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Title: A Phase 2 Randomized Discontinuation Trial in Patients with Hormone-Dependent Rising Prostate-Specific Antigen Progression After Local Therapy For Prostate Cancer Evaluating the Synergy of Metformin Plus Aspirin (PRIMA Trial).

Principal Investigator: [redacted]

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PI: [redacted]

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LIST OF ABBREVIATIONS

ADT	Androgen depravation therapy
AE	Adverse Event
AKT	Protein kinase B (PKB) also known as AKT
ALT	Alanine transaminase
AMPK	AMP-activated protein kinase
ASA	Aspirin
AST	Aspartate aminotransferase
ANC	Absolute neutrophil count
AR	Androgen receptor
BUN	Blood urea nitrogen
CBC	Complete blood count

[redacted]

CINJ	[redacted]
CINJOG	
CI	Confidence interval
COX	Cyclooxygenase
CT	Computer Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CXR	Chest x-ray
dL	Deciliter
DNA	Deoxyribonucleic acid
DSMP	Data Safety Monitoring Plan
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EGFR	Epidermal growth factor receptor
FDA	Food and Drug Administration
GI	Gastrointestinal
IGF-I	Insulin-like growth factor I
IRS	Insulin receptor substrates
IRB	Institutional Review Board
JAK	Janus kinase
LKB1	Liver kinase B1
MAPK	Mitogen-activated protein kinases
Mg	Milligrams
mL	Milliliters
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
NCI	National Cancer Institute
NF-kB	Nuclear factor kappa-light chain enhancer of activated B cells
Ng	Nanograms
NIH	[redacted]
OR	Odds ratio
OHRS	Office of Human Research Services
OHRP	Office of Human Research Protection
PI3K	Phosphoinositide-3-kinase
PI	Principal Investigator
Pca	Prostate Cancer
PSA	Prostate specific antigen
PSA DT	Prostate specific antigen doubling time
Rb	Retinoblastoma protein
SAE	Serious adverse event
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
STAT	Signal transducer and activator of transcription
WBC	White blood cell

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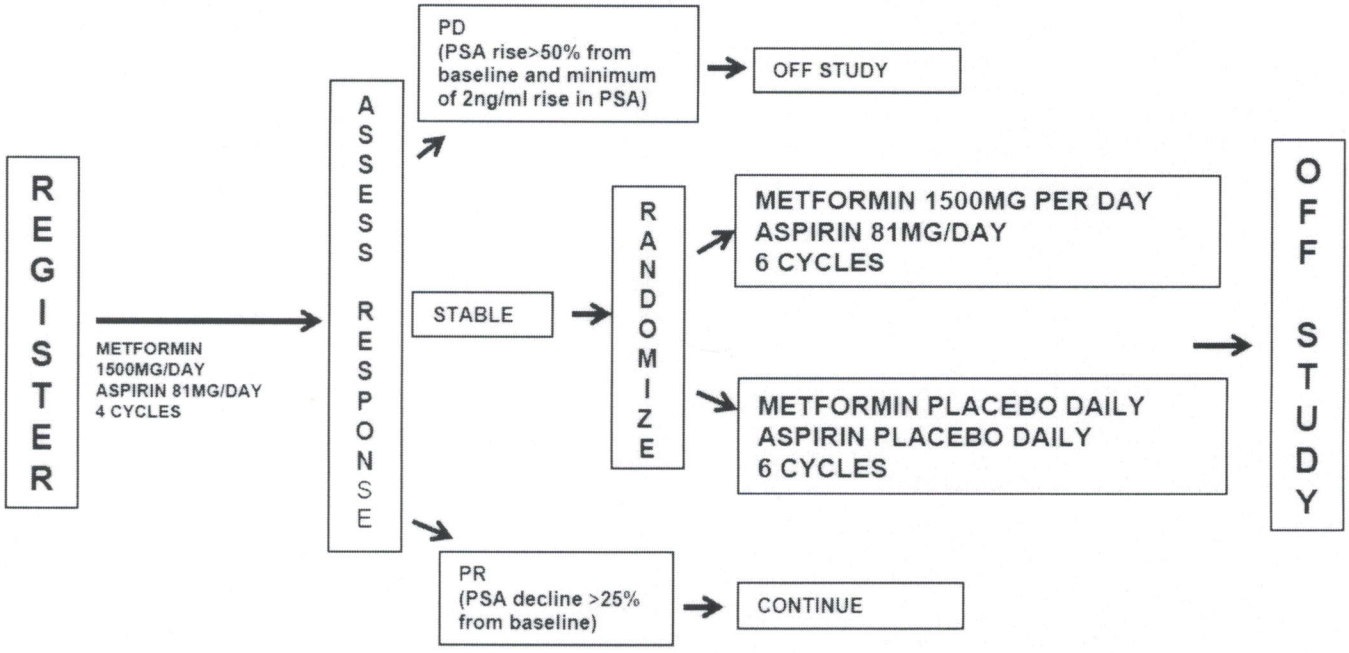
SYNOPSIS:



Primary endpoint:
Increase in 6 month rate of PSA and clinical progression in randomized patients from 40% in the treatment arm to 80% in the placebo arm.

TOTAL NUMBER OF REGISTERED PATIENTS= 66 (assuming 10 patients progress prior to randomization)

TOTAL NUMBER OF RANDOMIZED PATIENTS= 56
28 PATIENTS PER ARM

STATISTICAL ANALYSIS: With 28 patients randomized to treatment or placebo the study will have 80% power to detect an increase in the proportion of patients with progression at 6cycles from 40% to 80% with alpha =0.05

DEFINITION OF PSA PROGRESSION DURING RANDOMIZATION PERIOD:
Increase in PSA by >50%above and absolute rise of >2ng/ml

1. Purpose/Specific Objectives

In this phase II protocol we specifically propose the following aims:

1.1 Primary Endpoints

1.1.1 To determine the effect of metformin and aspirin on the change in PSA progression in men with rising PSA after definitive therapy for localized prostate cancer and stable disease during a run-in period with the study regimen.

1.2 Secondary Endpoints

1.2.1 To determine the feasibility and safety of administering metformin and aspirin

1.2.2 To determine the effect of metformin and aspirin on PSA levels and the serum obesity-related PCa biomarkers (insulin, IGF-1, IL-1β, IL-6, and TNF-α)

2. Background and Significance

Prostate cancer is the most commonly diagnosed cancer in males and the second leading cause of death from cancer in men. One in every six men is diagnosed with prostate cancer during their lifetime. The American Cancer Society estimates that during 2014 approximately 233,000 new cases of prostate cancer will be diagnosed and over 29,480 men will die from the disease in the U.S. Twenty to forty percent of patients undergoing radical prostatectomy [1, 2] and 30–50% of patients undergoing radiation therapy will experience biochemical recurrence within 10 years [3],[4].

While there is no current standard of care therapy in patients with PSA progression following local therapy without defined metastasis, it is of particular difficulty to make treatment decisions in this population of patients. Despite a clearer understanding of the usage of androgen depravation therapy (ADT) in patients with metastatic disease, there is no definitive clinical trial or approach to define when and if to initiate ADT in this population with only PSA progression after local therapy [5]. Given the eventual progression to resistance to, and toxicities of, ADT, however, additional options of less toxic therapies would be impactful to this large population of patients [6, 7].

Multiple mechanisms of tumor progression and drug resistance are now understood and provide potential therapeutic targets. Most rising PSA only prostate cancer patients are hormonal sensitive and depend mainly on the classical androgen receptor-signaling pathway. This classical pathway starts with an androgen binding to the androgen receptor (AR), the complex then transports from the cytosol into the cell nucleus and binds to a specific sequence of DNA and interact with other proteins, resulting in up- or down-regulation of specific gene transcription [8]. Alternative androgen receptor-signaling pathways, such as PI3K/Akt/mTOR [9-11], MAPK [12-14], Wnt/beta-catenin [15], JAK/STAT [16], have been implicated in prostate carcinogenesis. A number of clinical trials testing inhibitors targeting the alternative androgen receptor-signaling pathways, such as Akt, EGFR inhibitors, in treating rising PSA only prostate cancer have been performed or are currently ongoing.

Metformin activity in cancer: Metformin is the most widely used antidiabetic drug because of its proven efficacy and limited side effects. Interestingly, recent studies have reported that metformin can block the growth of different tumor types [17, 18]. Metformin causes secondary inhibition of

the mTOR complex via activating AMP-activated protein kinase (AMPK) [19]. Insulin and Insulin-like growth factor I (IGF-I) are key factors in promoting cancer development. Binding of insulin/IGF-I to their receptors results in receptor autophosphorylation and activation of receptor tyrosine kinase, followed by tyrosine phosphorylation of insulin receptor substrates, which further propagates the downstream PI3K/AKT/mTOR signaling pathway [20]. Metformin is observed to reduce hyperglycemia, improve glucose utilization, and reduce free fatty acid utilization and gluconeogenesis. Subsequently, metformin reduces insulin and IGF-1 production. Metformin may abolish mTOR activation through AMPK-independent pathway - inhibition of insulin/IGF-I signaling.

