888.

- Rates of PSA decline will be exhibited using waterfall plots of 12 week PSA decline and maximum PSA decline by treatment arm. Additionally, the rate of PSA decline will be explored using a repeated measures mixed model with the natural log of PSA as the outcome. Fixed effects likely will include treatment, Fusion status, time and the interactions. A second order of time may be necessary as PSA will increase with progression after the decline.
- Objective response proportions with corresponding binomial confidence intervals will be reported by treatment.
- Progression-free survival (PFS) events are defined as the first event of disease progression or death. Patients who do not have an event will be censored at their last disease assessment. Kaplan-Meier methods will be used to describe progression free survival by arm.
- Toxicity will be described by type, grade and frequency for each treatment and arm.

Correlative Analysis Plan:
Correlative aims include evaluating circulating tumor cells (CTCs) at baseline, during therapy, and at progression in all patients, concordance in fusion status among prostate cancer samples from the primary site, biopsied metastasis, and CTCs, ability of expression levels of ETS fusion products in the primary tumor, metastasis, and CTCs to predict response to ETS-directed therapies, and next-generation sequencing for discovery of novel gene fusions in prostate cancers negative for ETS fusions.

- CTCs at baseline will be described by treatment arm and strata. Models may be used to explore early baseline CTCs enumeration and molecular characterization to predict response and PFS.
- Concordance of the ETS fusion status between samples (primary, metastatic, and CTCs) from each patient will be described and tested between the three populations using a generalized kappa. The three pairwise comparisons will also be investigated and McNemar's test and kappa statistic for each will be reported.
- Logistic models with response as the outcome and loss of PTEN in the specimen will be used to determine if PTEN loss predict response to Abiraterone +/- ABT-888 Treatment arm may be controlled for or used as a stratification factor in the model.
- Logistic models with response as the outcome and expression levels of PAR in the specimen will be used to determine if PARP activity predict response to Abiraterone +/- ABT-888 Treatment arm may be controlled for or used as a stratification factor in the model.
- Next-generation sequencing for discovery of novel gene fusions in prostate cancers negative for ETS fusions will be performed. In general, genes fusions that are highest and are found in 2 or more patients will be explored further. The best methods that are recommended for this exploration at the time that all tissue is collected and available will be used. As these methods are rapidly changing, this is difficult to predict at this time.

- Standard quality control statistical analyses (i.e. Hardy-Weinberg Equilibrium testing) will be performed to analyze SNP assay results. Univariable analyses will be performed to identify candidate SNPs associated with favorable abiraterone outcomes. SNPs identified in the univariable analyses will be tested for significance in a multivariable analysis with inclusion of relevant clinical patient parameters (i.e. ETS fusion status, ABT-888 treatment status, performance status, prior ketoconazole therapy, etc.).
- Blood TMPRSS2:ERG mRNA levels at baseline and change from baseline during therapy will be described by treatment arm and strata. Logistic and Cox regression models may be used to explore baseline RNA levels or early changes in RNA levels to predict response and PFS respectively.

13.5 Reporting and Exclusions

13.5.1 Evaluationoftoxicity – All patients will be evaluable for toxicity from the time of their first treatment with Abiraterone or Abiraterone + ABT-888.

13.5.2 Evaluationofresponse – All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

All conclusions will be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses will not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals will be provided.

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APPENDICES:
APPENDIX A

Performance Status Criteria

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

APPENDIX B

CTEP MULTICENTER GUIDELINES

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

ResponsibilityoftheProtocolChair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

ResponsibilitiesoftheCoordinatingCenter

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.

- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

InclusionofMulticenterGuidelinesintheProtocol

- The protocol must include the following minimum information:
  - The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
  - The Coordinating Center must be designated on the title page.
  - Central registration of patients is required. The procedures for registration must be stated in the protocol.
  - Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms to the Coordinating Center should be stated.
  - Describe how AEs will be reported from the participating institutions, either directly to CTEP or through the Coordinating Center.
  - Describe how Safety Reports and Action Letters from CTEP will be distributed to participating institutions.