Metformin has also been shown to inhibit cyclin D1 expression and retinoblastoma protein (Rb) phosphorylation, which results in the inhibition of cell proliferation [21]. Metformin inhibits androgen-induced IGF-IR up-regulation in prostate cancer cells by disrupting membrane-initiated androgen signaling [22]. These indicate metformin might have direct inhibitory effect on prostate cancer cells.

Aspirin activity in cancer: Aspirin (ASA) is a salicylate drug, often used as an analgesic, an antipyretic and anti-inflammatory medication. ASA also has been widely used as an antiplatelet in the prevention of cardio-cerebrovascular diseases. ASA is non-selective and irreversibly inhibits both COX1 and COX2. ASA clearly demonstrated its role in preventing cancer death as described in a meta-analysis of eight randomized clinical trials - the largest drop in risk was for colorectal cancer and some more modest risk reductions for several other common cancers, including lung and prostate [23]. The mechanisms for its chemopreventative effects are, however, not clear yet. Various hypotheses have been proposed; the most accepted among these proposes that its anti-cancer action is mediated through COX-2 inhibition [24, 25]. Other possible mechanisms includes inhibition of NF-kB [26], induction of polyamine catabolism [27], inhibition of mTOR signaling [28] and activation of AMPK [29], effects on PI3K pathway [30] or its crosstalk with COX-2, and induction of apoptosis through other pathways. We recently demonstrated that COX/beta-catenin signaling pathway is activated in hematopoietic stem cell self-renewal and leukemogenesis [31]. ASA might exert its direct anti-prostate cancer effects through inhibitions of COX2, mTOR, AMPK, Wnt/beta-catenin and PI3K pathways and might be synergistic when combined with metformin.

Metformin, aspirin and PCa tumorigenesis: A recent non-randomized study showed that metformin use was associated with a 24% reduction in PCa mortality for every additional 6-months of post PCa diagnosis exposure (odds ratio [OR] 0.76; 95% confidence interval [CI], 0.64-0.79) compared with other antidiabetic drug use among diabetics [32]. Consistent with this finding, metformin use is also associated with a reduced risk of distant metastasis, development of castration-resistant PCa (OR=0.07, 95% CI 0.008-0.55) following biochemical recurrence, and overall mortality among men receiving external beam radiation [33]. A randomized trial demonstrated that metformin use (500mg three times a day for 41 days) before surgery reduced cancer proliferation rate by 32% in the surgical specimen compared with pre-surgical biopsy specimen [34]. In addition, a recent phase 2 trial in patients with castration-resistant PCa showed metformin treatment for 24 weeks may induce disease stabilization and prolong the prostate-

specific antigen (PSA) doubling time [35]. Furthermore, metformin significantly inhibited the tumor growth in LNCaP xenograft mice [36] and reduced PIN development and PCa lesions in Hi-Myc mice [37]. For aspirin, there is some evidence for a protective effect against PCa [38-40], however, controversy data suggesting no association has been reported [41]. In a longitudinal cohort study of 5955 patients with localized PCa who were followed for up to 10 years, Choe *et al.* recently reported that aspirin use was independently associated with a lower risk of PCa-specific mortality (adjusted hazard ratio, 0.43; 95% CI 0.21-0.87) [42]. A systematic meta-analysis showed that aspirin use decreased the total risk of PCa by 17% (95% CI 0.77-0.89) and decreased the risk of advanced PCa by 19% (95%CI 0.72-0.92) [43, 44]. **These results suggest that metformin and aspirin could be used in preventive and/or therapeutic strategies for PCa.**

Metformin, aspirin and the NF-κ B/STAT3 signaling pathway: NFκB, a mediator of inflammatory response, plays a significant role in carcinogenesis and is now emerging as a link between inflammation and cancer. Abundant data supports a key role for the NFκB signaling pathway in controlling the initiation and progression of human cancer. The NFκB pathway is an important contributor to PCa metastasis [45-47] and activation of NFκB signaling promotes castrate-resistant growth of PCa [48, 49]. STAT3 mediates a complex spectrum of cellular responses including inflammation, cell proliferation and apoptosis. Constitutively activated STAT3 has been implicated in a number of cancers, including PCa [50-52]. More important, inhibition of STAT3 has been shown to induce apoptosis in PCa cells [51-53], suggesting STAT3 may be an excellent molecular target for PCa prevention and treatment. Aspirin has been extensively studied as a COX inhibitor (Figure 1). It has also been shown to inhibit NFκB activation [54, 55], and to induce apoptosis through down-regulation of interleukin (IL)-6-STAT3 signaling pathways [56, 57]. These data indicate that NFκB/STAT3 play a critical role in the prevention of aspirin against PCa. Metformin has also been shown to inhibit STAT3 phosphorylation and downstream signaling in triple-negative breast cancer cells [58], and increase the sensitivity of resistant cells to cisplatin by suppressing STAT3 activity without activation of AMPKα, suggesting STAT3 is a critical regulator of metformin action [59].

Metformin, aspirin and AMPK/mTOR signaling pathway: Metformin may diminish the effects of insulin on tumor development and growth through inhibition of hepatic gluconeogenesis or increase insulin sensitivity, thus leading to reduced circulating insulin and IGF-1 levels (Figure 1) [60, 61]. Binding of insulin/IGF-1 to their receptors results in receptor autophosphorylation and activation of receptor tyrosine kinase, followed by tyrosine phosphorylation of insulin receptor substrates (IRS 1-4), which further propagates the downstream phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR signaling pathway [20]. Metformin may abolish mTOR activation through inhibition of insulin/IGF-1 signaling an AMPK-independent mechanism, or through liver kinase B1 (LKB1)-mediated activation of AMPK an AMPK-dependent mechanism [62]. While AMPK-dependent suppression of mTOR signaling remains the key candidate mechanism of antitumor action of metformin, of particular note is that metformin may also target the inflammatory component, leading to tumor reduction [63, 64]. Salicylate, as a main metabolite of aspirin, was recently reported to directly activate AMPK activity [65]. Din *et al.* recently showed that aspirin alone or combination of aspirin and metformin activates AMPK and inhibits mTOR signaling in

colorectal cancer cells [66]. Potential mechanisms of synergistic action of these two agents in pancreatic cancer have been reviewed comprehensively [67].

In vitro mechanism studies: Recent work in the laboratory of Dr. Tan and Rutgers CINJ demonstrates that the combination of metformin and aspirin at low concentrations had significant synergistic effects on the inhibition of cell viability and colony formation (Figure 3), and resulted in a significant decrease in the phosphorylation of mTOR and STAT3 (Figure 4) in two PCa cell lines LnCap and Du145.

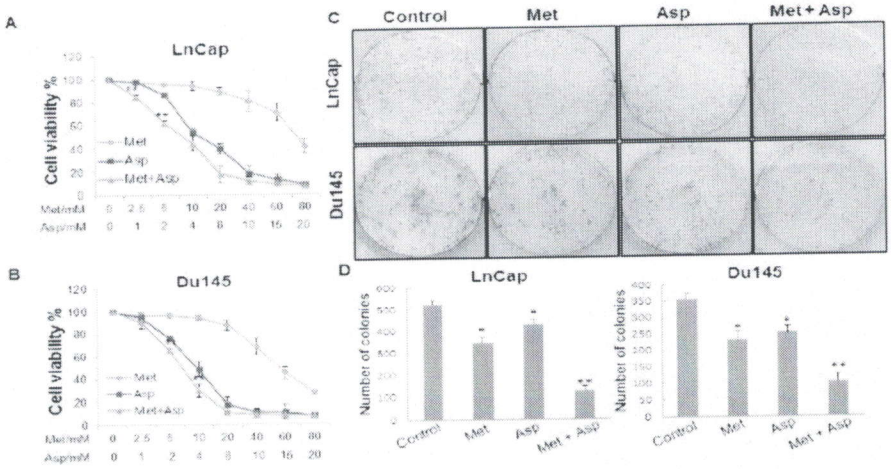


Fig. 1. Metformin and aspirin synergistically inhibit cell viability and colony formation in both LnCap and Du145 cells. A and **B,** MTT assay for the cell viability of LnCap and Du145, respectively. Cells were treated with indicated concentrations of metformin and aspirin, alone or in combination for 72 h; **C.** Clonogenic assay for LnCap and Du145. Cells were treated with metformin (5 mM) and aspirin (2 mM), alone or in combination for 72 h; then 1000 treated or untreated cells per well were seeded in 6-well plates and cultured for another 10 days; **D.** Quantification of colony formation assay in LnCap and Du145 cells, respectively. Met, Metformin; Asp, Aspirin: *P < 0.05, compared to the control, **P < 0.05 for the test of synergy.