AgentOrdering

- Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP. Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

APPENDIX C

Standard Operating Procedures for CT-guided Biopsy of Metastatic Prostate Cancer Lesion in Bone

Patients will be scheduled for a CT-guided biopsy of a bone or soft tissue abnormality consistent with prostate metastasis. The site of biopsy will be determined after review of available radiographs, CT, MRI, and/or bone scan (where applicable). The decision regarding which metastatic lesion to biopsy should depend on three main factors:

1) Size of lesion (with preference towards the largest lesion)
2) Safety (with obvious preference towards least risk to the patient)
3) For bone lesions, the intensity of lesion activity on bone scan (with preference towards the most intense activity)

Verbal and written informed consent will be obtained after discussion of risks (bleeding, infection, soft tissue and bone injury, inconclusive biopsy results) of the procedure. Standard laboratory tests to assess coagulation and bleeding will also be completed prior to the procedure. Conscious sedation will be used as necessary using intravenous Fentanyl and Midazolam with appropriate nursing support. In order to ensure adequate tumor sample as long as it is deemed safe and based on accessibility, target lesion size, specimen integrity, specimen appearance, intra-procedural bleeding, tumor biopsy should attempt to obtain 2-8 bone biopsy samples in addition to possible bone marrow aspirate, to be determined at the time of biopsy.

The patient will be initially scanned with a clinically-available multislice CT scanner for the purpose of biopsy planning. Biopsies will be performed using sterile technique and lidocaine local anesthetic. For bone lesions, a clinically-available 11-to-14 gauge bone cutting needle, with or without a co-axial trocar, will be used to obtain **two to eight** biopsy samples of the selected lesion. Options for obtaining multiple samples of the biopsy can include:

a) Sequential advancement of needle passes through a fixed trocar, such that each needle pass progresses more deeply into the bone lesion
b) Re-insertion and re-direction of the needle and/or trocar through another region of the bone lesion
c) Aspiration of the lesion will be attempted in conjunction with biopsy, by attaching a 20 cc sterile syringe to the cannula of the biopsy needle and aspirating into this syringe, with repeat aspirations as needed.

At the completion of the procedure, CT imaging will be repeated to assess for immediate complications. The patient will be observed for 2 – 4 hours before discharge.

APPENDIX D

Standard Operating Procedures for CT-guided Biopsy of Metastatic Prostate Cancer Lesion in Soft Tissue

The patient's imaging will be reviewed and all soft tissue or nodal lesions deemed suspicious for metastasis will be considered. A target for sampling will be selected based on: 1) perceived percutaneous access with respect to safety and technical feasibility, and 2) suspected likelihood of obtaining malignant tissue. In the cases where both bone and soft tissue lesions are safely accessible and have high likelihoods of containing malignant tissue, it would be preferable to biopsy the soft tissue lesion.

- For lymph nodes or soft tissue lesions a CT scan will be utilized. An ultrasound may be utilized, provided that the target lymph node or soft tissue mass is visible and accessible.

- In order to ensure adequate tumor sample as long as it is deemed safe and based on accessibility, target lesion size, specimen integrity, specimen appearance, intra-procedural bleeding, tumor biopsy should attempt to obtain:
    - at least 6 cores if obtaining a 1-cm-long 18-gauge core specimen.
      or
    - at least 4 cores if obtaining a 2-cm-long 18-gauge core specimen. The needle should be directed into the center of the lesion.

Options for obtaining multiple samples include similar principles as described for bone lesions, including sequential advancement through the lesion, re-insertion/re-direction, or aspiration where appropriate.

Mitigating factors may include but are not limited to: 1) safety of obtaining multiple core specimens, 2) friability of the core specimens on visual inspection, 3) target size, 4) perceived likelihood of obtaining additional diagnostic tissue by continued sampling. After biopsy is performed, the biopsy would be snap frozen, as described in Appendix E. No aspirate will be performed for soft tissue biopsies.

APPENDIX E:

Standard Operating Procedure (SOP) for Handling Samples

1.0 PURPOSE

To standardize the method for handling frozen needle tumor biopsies, to optimize specimen use for molecular analyses.

2.0 MATERIALS AND EQUIPMENT REQUIRED

2.1 Sample kit containing:

Dual sample kit will be sent to each enrolling site immediately following patient registration

A) Cell Free DNA 1 tube
B) EDTA 1 tube
C) PAXgene tubes 2
D) Sheet for recording biopsy details

This sample kit will be sent to each enrolling site immediately following patient registration.