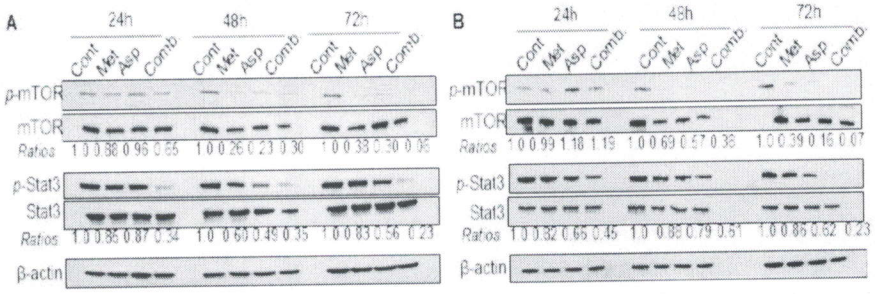


Fig.2. Metformin and aspirin synergistically inhibit the phosphorylation of mTOR and STAT3 in both LnCap (A) and Du145 (B) cells. Cells were treated with 5 mM metformin, 2 mM aspirin or a combination for 24, 48, or 72 h, respectively. Protein expression levels of mTOR, *p*-mTOR, STAT3 and p-STAT3 were determined by Western blot. The ratios of p-mTOR/mTOR and psSTAT3/STAT3 we[redacted] calculated and normalized to the control. Cont, Control; Met, Metformin; Asp, Aspirin; Com[redacted] Combination.

Clinical trial:

Based on compelling epidemiological and preclinical data suggesting that metformin and aspirin may act synergistically to inhibit prostate cell growth, we will conduct a placebo-controlled Phase II intervention study, using a randomized discontinuation design. The primary objective is to determine if metformin plus aspirin prolongs the PSA doubling time compared to use of metformin alone in patients who have stable PSA values during a 4 month run in period of treatment with both agents.

In this study a randomized discontinuation design will be used in order to address some of the unique considerations in testing a regimen that would be considered effective if it leads to disease stabilization and that depends on modulation of a blood based biomarker as the primary determinant of efficacy.

As noted by Ratian et.al, "a randomized discontinuation design initially treats all patients with the study agent (stage 1) and then randomizes in a double-blind fashion to continuing therapy or placebo only those patients whose disease is stable (stage 2). This design allows the investigators to determine if apparent slow tumor growth is attributable to the drug or to selection of patients with naturally slow-growing tumors. By selecting a more homogeneous population, the randomized portion of the study requires fewer patients than would a study randomizing all patients at entry. The design also avoids potential confounding because of heterogeneous tumor growth. Because the two randomly assigned treatment groups each comprise patients with apparently slow growing tumors, any difference between the groups in disease progression after randomization is more likely a result of the study drug and less likely a result of imbalance with respect to tumor growth rates". Utilization of this design allowed investigators to determine that sorafenib caused statistically meaningful disease stabilization that was subsequently confirmed in a phase III trial leading to drug approval.

In patients with rising PSA, drugs causing disease stabilization would be expected to lead to a slowing in the rate of rise in the PSA, which can also be expressed as a lengthening of the PSA doubling time. Multiple prior phase II trials for men with rising PSA, including several randomized placebo controlled trials, have assessed changes in PSA doubling time as an endpoint. In these trials it was noted that patients in the placebo arm often have an increase in the PSADT after going on study. As noted by Paller et al., in a Rosiglitazone trial involving 106 patients, 73% of patients on placebo had an increase of PSADT in excess of 100%, and 31% exceeded 200% in PSADT lengthening. In an Atrasentan trial involving 222 patients, 78% of the patients on placebo had a lengthening of PSADT. In a celecoxib trial involving 78 patients, 20% of the patients on placebo had >200% increase of PSADT. In these studies, the increase in pre and on-treatment PSA doubling time has been attributed to difference between spacing interval and number of PSA values collected in an uncontrolled "pre-study period compared to the regular intervals at which PSA values are collected once on study".

In the current design all subjects will receive study therapy for 4 cycles. During this period PSA will be measured at monthly intervals in order to determine a PSA doubling time in a uniform manner, and to obtain a relatively homogeneous population of patients for randomization. Patients

meeting the study specified definition of stable disease will then proceed to randomization while patients meeting criteria for PSA progression will come off study and those patients with PSA response will continue on therapy until the study definition of disease progression is met.

After the run in phase patients meeting the criteria for stable disease will be randomized to continue therapy with metformin and aspirin or to placebo metformin, and placebo aspirin.

The secondary objective of this study is to determine whether the intervention may modify the PSA levels and the serum obesity-related PCa biomarkers. The tolerability of and compliance of metformin and aspirin treatment in these subjects will also be evaluated.

3. Participating Institutions

Participants for this study will be recruited from the patient population evaluated and treated at the [redacted]. Members of the CINJOG network may also participate in [redacted]tudy, and patients may be evaluated and treated at these respective institutions as well.

4. Experimental Design and Methods

Eligible patients with rising PSA after definitive therapy of localized prostate cancer, no visible metastatic disease on conventional imaging studies including CT scan of the abdomen and pelvis and bone scan, and a PSA doubling time of greater than 6 cycles will be treated with 4 cycles of metformin 500mg/1000mg BID and aspirin 81mg/day.

4.1 Disease Assessment After Run In Stage:

After 4 cycles of treatment patients with disease progression (PSA increase of >50% and minimum of 2ng/ml rise in PSA) will come off study. Patients with disease response (>25% decline in PSA) will continue on study agents.

Patients with stable disease (not meeting criteria for progression or stable disease) will be randomized to continue on the study regimen or receive placebo therapy for 6 cycles.

4.2 Run In Stage Treatment:

Metformin treatment will be started at 500 mg twice daily, if no GI toxicity grade greater than 1 is noted after 2 weeks it will be increased to 500 mg with breakfast/1000 mg at bedtime, which is the target dose for the remainder of the study. Aspirin 81 mg will be taken once daily with food.

4.3 Randomization Stage Treatment:

Patients meeting criteria for randomization will be randomized to continue therapy with metformin 500 mg in the morning and 1000 mg at night and aspirin 81 mg per day and or to placebo metformin and, placebo aspirin.

Randomization will be performed based on a randomization schedule generated by statistician (Dr. Lin) using a stratified randomization method in order that the numbers of obese patients (BMI $\geq$ 30) on each group are balanced.

4.4 PSA Responder Treatment:

Patients meeting criteria for PSA response (>25% decline in PSA during the run-in phase) randomization will be assigned to continue therapy with metformin 500 mg in the morning and 1000 mg at night, and aspirin 81 mg per day until disease progression.

4.5 Disease Progression Criteria During Randomization Stage Treatment

Disease progression during the randomization is defined as rise in PSA by >50% above baseline and absolute increase of 2ng/mL or radiographic progression

4.6 Duration of Follow-up

For this protocol, all patients, including those who discontinue protocol therapy early, will be followed for response (or progression) and for survival for 1 year from the date of registration. All patients must also be followed through completion of all protocol therapy.

5. Patient Selection Criteria

5.1 Inclusion Criteria

A patient is eligible for enrollment if all of the following inclusion criteria are met:

5.1.1 Patients with histologically proven prostate cancer treated with surgery, radiation, or the combination of surgery and radiation or prostate cancer (metastatic to regional lymph nodes) with resection of the nodes, who now has a rising PSA value after definitive local therapy, and no visible metastatic disease on conventional imaging studies

5.1.2 Patients must have undergone local treatment via prostatectomy or radiation therapy.

5.1.3 Patients must have PSA progression after local treatment:

a. PSA values for patients after surgery (or surgery and salvage/adjuvant radiation) must be greater than or equal to 0.2 ng/mL, determined by two measurements, at least 1 month apart and at least 6 months after prostatectomy

OR

b. PSA values for patients after radiation must be greater than or equal to 2.0 ng/ml greater than the nadir achieved after radiation, determined by two measurements at 1 month apart and at least 6 months after the radiation treatment is completed. (Patients who received adjuvant or salvage radiation after prostatectomy must have PSA of greater than or equal to 0.2)

c. The first two PSA values (in 5.1.3a or 5.1.3b), along with a third (pre-study) value must all be rising (i.e., there must be an overall rising trajectory, such that the third value cannot be lower than the first value).

d. PSA doubling time using the mkscc.org PSA doubling time calculator must be greater than 4 months

5.1.4 Baseline bone scan, chest x-ray and CT/MRI of abdomen/pelvis demonstrating no metastatic disease.

5.1.5 A bone scan and a CT or MRI abdomen/pelvis and chest x-ray (CXR) or chest CT scan, will have been performed within 12 weeks of treatment start. Radiographic assessments will be selected by the attending physician as clinically indicated.

5.1.6 Age ≥ 18 years

5.1.7 Estimated life expectancy of at least 6 months.

5.1.8 ECOG performance status ≤ 2. (see Appendix A)

5.1.9 A WBC > 3500/µl, ANC >1500/µl, hemoglobin > 10 g/dl, and platelet count >100,000/µl are required.

5.1.10 Adequate renal function with estimated GFR by Cockcroft Gault of greater than 40 ML per minute

5.1.11 Total bilirubin must be within 1.5X the normal institutional limits. If total bilirubin is outside the normal institutional limits, assess direct bilirubin. The direct bilirubin must be within normal parameters. Transaminases (SGOT and/or SGPT) must be less than 2.5X the institutional upper limit of normal.