2.2 Other materials which will be sent to each enrolling site at the initiation of the clinical trial (to be kept for use over serial cases):

a) One box Tissue Tek™ cryomolds
b) Large bottle of Tissue Tek™ OCT
c) Blue Pads, Markers
d) Sterile tweezers
e) Shipping labels
f) A supply of additional Cell-Free DNA tubes (for CTC samples at the end of cycle 3 and at disease progression) (see Appendix G for handling of CTC samples)
g) Boxes for shipment of CTC sample (see Appendix G)

2.3 Materials that will be provided by the enrolling site:

a) Dry ice
b) -80°C freezer (particularly in the case of Friday biopsies)

3.0 OPERATING PROCEDURES

3.1 At least 24 hours prior to the biopsy, the research coordinator is to notify the institutional research team involved in this protocol, of the scheduled sample collections. A laboratory technician should pick up the sample kit (see 2.1 above), and prepare cryomolds prior to the biopsy, by labeling them, using the provided alcohol-proof marker, with the following

information:

Clinical protocol number
Specimen ID
Biopsy time and Date

The research coordinator and the laboratory technician should also confirm, on the day prior to biopsy, the availability of materials needed for handling the biopsies, as specified in Section 2 above.

3.2 The laboratory technician should arrive at the biopsy collection site at least 15 min ahead of the scheduled biopsy to allow sufficient time to set up laboratory supplies and ensure rapid transport of specimens to the laboratory after collection. He or she should also re-confirm, at that time, the availability of all specimens from Section 2. He should also, immediately before the biopsy, fill the insulated bucket with dry ice and isopentane.

3.3 **Immediately after the biopsy is performed, the freshly collected specimen should be placed in the cryomold. A single drop of Tissue Tek™ OCT should be placed on the specimen, and the sterile tweezers should be used to gently hold one end of the freshly collected needle biopsy and to push the biopsy to the bottom of the cryomold cassette with forceps. Make sure biopsy is as flat as possible. The cryomold should then be filled with OCT, and the cryomold should be immediately placed in direct contact with the dry ice/isopentane cocktail until the bottom of the OCT freezes and turns white. Only the bottom of the cryomold should contact the dry ice/isopentane--none of the dry ice/isopentane should spill inside the cryomold itself and contact the specimen.**
This process can be repeated using separate cryomold cassettes for separate biopsy samples.

3.4 **Once frozen, place cryomolds on dry ice for transport. The used isopentane should be poured back into its bottle using funnel.**

3.5 **For bone lesions, an aspirate of the site of bone biopsy may be performed, as described in Section C, using a 20 cc sterile syringe. The contents of this syringe (clotted aspirate) should be transferred into a 1.7 mL eppendorf tube. Several drops of Tissue Tek™ OCT should be added to the tube, such that the clotted aspirate is covered by OCT. The eppendorf tube should then be closed, and placed on dry ice for snap freezing.**

3.6 The cryopreserved biopsy and aspirate specimen(s) should be stored at -80°C until shipment. Ideally, shipment should occur on the same day as the biopsy, unless the biopsy occurs on a Friday, in which case the specimen should be preserved at -80°C until shipment on the following Monday.

4. Quality Assurance Process

After completion of each biopsy, the following information should be recorded on the information sheet shipped with each sample kit:

<u>Biopsycollection</u>

Date:
Specimen ID:
Time guide needle placement confirmed:

Needle Type:
Needle diameter: gauge; and length: cm
Time biopsy needle introduced:
Time biopsy snap-frozen on dry ice:
Number of specimens:
Time aspirate performed (for bone lesions):
Time aspirate snap-frozen on dry ice:
Date/time of biopsy specimen(s) (and aspirate specimens, if applicable) placed at -80°C:
Date/time of biopsy specimen(s) (and aspirate specimens, if applicable) shipped:
Notes, including any deviations from the standard operating procedure:

APPENDIX F:
Standard Operating Procedure for Shipment of non-CTC Clinical Samples

I. PreparingforShipment

A. Send an e-mail to Siddiqui@med.umich.edu prior to shipping to advise recipient of scheduled shipping time.

B. Generate a shipping list recording the number of samples being shipped, type of sample (frozen biopsy, blood, or archived formalin-fixed biopsy), and clinical protocol number of the patient