5.1.12 Patients must have a serum total testosterone level ≥150 ng/dL at the time of enrollment within 12 weeks prior to randomization.

5.1.13 Patients must sign informed consent.

5.2 Exclusion Criteria

A patient will not be eligible for this study if any of the following exclusion criteria are met:

5.2.1 Serious concomitant systemic disorder that would compromise the safety of the patient or compromise the patient's ability to complete the study, at the discretion of the investigator.

5.2.2 Patients may have received prior ADT in the neoadjuvant, adjuvant and/or salvage setting, but must be off therapy for at least 3 months and have a testosterone level > 150 ng/dl.

5.2.3 Second primary malignancy except most situ carcinoma (e.g. adequately treated non-melanomatous carcinoma of the skin) or other malignancy completely resected with no recurrence.

5.2.4 Patients with type II diabetes currently already on metformin.

5.2.5 Patients taking aspirin for previously diagnosed cardiovascular disease.

5.2.6 Patients who received aspirin or metformin within the past 28 days.

5.2.7 Patients taking medications with known interactions with metformin or aspirin.

5.2.8 Patients taking warfarin or platelet inhibitors.

5.2.9 Patients requiring chronic use of NSAIDS.

5.2.10 Other concurrent experimental or investigational drugs.

5.2.11 Prior history of lactic acidosis or metabolic acidosis.

5.2.12 Patients with history of GI bleeding and peptic ulcer disease within the past 6 months.

5.2.13 Any unstable, serious co-existing medical conditions including but not limited to myocardial infarction, coronary bypass surgery, unstable angina, cardiac arrhythmias, clinically evident congestive heart failure, or cerebrovascular accident within 6 months prior to screening.

5.3 Women and Minorities

Men from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. Women will not be eligible for this study as prostate cancer does not occur in women.

5.4 Children

Only patients 18 years of age or older will be enrolled on this study, since prostate cancer does not occur in children.

5.5 Study Enrollment Procedures

A copy of the institution's IRB-approved informed consent document and written justification [redacted] Research Services (OHRS) before any participating institution may enter patients. Consent forms proposed for use at a participating institution must be reviewed and approved by the OHRS Regulatory Affairs Manager and all documents must be received (i.e., IRB approved documentation, IRB approved consent form, See Section 15.2 for a complete list of regulatory items).

Participating institutions will register patients through the [redacted] Registration Desk telephone [redacted]. Patient demographic information, the signed and dated study-specific eligibility checklist and completed signature page of the consent form and additional source documents if requested by OHRS must be sent to the registration desk. Once the OHRS Registration Desk verifies eligibility, a unique patient study number will be issued. The patient will not be identified by name. This is the point that the patient is considered on study.
Patients must <u>not</u> start protocol treatment prior to registration.

If a patient does not receive any protocol therapy, baseline data will be collected and submitted on the pre-study and follow-up electronic case report forms (eCRF) through Oncore®. The reason for not starting protocol therapy will be documented in the "follow-up eCRF". Case report form completion instructions and training will be provided to each participating institution prior to study activation at the participating institution.

6. Study Parameters

The following tests and evaluations will be performed according to the schedule below. Baseline (i.e., pre-study) evaluations must be performed within 4 weeks (+/- 3 days) after enrollment, and prior to therapy, unless otherwise indicated in one of the footnotes below in the table. The study drugs, correlative studies, and salicylate levels will be paid for by [redacted] will be billed to the subject's insurance company.

Evaluations	Pre-Study	On Treatment (every 4 weeks ± 3 days)	Randomization Day 1 of 1st Cycle Post 4 Month Lead-In	Off Treatment (+30 days)	Follow Up[7]
Initial History & Physical[1]	X				
Interim History & Physical		X[10]		X	
Toxicity Assessment	X	X[9]		X	
ECOG Performance Status	X	X[10]		X	
Weight and Vitals	X	X		X	
CBC, differential, platelets	X[1]	X[10]		X	
Serum Chemistries[2]	X	X[10]		X	
Liver Enzymes[3]	X			X	
Radiographic Assessments[4]	X				
Correlative Studies[5]	X	X		X	
Compliance Assessment		X		X	
Salicylate levels[6]	X	X[6]			
Prostate-specific antigen (PSA) *	X[1]	X	X[8]	X	X

1. Within 4 weeks of enrollment. Second PSA will be obtained within a week of starting C1D1 (this PSA will establish a baseline to assess response and will not determine eligibility).
2. Includes: Electrolytes, Calcium, BUN, Creatinine, Glucose.
3. Includes: Total and Direct Bilirubin, AST/ALT, Alkaline Phosphatase, Albumin, Total Protein.
4. A bone scan and a CT or MRI abdomen/pelvis and chest x-ray (CXR) or chest CT scan, will have been performed within 12 weeks of treatment start. Radiographic assessments will be selected by the attending physician as clinically indicated.
5. See Section 11 for details of laboratory evaluations. These analysis are optional however, every effort should be made to collect samples for analysis.
6. Salicylate levels will be drawn at baseline and through Cycle 5. Salicylate levels will NOT be collected after Cycle 5. Salicylate levels drawn at C1D1 are acceptable as baseline Salicylate levels as long as they are drawn prior to drug administration.
7. All patients, including those who discontinue protocol therapy early, will be followed for response (or progression) with PSA every 12-16 weeks and for survival for 1 year from the date of registration.
8. PSA will be drawn within 1 week of 1st Cycle of Treatment.
9. The research nurse will call the patient 2 weeks after starting metformin to assess for GI toxicity.
10. The H&P, ECOG and labs do not need to be repeated on C1D1 if these were completed within the pre-study time point.

* PSA should preferably be collected at the same lab for all time-points to prevent PSA fluctuations between different labs.

7. Treatment Plan

7.1 General Considerations

Treatment will be administered on an outpatient basis. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy. Patients may discontinue therapy at any time for any reason.

7.2 Study Agent

Metformin treatment will be started at 500 mg twice daily, if no GI toxicity grade greater than 1 is noted after 2 weeks it will be increased to 500 mg with breakfast/1000 mg at bedtime, which is the target dose for the remainder of the study. If GI toxicity grade greater than 1 occurs during the first 4 weeks, the subject will be evaluated every 2 weeks until resolution of toxicity to grade less than or equal to 1 and, then, the metformin dose will be increased to the next dose level. Metformin will be taken with food in order to minimize gastrointestinal side effects.

Aspirin will be taken at a dose of 81 mg once daily. Aspirin must be taken with food.

Missed Doses

If patients vomit after taking medications, patients should be instructed not to retake the dose. Patients should take the next dose at the scheduled time.

If a scheduled dose is missed, patients should be instructed to take the dose as soon as remembered. If it is within 4 hours of their next dose they should be instructed to skip the dose and resume their usual dosing schedule. Patients should not double their dose to catch up missed doses.

7.3 Dose Modifications or Escalations

7.3.1 Toxicity

Any Grade 3 or 4 non-hematologic adverse event, or Grade $\geq$ 2 renal toxicity, thought to be drug-related will lead to cessation of medications. If any of these occur, the patient will be removed from the study.

7.3.2 Dose Reduction

Dose modification will not occur for any reason.

7.4 Concomitant Medications

Patients may take any type of FDA approved pharmacologic agents while enrolled on this clinical trial.

7.5 Supportive Care Guidelines

Patients are allowed to receive full supportive care therapies concomitantly during the study. No other chemotherapy, immunotherapy, hormonal cancer therapy, radiation therapy, surgery for cancer, or experimental medications will be permitted while the patients are participating in

this study. It is not anticipated that patients will require significant supportive care. They may experience nausea and vomiting and the administration of antiemetic agents to control these symptoms would be acceptable.

7.6 Adherence/Compliance

7.6.1 Patients will be given a medication diary and instructed to initial it each time a dose is taken. Patients will also note the time the dose was taken, and if any side effects were experienced.

7.6.2 The desired level of compliance is ≥ 90%. Patients who cannot maintain this level of compliance will be removed from the study.

7.7 Emergency Unblinding procedure

Emergency Unblinding: In the event of an emergency, please contact [redacted] PharmD, FCCP, BCOP, Monday through Friday between 9:00am and 5:00pm Eastern Time. For unblinding outside of these hours, please contact [redacted] for further instruction. **Remember, this is only in the event of an emergency. Please note that, if a patient is emergently unblinded, they are considered to be off treatment, but should continue to be followed for outcome evaluations.**

8. Toxicity Monitoring and Adverse Event Reporting

All patients who receive one dose of protocol therapy will be evaluable for toxicity. Each month the treating physician will fully assess the patient's condition with respect to possible treatment related toxicities. All adverse events, whether observed by the physician or reported by the patient, occurring during the active portion of therapy, or up to 30 days after the last dose of treatment will be graded by a numerical score according to the NCI's Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0 (http://ctep.cancer.gov/reporting/ctc.html) and recorded in the patient's medical record. Toxicities will be reported as outlined in the study data capture plan. The type, grade, and toxicity attribution information will be recorded.