C. Make sure that the following packing materials are available:

- Cardboard shipping box with Styrofoam insert.
  --Shipper Boxes 13.38L x 9.25W x 6 in.D; Outside Dimensions: 15.5L x 11.5W x 8 in.H; Wall Thickness: 1 in. Fischer Scientific Catalog # 03-528-27
- Dry ice (amount varies depending on size of Styrofoam insert; usually about 15 kg's)
- 6 in x 6 in cardboard specimen box, with fiberboard box dividers- holds 81, 16mm vials
  --Revco Fiberboard Storage Boxes, 12/PAK
  --Revco Fiberboard Box Dividers, Holds 81 16mm vials, 12/PAK
  Fischer Scientific Catalog # 11-678-24A, 13-989-218 (resp.)
- Plastic bag (to go over the cardboard box –in case of leakage)
- Absorbent strip (to go inside the plastic bag that the boxes are in; can soak up to 250 cc of liquid)
  --Absorbent Strips 250/Cs
  --Fischer Scientific Catalog # NC9193000
- UN 3373 label (2 in x 2 in)
  --Labelmaster UN3373 Labels > 2W x 2.75 In. L
  --Labels; Biological Substance; 2W x 2.75 In. L; Paper; 500/RL
  Fischer Scientific Catalog # NC9493045
- Dry Ice Label with designated UN 1845 diamond (can also be ordered from Fischer Scientific)
  --5 1/2 x 5 1/2 in.; complies with DOT (49 CFR 173.217) and IATA (Packing Instructions 904)
  --Fischer Scientific Catalog #22-130-065
- Packing tape

II. Packagingthespecimens

1. Pull sample tubes from temporary storage freezers and place in dry ice, make sure the labels are securely placed on the tube and tubes properly labeled and easy to read.
2. Ensure that caps are tightly secured and place in 6 in x 6 in cardboard specimen box (which should be kept on dry ice during this transfer).

3. Be sure to **check off** EACH specimen being shipped and verify the contents of the package match the shipping list
4. Label the top of the cardboard specimen box with number of samples; clinical protocol/specimen number, and contact information of sender, as well as contact of receiver.
5. Cover the specimen box, and either tape the sides down or place a rubber band around the box, to ensure that the cover will not come off.
6. Place inside the plastic Ziploc bag with the absorbent strip and seal. Keep on dry ice until ready to transfer to the larger shipping box. (can fit two of these specimen boxes in a shipper box that is the designated size above)
7. Fill the shipper box about half way with dry ice (about 1-2 inches in height) and place the specimen box inside with shipper.
8. Add more dry ice to cover the specimen boxes. (about 1 or 2 inches in height again and 1-2 inches on the sides the boxes as well). Note: there should be sufficient dry ice to maintain the samples at -20°C for at least 72 h.
9. DO NOT place dry ice inside the specimen boxes or inside the plastic bag.
10. Close the Styrofoam box, and tape the packing list to the top. DO NOT seal the Styrofoam box with tape.
11. Ship the specimens with a copy of the shipping list and the completed quality assurance record (see Appendix D) for all specimens. Retain copies of the completed shipping list and quality assurance record in your records.
12. Seal the shipping box by taping the flaps of the insulated box along the top edges.
13. At this point, you should check that your final packaging consists of three components (triple packaging):
    (a) a primary receptacles (the specimen tubes)
    (b) a secondary packaging (6 in x 6 in cardboard specimen box)
    (c) a rigid outer packaging (larger cardboard shipping box)
14. Should you decide to use alternative packaging materials instead of those listed in IC above, please note the following requirements:
    The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packagings must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging. The primary container (i.e. test tube) must be leak proof, as should the secondary container. The outer packaging (tertiary container) does not have to be leak proof. Packaging must comply with the IATA packing instructions 650 which are summarized below; for further questions, please refer to the IATA website:
    http://www.iata.org/SiteCollectionDocuments/DGR51_PI650_EN.pdf

III. Labellingandsendingtheshippingbox

A. Fill out the black and white dry ice label to reflect the weight of the dry ice in the box, note 2lbs= 1kg. Place completed black and white dry ice label on the shipping box

B. All shipping boxes must be affixed with two labels:
"DIAGNOSTIC SPECIMENS PACKED IN COMPLIANCE WITH IATA PACKING INSTRUCTION 650"
and
"UN 3373 Biological Substance Category B"

C. Complete the Fed Ex air bill online and attach packing labels to the outside of the shipper. Schedule a pick up time and ship overnight to:
Javed Siddiqui, MS, MT(ASCP, CLsp (MB))
Michigan Center for Translational Pathology
Room 1138
2900 Huron Parkway Suite 100
Ann Arbor, MI 48105
Phone: (734) 232-0829
Fax: (734) 232-0805
siddiqui@med.umich.edu

D. All shipping boxes are required to ship out FedEx Priority Overnight (or another comparable shipping service that results in delivery by 10:30 AM the following day).

E. Biopsies on Monday through Thursday should be shipped on the same day as the biopsy. Biopsies on Friday should be stored at -80 degrees Celsius until the following Monday, and then should be shipped via FedEx Priority Overnight (or a comparable service).

F. The sender would need to complete an "air waybill" form

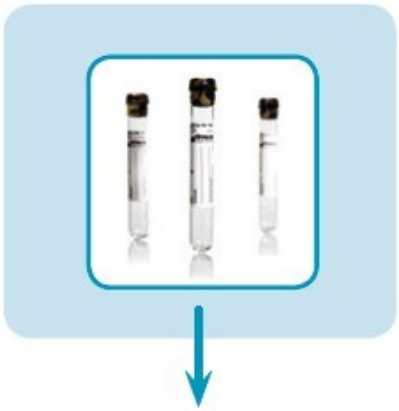
G. All diagnostic or investigational specimens shipped on this trial should be classified as "Diagnostic Specimens" not "Infectious Substances" when shipping via a standard carrier. (i.e. UPS, FED-EX, DHL/AIRBORNE). Diagnostic Specimens DO NOT require a Shipper's Declaration of Dangerous Goods form.

IV. Questions

For any questions regarding shipping, please contact Javed Siddiqui at siddiqui@med.umich.edu or phone: (734) 232-0829.

APPENDIX G: Standard Operating Procedure for Shipment of CTC Clinical Samples

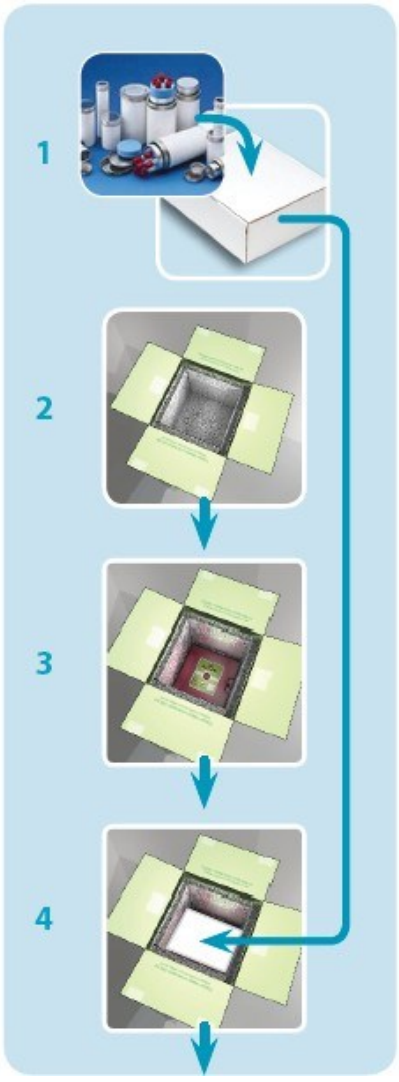
BLOOD SPECIMEN COLLECTION AND SHIPPING INSTRUCTIONS



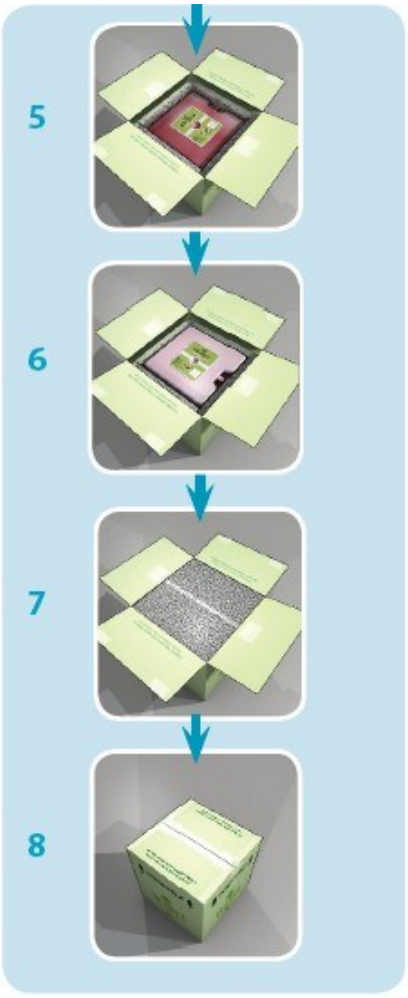
Blood Specimen Collection

- Collect blood in Cell-Free DNA blood collection tubes and invert twice after the blood draw to mix the anti-coagulatant
- Be sure each tube is at least half way filled or cell preservative will not work optimally
- Keep blood tubes at room temperature with no agitation until shipment