A preexisting condition is one that is present at the start of the study drug. A preexisting condition should only be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

8.1 Adverse Event Reporting Requirements

An adverse experience is defined as any unintended or abnormal clinical observation that is not of benefit to the patient. Either the condition was not present prior to exposure to the study therapy, or it has worsened in intensity or frequency following exposure to the study therapy.

All "unexpected" (defined below) and/or "serious" (defined below) adverse events occurring during the active portion of therapy, or up to 30 days after the last dose of treatment, will be reported to OHRS at [redacted]. Events will be promptly reported, in writing, to the local IRB. If a death occurs the IRB will be notified within 24 hours of initial receipt of information. All other SAEs must be reported to the IRB within three to ten days of initial

receipt of information. Written follow-up reports are required when additional information is obtained to fully characterize the event. Copies of each report sent to the IRB will be kept in the study regulatory file.

In addition to reporting to the local IRB, reporting to external bodies such as the FDA must also occur.

8.2 Reporting SAEs

The PI shall notify the FDA of any adverse experience associated with the use of the drug that is both serious and unexpected, as soon as possible and in no event later than 15 calendar days after the PI's discovery of the event. Each written notification may be submitted on FDA Form MedWatch 3500A http://www.fda.gov/medwatch/safety/3500a.pdf (fax # 1-800-FDA-0178).

The PI shall also notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experiences associated with the use of the drug, as soon as possible but no later than 7 calendar days from the PI's discovery of the event information.

8.3 Definition of Serious Adverse Events (SAEs)

A serious adverse event (experience) is one occurring at any dose level that results in any of the following outcomes:

- Death
- Life-threatening- immediate risk of death from the reaction.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in a congenital anomaly/birth defect.
- Requires intervention to prevent one of the outcomes listed in this definition.

The definition of serious adverse event (experience) also includes *important medical events*. Medical and scientific judgment will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These events will usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

8.4 Definition of Related Adverse Event

There is a reasonable possibility that the drug caused the adverse experience. That is, the event is judged by the investigator to be possibly, probably or definitely related to the treatment.

8.5 Definition of Unexpected Adverse Event

Any adverse drug experience and/or specificity or severity, that is not included in the current investigator's brochure and/or package insert.

9. Treatment Evaluation/Criteria for Response

9.1 Biochemical Response/progression:
Criteria for response are indicated in section 4.

9.2 Clinical Progression
The appearance of new lesions on examination or radiographs (CXR, CT scan, MRI scan, or bone scan) will be evidence for clinical progression. The development of symptoms consistent with metastatic disease (i.e., bone pain) with a concurrent increase in serum PSA from baseline will also be grounds for evaluating for clinical progression via radiographs.

9.3 Survival
Survival will be measured starting from the date of entry on study. Patients will be followed for one year after initial enrollment.

10. Removal of Patients from Study/Off Study Criteria
In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

a) Intercurrent illness that prevents further administration of treatment,
b) Unacceptable adverse event(s),
c) In the event of any drug-related life-threatening toxicity or laboratory abnormality the patient will be withdrawn from further treatment,
d) Patient decides to withdraw from the study,
e) Noncompliance with treatment plan,
f) General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator, or
g) Protocol violation - any patient found to have entered this study in violation of the protocol might be discontinued from the study at the discretion of the Principal Investigator.
h) PSA progression after at least 30 days of treatment (see Section 4.5).

11. Laboratory Evaluations and Correlative Studies
These analysis are optional however, every effort should be made to collect samples for analysis. . Metformin treatment has been reported to not only lower blood glucose but also to reduce serum IGF-1 levels and insulin levels [68]. IGF-1 plays an important role in both cellular mutagenesis and human malignancies. Aspirin may inhibit the activation of NFκB, which further suppresses the expression of tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6) and IL-1β [54]. Therefore, in this proposed study, we are particularly interested in determination of the serum factors, insulin, IGF-1, IL-6, IL-1β, and TNF-α. Additionally salicylate levels will be measured at baseline and through Cycle 5. Salicylate levels will NOT be collected after Cycle 5..

- At baseline, cycle 2, cycle 4, cycle 5, cycle 6, cycle 8, cycle 10 and end of treatment cycle, 7.5mL of blood will be collected in a red top tube. Serum will be distributed into eppendorf tubes and frozen for batched analysis by ELISA.
- To monitor aspirin levels in patients, salicylate levels will be monitored at baseline and every other cycle.

11.1 Collection and Handling Procedures

Blood specimens will be handled by the Cancer Institute's Tissue Analytical Service (TAS).

Documentation

Please have the name, address and the appropriate laboratory or office phone number of the institution that the patient specimens were shipped from. For any questions relating to shipment of samples please call [redacted]

[redacted]

11.2 Storage & Future Use of Specimens

Subjects will provide consent for retention and future use of specimens. At the time of consent, subjects will be informed that they may also refuse permission for future research use of their specimens without affecting their participation in the study or care by the health provider.

Specimens will be collected and processed following standard Biospecimen Repository Service (BRS) procedures. All specimens will be de-identified and given a unique BRS#. Only pathological information for each corresponding blood samples will be recorded.

Specimens will be stored at [redacted]
Service (BRS), Room # 2050, [redacted]
Specimens will be labeled by a unique identification number and initials only; there will be no other link to the subject. This link, along with data and health information collected for the main protocol , will be retained for a period of 6 years in the Office of Human Research Services (OHRS) on behalf of the principal investigator.

12. Pharmaceutical Information

12.1 Metformin

Product Description: tablet
Product Name: Metformin
Product Dosing: 1000mg BID.
How Supplied: commercial supply and distributed by CINJ pharmacy
Storage Requirements/Stability: room temperature
Route of Administration: Oral
Adverse Reactions: Most common: diarrhea, nausea, vomiting, flatulence, asthenia, indigestion, abdominal discomfort and headache.

12.2 Aspirin

Product Description: tablet
Product Name: Aspirin
Product Dosing: 81mg.
How Supplied: commercial supply and distributed by [redacted]armacy
Storage Requirements/Stability: room temperature
Route of Administration: Oral
Adverse Reactions: Most common: Rash, gastrointestinal ulcerations, abdominal pain, cramping, nausea, gastritis, lactic acidosis, and bleeding.

12.3 Aspirin and Metformin Placebo

Aspirin and metformin placebo tablets will be produced by the Rutgers School of Pharmacy and distributed by th[redacted]nacy.

13. Data Collection and Records to be Kept

13.1 Case Report Forms

A subset of the [redacted] CRFs, in electronic format, will be utilized. Completion of the electronic CRFs (eCRFs) will be done in accordance with the instructions in a study specific data capture plan. All eCRFs will be completed by clinical research coordinators of OHRS at the [redacted] The eCRFs will be maintained in a confidential format in Oncore®.

13.2 Data Submission Timeline and Forms

Completion of eCRFs will occur in accordance with NCI guidelines. Baseline (pre-study) eCRFs (e.g., enrollment, medical history, concomitant medications, disease assessment, etc.) will be completed no later than 14 days after the start of treatment. Treatment eCRFs (e.g., drug administration, adverse events, chemistries, etc.) will be completed no later than 14 days following each cycle of treatment. Off-treatment information (e.g., follow-up, best response, etc.) will be completed no later than 14 days after the end of protocol treatment.

13.3 Research Charts

A research chart is maintained at OHRS for each patient enrolled. Copies of significant study source documents will be maintained in the research chart. Examples of source document copies that will be maintained in the research chart include: signed informed consent form, documents that verify eligibility and treatment and documents that verify Grade 3-4 adverse events and response. This information will be updated on a prospective basis and will be confidentially maintained in OHRS.

13.4 Reports

Publications and annual reports for submission to the IRB and FDA will be written by the C[redacted] sing the data captured on the eCRFs.

14. Data and Safety Monitoring

Monitoring of this study will occur in accordance with the [redacted] approved Data and Safety Monitoring Plan (DSMP). An "initiation audit" will be conducted at [redacted] in accordance with the DSMP following enrollment of the first two (2) or three (3) patients. Subsequent audits will occur on an annual basis prior to annual IRB continuing review, if the findings from the initiation audit were satisfactory. More frequent audits of patient data and study conduct will occur if necessary. Prior audit findings and/or situations that may arise during the course of the study will determine the need for more frequent auditing. All audit findings will be discussed with the principal investigator and reported to the [redacted]

15. Multi-Institutional Guidelines

15.1 IRB Approvals

As the Coordinating Center for a trial, it is the [redacted] responsibility to ensure that no patients are entered on the trial at a participating institution without full IRB approval. Thus, OHRS will approve the addition of each participating institution to the study. A copy of the IRB approval document from each participating institution will be obtained by the OHRS prior to activation of the study at the participating institution.

15.2 Other Pre-Study Documents

Each participating center is required to have the following documents on file at OHRS:

- Curricula Vitae of all physician Investigators
- Signed FDA form 1572 of all physician Investigators (if applicable)
- [redacted] of all physician Investigators
- Documentation of Human Subjects Protection training from all Investigators
- Signed Investigator agreement from the Principal Investigator at each participating center
- Medical license from each Investigator

15.3 Initiation Meetings

A study initiation meeting will be conducted with each participating institution prior to enrollment of patients from the institution. OHRS staff will conduct the study initiation meeting in close proximity to IRB approval of the study at the participating center. In most situations the study initiation meeting will be conducted via teleconference. Web-based training regarding e-CRF completion will be utilized as appropriate.

15.4 IRB Continuing Approvals

Investigators from participating institutions must provide the [redacted], through OHRS, a copy of the institution's approved continuing review documentation. Registration will be halted

at any participating institution in which a current continuing approval is not on file at OHRS. Centers that are approved to utilize the Rutgers University central IRB will not be required to file continuing review documentation.

15.5 Amendments and Consents

OHRS will maintain a copy of all amendments, consent forms and approvals from each participating institution. Consent forms will be reviewed and approved by OHRS to ensure consistency with the IRB approved consent. Should changes to the protocol become necessary, protocol amendments will be submitted in writing to the [redacted] and local IRB for approval prior to implementation; unless the patient's best interest is endangered. In that case, notification to [redacted] and local IRB will be made as soon as possible.

15.6 Patient Registration

All patients from participating institutions must register patients with the OHRS central Registration Desk, as described in Section 5.7 of this protocol.

15.7 Data Collection and Toxicity Reporting

The PI at each institution will be responsible for assuring that all the required data is collected and entered onto the eCRFs accurately and completed eCRFs submitted as described in Section 13.

15.8 Data Monitoring and Source Document Verification

[redacted] staff will conduct monitoring visits to participating institutions of [redacted] Oncology Group no less frequently than every 3 months. The monitoring visits will focus on verifying eCRF data with source documents. Adherence to the protocol(s), including the prompt reporting of serious adverse events, will be assessed. Findings of all monitoring visits are recorded in a [redacted] Monitors Report, which is kept on file in the OHRS regulatory file. Issues concerning study compliance are at regular meetings of the [redacted] Human Research Oversight Committee.

15.9 Data and Center Audits

[redacted] staff will conduct annual audits to participating centers in accordance with OHRS Standard Operating Procedures (SOPs). The audit guidelines are in accordance with the [redacted] and Safety Monitoring Plan.

16. Statistical Considerations

16.1 Primary Hypothesis

The primary hypothesis is that metformin and aspirin will result in persistent stable disease in patients randomized to receive these medications after a run in period. By comparison patients with stable disease after a four-month run in period, who are randomized to placebo will experience a greater rate of disease progression

16.2 Outcome Measures

Difference in the stable PSA rates after 6 cycles of metformin and aspirin or placebo therapy will be calculated in patients who have received 4 cycles of open label treatment during the "run in phase"

16.3 Sample Size and Statistical Analysis

In this study, we hypothesize that patients with stable PSA after 4 cycles of open label treatment who are then treated with 6 cycles of metformin and aspirin will have a 40% rate of PSA or clinical progression compared to 80% rate of progression in in patients treated with placebo. With 28 patients randomized to treatment or placebo the study will have 80% power to detect an increase in the proportion of patients with progression at 6 cycles from 40% to 80% with alpha =0.05. Assuming that approximately 10 patients progress prior to randomization, a total of 66 patients will be entered on this study.

17. Human Subjects

17.1 Subject Population

Men with clinical stage D0 prostate cancer as evidenced by rising PSA values despite have received definite local therapy for prostate cancer (e.g., prostatectomy or radiation therapy)

17.2 Potential Risks

Potential risks to the patient secondary to metformin include nausea, vomiting, diarrhea, and lactic acidosis. Potential risks for aspirin include peptic ulcer disease and GI bleeding. These will be monitored as detailed in Sections 6-8. In terms of the individual's prostate cancer, there is a risk of treatment not being effective. Furthermore, if the drug is not effective, there is the possibility of disease progression (either PSA rising further or by observable metastasis).

17.3 Consent Procedures

Informed consent must be obtained prior to commencing any research procedures. The PI shall seek such consent only under such circumstances that provide the prospective patient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information given to the patient, or the representative, shall be in a language understandable to the subject or representative. The informed consent document may not include any exculpatory language through which the subject or representative is made to waive any of the subject's legal rights or releases, or appears to release the investigator, the sponsor or the institution from liability for negligence.

17.4 Potential Benefits

The benefits of participating in this study may be improvement in a patient's cancer either as a measure of disease activity or quality of life improvements.

17.5 Risk-Benefit Ratio

All risks and benefits of this treatment will be discussed with the patient prior to enrollment on this study. Specifically, this includes that the patient's rising PSA value likely represents some

micrometastasis, and the possibility of further progression without some form of intervention. Along these lines, the basic principles of what we are hoping to accomplish with metformin and aspirin will be explained. The potential risks detailed in Section 17.2 will be made clear as well, along with what measures are in place to minimize these risks.

Alternative treatment will also be discussed. These options include beginning androgen ablation therapy, monitoring off therapy, or enrolling in another clinical trial. Additionally, the importance of the knowledge gained through this study will be discussed with the patient.

All told, with the combination of employing proper patient selection and study termination criteria, close clinical and lab follow-up (both of the prostate cancer as well as other relevant body systems), and with the medical knowledge of metformin and aspirin are being utilized in patient treatment (as an FDA-approved medication, for non-oncologic medical purposes). Therefore we believe that the potential benefits outweigh the risks of the trial.

17.6 Gender and Minorities

The National Institutes of Health and NCI have stressed the importance of gender and minority inclusion in clinical services and research. In the past two years, African Americans made up 6%, Hispanics 6%, and Asians 3% of the [redacted]espectively. The percentage of minority patients enrolled onto clinical research trials were 7% African-American, 6% Hispanic, and 4% Asian, respectively.

Since the prostate cancer study only involves the male population our focus of enrollment of minorities would be directed to minority male participants.

No person shall, on the grounds of race, color, or national origin, be excluded from participation in, be denied the benefits of, enrollment in this protocol. Minorities are prevalent in the New Brunswick area. Therefore, efforts to include the broadest possible representation of minority groups will be focused on accrual from the community as well as distant referrals. This will be accomplished by conducting educational programs with community physicians.

18. Economic/Financial Considerations

Patients and/or their insurance carriers will be expected to pay for costs related to monitoring and follow-up. Patients will be expected to pay for any costs not paid by their insurance carrier. Patients will be responsible for any and all transportation costs.

19. Publication of Research Findings

The policies and procedures of [redacted] department (see: Investigator's Handbook) will govern publication of the trial. The Cancer Institute's PI, and all co-authors prior to submission or use, must review any abstract or manuscript.

References

1. Freedland SJ, Humphreys EB, Mangold LA, Eisenberger M, Dorey FJ, Walsh PC, and Partin AW, *Risk of Prostate Cancer-Specific Mortality Following Biochemical Recurrence after Radical Prostatectomy.* JAMA, **2005**. 294(4): p. 433-9.
2. Roehl KA, Han M, Ramos CG, Antenor JA, and Catalona WJ, *Cancer Progression and Survival Rates Following Anatomical Radical Retropubic Prostatectomy in 3,478 Consecutive Patients: Long-Term Results.* J Urol, **2004**. 172(3): p. 910-4.
3. Kupelian PA, Mahadevan A, Reddy CA, Reuther AM, and Klein EA, *Use of Different Definitions of Biochemical Failure after External Beam Radiotherapy Changes Conclusions About Relative Treatment Efficacy for Localized Prostate Cancer.* Urology, **2006**. 68(3): p. 593-8.
4. Freedland SJ, Sutter ME, Dorey F, and Aronson WJ, *Defining the Ideal Cutpoint for Determining Psa Recurrence after Radical Prostatectomy. Prostate-Specific Antigen.* Urology, **2003**. 61(2): p. 365-9.
5. Widmark A, Klepp Or, Solberg A, Damber J-E, Angelsen A, Fransson P, Lund J-Ö, Tasdemir I, Hoyer M, Wiklund F, and Foss√• SD, *Endocrine Treatment, with or without Radiotherapy, in Locally Advanced Prostate Cancer (Spcg-7/Sfuo-3): An Open Randomised Phase Iii Trial.* The Lancet. 373(9660): p. 301-08.
6. Yang W and Levine AC, *Androgens and Prostate Cancer Bone Metastases: Effects on Both the Seed and the Soil.* Endocrinol Metab Clin North Am, **2011**. 40(3): p. 643-53.
7. Seruga B, Ocana A, and Tannock IF, *Drug Resistance in Metastatic Castration-Resistant Prostate Cancer.* Nat Rev Clin Oncol, **2011**. 8(1): p. 12-23.
8. Heemers HV and Tindall DJ, *Androgen Receptor (Ar) Coregulators: A Diversity of Functions Converging on and Regulating the Ar Transcriptional Complex.* Endocr Rev, **2007**. 28(7): p. 778-808.
9. Sarker D, Reid AH, Yap TA, and de Bono JS, *Targeting the Pi3k/Akt Pathway for the Treatment of Prostate Cancer.* Clin Cancer Res, **2009**. 15(15): p. 4799-805.
10. Yoshimoto M, Cunha IW, Coudry RA, Fonseca FP, Torres CH, Soares FA, and Squire JA, *Fish Analysis of 107 Prostate Cancers Shows That Pten Genomic Deletion Is Associated with Poor Clinical Outcome.* Br J Cancer, **2007**. 97(5): p. 678-85.
11. Schmitz M, Grignard G, Margue C, Dippel W, Capesius C, Mossong J, Nathan M, Giacchi S, Scheiden R, and Kieffer N, *Complete Loss of Pten Expression as a Possible Early Prognostic Marker for Prostate Cancer Metastasis.* Int J Cancer, **2007**. 120(6): p. 1284-92.
12. Abreu-Martin MT, Chari A, Palladino AA, Craft NA, and Sawyers CL, *Mitogen-Activated Protein Kinase Kinase Kinase 1 Activates Androgen Receptor-Dependent Transcription and Apoptosis in Prostate Cancer.* Mol Cell Biol, **1999**. 19(7): p. 5143-54.
13. Di Lorenzo G, Tortora G, D'Armiento FP, De Rosa G, Staibano S, Autorino R, D'Armiento M, De Laurentiis M, De Placido S, Catalano G, Bianco AR, and Ciardiello F, *Expression of Epidermal Growth Factor Receptor Correlates with Disease Relapse and Progression to Androgen-Independence in Human Prostate Cancer.* Clin Cancer Res, **2002**. 8(11): p. 3438-44.
14. Traish AM and Morgentaler A, *Epidermal Growth Factor Receptor Expression Escapes Androgen Regulation in Prostate Cancer: A Potential Molecular Switch for Tumour Growth.* Br J Cancer, **2009**. 101(12): p. 1949-56.