Blood Specimen Shipment



1. Place blood tube(s) into foam insert and place in aluminum canister. Place canister into supplied cardboard box
2. Open pre-assembled reusable specimen shipping container, and remove insulation panel
3. Place two liquid (red) E23 panels that have been stored at room temperature in the bottom of the shipping container rotated 90 degrees from each other
4. Place box containing canister with blood specimen(s) directly on top of previously placed panel



5 Place one liquid (red) E23 panel that has been stored at room temperature on top of the box rotated at 90 degrees from the panel below it

6 Place one solid (red) E23 panel that has been stored at 4°C on top of box

7 Place locking lid on top of the 4th panel with the seam facing up to ensure insulation

8 Close shipping box and tape appropriately

9 **Ship to:**

Epic Sciences
c/o Dena Marrinucci
10975 N Torrey Pines Road
La Jolla CA 92037
tel: 858.356.6610

Samples may be mailed to Epic Sciences for Monday–Saturday delivery

10 **Send Email to:**

partners@epicsciences.com

In the Email Include:

1. Tracking number
2. The number of samples being shipped
3. The date and time of each blood draw
4. Case report form including white blood cell count of patient(s)

APPENDIX H
PATIENT'SMEDICATIONDIARY

CTEP-assigned Protocol # 9012

Local Protocol # _

Today's date _____ **Agent: ABT-888**

Patient Name_____(*initials acceptable)* **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle of treatment.
2. You will take **ABT-888**
   Dose: ABT-888 ___50 mg tablets and ABT-888 ___100 mg tablets. Take ABT888 twice daily. Take it with or without meals. Do not crush, break, or chew it.
3. Record the date, the number of tablets of each size of tablet that you took, and when you took them.
4. If you have any comments or notice any side effects, please record them in the Comments column.
5. Please bring this form and your bottles of ABT-888 tablets when you return for each appointment.
6. Please record missed or skipped doses. Do not share your study drug supply, and wash your hands after touching the pills

Day	Date	Time of AM dose ABT-888	# of tablets taken ABT-888	Time of PM dose ABT-888	# of tablets taken ABT-888	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
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16						
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23						
24						
25						
26						
27						
28						

Patient's signature_____

Physician's Office will complete this section:

1. Date patient started protocol treatment _____
2. Date patient was removed from study _____
3. Patient's planned total daily dose _____
4. Total number of tablets taken this month _____
5. Physician/Nurse/Data Manager's Signature _____

APPENDIX I

PATIENT'S MEDICATION DIARY

Agent: Abiraterone/Prednisone Cycle 1 Arms IB and IIB

CTEP-assigned Protocol #9012
Local Protocol #

Today's date _____

Patient Name_____(*initials acceptable*) **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete this form for each cycle 1 of treatment.
2. You will take **Abiraterone and Prednisone**
   Dose: Abiraterone ___ 250 mg tablets. You should take the Abiraterone tablets on an empty stomach (1 hour before or 2 hours after a meal) at approximately the same time each day. Swallow the tablets whole with water. Do not break, crush, or chew it.
   Prednisone ___ 5 mg tablets. Take Prednisone twice daily with food.
3. Record the date, the number of tablets of each size of tablet that you took, and when you took them.
4. If you have any comments or notice any side effects, please record them in the Comments column.
5. Please bring this form and your bottles of Abiraterone/Prednisone tablets when you return for each appointment.
6. Please record missed or skipped doses. Do not share your study drug supply, and wash your hands after touching the pills

Day	Date	Time of AM dose Prednisone	# of tablets taken Prednisone	Time of PM dose Prednisone	# of tablets taken Prednisone	Time of dose Abiraterone	# of tablets taken Abiraterone	Comments
1	N/A							
2								
3								
4								
5								
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27								
28								

Patient's signature_____

Physician's Office will complete this section:

1. Date patient started protocol treatment _____
2. Date patient was removed from study _____
3. Patient's planned total daily dose _____
4. Total number of tablets taken this month _____
5. Physician/Nurse/Data Manager's Signature _____

APPENDIX J

PATIENT'S MEDICATION DIARY

Agent: Abiraterone/Prednisone (Not for Cycle 1 Arm IB or IIB)

CTEP-assigned Protocol #9012

Local Protocol #

Today's date _____

Patient Name_____(*initials acceptable)* **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete this form for each cycle of treatment.
2. You will take **Abiraterone and Prednisone**
   Dose: Abiraterone ___250 mg tablets. You should take the Abiraterone tablets on an empty stomach (1 hour before or 2 hours after a meal) at approximately the same time each day. Swallow the tablets whole with water. Do not break, crush, or chew it.
   Prednisone ___5 mg tablets. Take Prednisone twice daily with food.
3. Record the date, the number of tablets of each size of tablet that you took, and when you took them.
4. If you have any comments or notice any side effects, please record them in the Comments column.
5. Please bring this form and your bottles of Abiraterone/Prednisone tablets when you return for each appointment.
6. Please record missed or skipped doses. Do not share your study drug supply, and wash your hands after touching the pills

Day	Date	Time of AM dose Prednisone	# of tablets taken Prednisone	Time of PM dose Prednisone	# of tablets taken Prednisone	Time of dose Abiraterone	# of tablets taken Abiraterone	Comments
1								
2								
3								
4								
5								
6								
7								
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Patient's signature_____

Physician's Office will complete this section:

1. Date patient started protocol treatment _____
2. Date patient was removed from study _____
3. Patient's planned total daily dose _____
4. Total number of tablets taken this month _____
5. Physician/Nurse/Data Manager's Signature _____

spread of ETS positive cells. In preclinical models, PARP1 inhibition blocked the formation of liver metastases in an ETS-positive cell line but not an ETS-negative cell line. Moreover, using 8 different prostate cancer xenograft models (4 positive for ETS fusions, 4 negative for ETS fusions), PARP1 inhibition had a much more significant effect on xenograft growth in all of the ETS fusion-positive xenografts compared to the ETS fusion-negative xenografts. (Fig. 3)

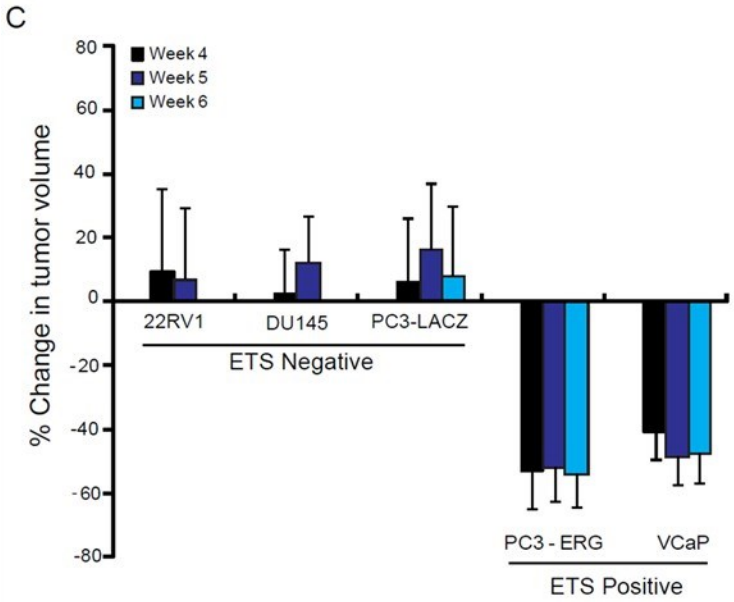

Fig. 3

Taken together, this data suggests that ETS-mediated oncogenic features, such as metastasis and tumor growth, depend on PARP1, and that inhibition of PARP1 can inhibit these phenotypes and specifically target ETS- positive prostate cancers.

2.3 ABT-888 (Veliparib)

ABT-888 is an orally available, small molecule inhibitor of poly(ADP-ribose) polymerase (PARP). PARP is an essential nuclear enzyme that plays a role in recognition of DNA damage and facilitation of DNA repair. Therefore, inhibition of PARP is expected to enhance the effects of DNA damage. Expression of PARP is higher in tumor cells as compared to normal cells. This overexpression has been linked to drug resistance and the ability of tumor cells to withstand genotoxic stress. Hence, it is anticipated that PARP inhibitors will function as sensitizing agents for chemotherapy and radiation therapy that are designed to cause DNA damage.