15. Yardy GW and Brewster SF, *Wnt Signalling and Prostate Cancer.* Prostate Cancer Prostatic Dis, **2005**. 8(2): p. 119-26.
16. Aaronson DS, Muller M, Neves SR, Chung WC, Jayaram G, Iyengar R, and Ram PT, *An Androgen-Il-6-Stat3 Autocrine Loop Re-Routes Egf Signal in Prostate Cancer Cells.* Mol Cell Endocrinol, **2007**. 270(1-2): p. 50-6.
17. Kourelis TV and Siegel RD, *Metformin and Cancer: New Applications for an Old Drug.* Med Oncol, **2012**. 29(2): p. 1314-27.
18. Liu B, Fan Z, Edgerton SM, Deng XS, Alimova IN, Lind SE, and Thor AD, *Metformin Induces Unique Biological and Molecular Responses in Triple Negative Breast Cancer Cells.* Cell Cycle, **2009**. 8(13): p. 2031-40.
19. Joshua AM, Zannella VE, Downes MR, Bowes B, Hersey K, Koritzinsky M, Schwab M, Hofmann U, Evans A, van der Kwast T, Trachtenberg J, Finelli A, Fleshner N, Sweet J, and Pollak M, *A Pilot 'Window of Opportunity' Neoadjuvant Study of Metformin in Localised Prostate Cancer.* Prostate Cancer Prostatic Dis, **2014**. 27(10): p. 20.
20. Taniguchi CM, Emanuelli B, and Kahn CR, *Critical Nodes in Signalling Pathways: Insights into Insulin Action.* Nat Rev Mol Cell Biol, **2006**. 7(2): p. 85-96.
21. Matsushime H, Quelle DE, Shurtleff SA, Shibuya M, Sherr CJ, and Kato JY, *D-Type Cyclin-Dependent Kinase Activity in Mammalian Cells.* Mol Cell Biol, **1994**. 14(3): p. 2066-76.
22. Malaguarnera R, Sacco A, Morcavallo A, Squatrito S, Migliaccio A, Morrione A, Maggiolini M, and Belfiore A, *Metformin Inhibits Androgen-Induced Igf-Ir up-Regulation in Prostate Cancer Cells by Disrupting Membrane-Initiated Androgen Signaling.* Endocrinology, **2014**. 155(4): p. 1207-21.
23. Mills EJ, Wu P, Alberton M, Kanters S, Lanas A, and Lester R, *Low-Dose Aspirin and Cancer Mortality: A Meta-Analysis of Randomized Trials.* Am J Med, **2012**. 125(6): p. 560-7.
24. Thun MJ, Henley SJ, and Patrono C, *Nonsteroidal Anti-Inflammatory Drugs as Anticancer Agents: Mechanistic, Pharmacologic, and Clinical Issues.* J Natl Cancer Inst, **2002**. 94(4): p. 252-66.
25. Chan AT, Arber N, Burn J, Chia WK, Elwood P, Hull MA, Logan RF, Rothwell PM, Schror K, and Baron JA, *Aspirin in the Chemoprevention of Colorectal Neoplasia: An Overview.* Cancer Prev Res, **2012**. 5(2): p. 164-78.
26. Chan AT, Ogino S, and Fuchs CS, *Aspirin and the Risk of Colorectal Cancer in Relation to the Expression of Cox-2.* New England Journal of Medicine, **2007**. 356(21): p. 2131-42.
27. Hubner RA, Muir KR, Liu JF, Logan RF, Grainge MJ, and Houlston RS, *Ornithine Decarboxylase G316a Genotype Is Prognostic for Colorectal Adenoma Recurrence and Predicts Efficacy of Aspirin Chemoprevention.* Clin Cancer Res, **2008**. 14(8): p. 2303-9.
28. Din FVN, Valanciute A, Houde VP, Zibrova D, Green KA, Sakamoto K, Alessi DR, and Dunlop MG, *Aspirin Inhibits Mtor Signaling, Activates Amp-Activated Protein Kinase, and Induces Autophagy in Colorectal Cancer Cells.* Gastroenterology, **2012**. 142(7): p. 1504-15.e3.
29. Hawley SA, Fullerton MD, Ross FA, Schertzer JD, Chevtzoff C, Walker KJ, Peggie MW, Zibrova D, Green KA, Mustard KJ, Kemp BE, Sakamoto K, Steinberg GR, and Hardie DG,

The Ancient Drug Salicylate Directly Activates Amp-Activated Protein Kinase. Science, **2012**. 336(6083): p. 918-22.

30. Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, Imamura Y, Qian ZR, Baba Y, Shima K, Sun R, Nosho K, Meyerhardt JA, Giovannucci E, Fuchs CS, Chan AT, and Ogino S, *Aspirin Use, Tumor Pik3ca Mutation, and Colorectal-Cancer Survival.* New England Journal of Medicine, **2012**. 367(17): p. 1596-606.
31. Zhang Y, Wang J, Wheat J, Chen X, Jin S, Sadrzadeh H, Fathi AT, Peterson RT, Kung AL, Sweetser DA, and Yeh JR, *Aml1-Eto Mediates Hematopoietic Self-Renewal and Leukemogenesis through a Cox/Beta-Catenin Signaling Pathway.* Blood, **2013**. 121(24): p. 4906-16.
32. Margel D, Urbach DR, Lipscombe LL, Bell CM, Kulkarni G, Austin PC, and Fleshner N, *Metformin Use and All-Cause and Prostate Cancer–Specific Mortality among Men with Diabetes.* Journal of Clinical Oncology, **2013**.
33. Spratt DE, Zhang C, Zumsteg ZS, Pei X, Zhang Z, and Zelefsky MJ, *Metformin and Prostate Cancer: Reduced Development of Castration-Resistant Disease and Prostate Cancer Mortality.* European Urology, **2013**. 63(4): p. 709-16.
34. Joshua A, Zannella V, Bowes B, Koritzinsky M, Sweet J, and Evans E. *A Phase Ii Study of Neoadjuvant Metformin in Prostatic Carcinoma.* in *AACR*. 2012. Chicago, IL: AACR.
35. Rothermundt C, Hayoz S, Templeton AJ, Winterhalder R, Strebel RT, Bärtschi D, Pollak M, Lui L, Endt K, Schiess R, Rüschoff JH, Cathomas R, and Gillessen S, *Metformin in Chemotherapy-Naive Castration-Resistant Prostate Cancer: A Multicenter Phase 2 Trial (Sakk 08/09).* EUROPEN UROLOGY, **2014**.
36. Ben Sahra I, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, Tanti JF, Le Marchand-Brustel Y, and Bost F, *The Antidiabetic Drug Metformin Exerts an Antitumoral Effect in Vitro and in Vivo through a Decrease of Cyclin D1 Level.* Oncogene, **2008**. 27(25): p. 3576-86.
37. Akinyeke T, Matsumura S, Wang X, Wu Y, Schalfer ED, Saxena A, Yan W, Logan SK, and Li X, *Metformin Targets C-Myc Oncogene to Prevent Prostate Cancer.* Carcinogenesis, **2013**.
38. Jacobs EJ, Rodriguez C, Mondul AM, Connell CJ, Henley SJ, Calle EE, and Thun MJ, *A Large Cohort Study of Aspirin and Other Nonsteroidal Anti-Inflammatory Drugs and Prostate Cancer Incidence.* J Natl Cancer Inst, **2005**. 97(13): p. 975-80.
39. Salinas CA, Kwon EM, FitzGerald LM, Feng Z, Nelson PS, Ostrander EA, Peters U, and Stanford JL, *Use of Aspirin and Other Nonsteroidal Antiinflammatory Medications in Relation to Prostate Cancer Risk.* Am J Epidemiol, **2010**. 172(5): p. 578-90.
40. Flahavan EM, Bennett K, Sharp L, and Barron TI, *A Cohort Study Investigating Aspirin Use and Survival in Men with Prostate Cancer.* Annals of oncology : official journal of the European Society for Medical Oncology / ESMO, **2014**. 25(1): p. 154-9.
41. Cardwell CR, Flahavan EM, Hughes CM, Coleman HG, O'Sullivan JM, Powe DG, and Murray LJ, *Low-Dose Aspirin and Survival in Men with Prostate Cancer: A Study Using the Uk Clinical Practice Research Datalink.* Cancer causes & control : CCC, **2014**. 25(1): p. 33-43.

42. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, and Meade TW, *Effect of Daily Aspirin on Long-Term Risk of Death Due to Cancer: Analysis of Individual Patient Data from Randomised Trials*. Lancet, **2010**.
43. Larsson SC, Giovannucci E, Bergkvist L, and Wolk A, *Aspirin and Nonsteroidal Anti-Inflammatory Drug Use and Risk of Pancreatic Cancer: A Meta-Analysis*. Cancer Epidemiol Biomarkers Prev, **2006**. 15(12): p. 2561-4.
44. Capurso G, Schunemann HJ, Terrenato I, Moretti A, Koch M, Muti P, Capurso L, and Delle Fave G, *Meta-Analysis: The Use of Non-Steroidal Anti-Inflammatory Drugs and Pancreatic Cancer Risk for Different Exposure Categories*. Aliment Pharmacol Ther, **2007**. 26(8): p. 1089-99.
45. Inoue J, Gohda J, Akiyama T, and Semba K, *Nf-Kappab Activation in Development and Progression of Cancer*. Cancer science, **2007**. 98(3): p. 268-74.
46. Lee CH, Jeon YT, Kim SH, and Song YS, *Nf-Kappab as a Potential Molecular Target for Cancer Therapy*. BioFactors, **2007**. 29(1): p. 19-35.
47. Jiang J, Slivova V, Jedinak A, and Sliva D, *Gossypol Inhibits Growth, Invasiveness, and Angiogenesis in Human Prostate Cancer Cells by Modulating Nf-Kappab/Ap-1 Dependent- and Independent-Signaling*. Clinical & experimental metastasis, **2012**. 29(2): p. 165-78.
48. Jin RJ, Lho Y, Connelly L, Wang Y, Yu X, Saint Jean L, Case TC, Ellwood-Yen K, Sawyers CL, Bhowmick NA, Blackwell TS, Yull FE, and Matusik RJ, *The Nuclear Factor-Kappab Pathway Controls the Progression of Prostate Cancer to Androgen-Independent Growth*. Cancer research, **2008**. 68(16): p. 6762-9.
49. Jin R, Sterling JA, Edwards JR, DeGraff DJ, Lee C, Park SI, and Matusik RJ, *Activation of Nf-Kappa B Signaling Promotes Growth of Prostate Cancer Cells in Bone*. PloS one, **2013**. 8(4): p. e60983.
50. Dhir R, Ni Z, Lou W, DeMiguel F, Grandis JR, and Gao AC, *Stat3 Activation in Prostatic Carcinomas*. The Prostate, **2002**. 51(4): p. 241-6.
51. Mora LB, Buettner R, Seigne J, Diaz J, Ahmad N, Garcia R, Bowman T, Falcone R, Fairclough R, Cantor A, Muro-Cacho C, Livingston S, Karras J, Pow-Sang J, and Jove R, *Constitutive Activation of Stat3 in Human Prostate Tumors and Cell Lines: Direct Inhibition of Stat3 Signaling Induces Apoptosis of Prostate Cancer Cells*. Cancer research, **2002**. 62(22): p. 6659-66.
52. Barton BE, Karras JG, Murphy TF, Barton A, and Huang HF, *Signal Transducer and Activator of Transcription 3 (Stat3) Activation in Prostate Cancer: Direct Stat3 Inhibition Induces Apoptosis in Prostate Cancer Lines*. Molecular cancer therapeutics, **2004**. 3(1): p. 11-20.
53. Ni Z, Lou W, Leman ES, and Gao AC, *Inhibition of Constitutively Activated Stat3 Signaling Pathway Suppresses Growth of Prostate Cancer Cells*. Cancer research, **2000**. 60(5): p. 1225-8.
54. Kopp E and Ghosh S, *Inhibition of Nf-Kappa B by Sodium Salicylate and Aspirin*. Science, **1994**. 265(5174): p. 956-9.
55. Yin MJ, Yamamoto Y, and Gaynor RB, *The Anti-Inflammatory Agents Aspirin and Salicylate Inhibit the Activity of I(Kappa)B Kinase-Beta*. Nature, **1998**. 396(6706): p. 77-80.
56. Tian Y, Ye Y, Gao W, Chen H, Song T, Wang D, Mao X, and Ren C, *Aspirin Promotes Apoptosis in a Murine Model of Colorectal Cancer by Mechanisms Involving*

Downregulation of Il-6-Stat3 Signaling Pathway. International journal of colorectal disease, **2011**. 26(1): p. 13-22.

57. Kim SR, Bae MK, Kim JY, Wee HJ, Yoo MA, and Bae SK, *Aspirin Induces Apoptosis through the Blockade of Il-6-Stat3 Signaling Pathway in Human Glioblastoma A172 Cells.* Biochemical and biophysical research communications, **2009**. 387(2): p. 342-7.
58. Deng XS, Wang S, Deng A, Liu B, Edgerton SM, Lind SE, Wahdan-Alaswad R, and Thor AD, *Metformin Targets Stat3 to Inhibit Cell Growth and Induce Apoptosis in Triple-Negative Breast Cancers.* Cell Cycle, **2012**. 11(2): p. 367-76.
59. Lin CC, Yeh HH, Huang WL, Yan JJ, Lai WW, Su WP, Chen HH, and Su WC, *Metformin Enhances Cisplatin Cytotoxicity by Suppressing Stat3 Activity Independently of the Lkb1-Ampk Pathway.* American journal of respiratory cell and molecular biology, **2013**.
60. Schneider MB, Matsuzaki H, Haorah J, Ulrich A, Standop J, Ding XZ, Adrian TE, and Pour PM, *Prevention of Pancreatic Cancer Induction in Hamsters by Metformin.* Gastroenterology, **2001**. 120(5): p. 1263-70.
61. Algire C, Amrein L, Bazile M, David S, Zakikhani M, and Pollak M, *Diet and Tumor Lkb1 Expression Interact to Determine Sensitivity to Anti-Neoplastic Effects of Metformin in Vivo.* Oncogene, **2011**. 30(10): p. 1174-82.
62. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, and Andreelli F, *Cellular and Molecular Mechanisms of Metformin: An Overview.* Clin Sci (Lond), **2012**. 122(6): p. 253-70.
63. Grisouard J, Dembinski K, Mayer D, Keller U, Muller B, and Christ-Crain M, *Targeting Amp-Activated Protein Kinase in Adipocytes to Modulate Obesity-Related Adipokine Production Associated with Insulin Resistance and Breast Cancer Cell Proliferation.* Diabetology & metabolic syndrome, **2011**. 3: p. 16.
64. Martin M and Marais R, *Metformin: A Diabetes Drug for Cancer, or a Cancer Drug for Diabetics?* Journal of clinical oncology : official journal of the American Society of Clinical Oncology, **2012**. 30(21): p. 2698-700.
65. Hawley SA, Fullerton MD, Ross FA, Schertzer JD, Chevtzoff C, Walker KJ, Peggie MW, Zibrova D, Green KA, Mustard KJ, Kemp BE, Sakamoto K, Steinberg GR, and Hardie DG, *The Ancient Drug Salicylate Directly Activates Amp-Activated Protein Kinase.* Science, **2012**. 336(6083): p. 918-22.
66. Din FV, Valanciute A, Houde VP, Zibrova D, Green KA, Sakamoto K, Alessi DR, and Dunlop MG, *Aspirin Inhibits Mtor Signaling, Activates Amp-Activated Protein Kinase, and Induces Autophagy in Colorectal Cancer Cells.* Gastroenterology, **2012**. 142(7): p. 1504-15 e3.
67. Yue W, Yang CS, DiPaola RS, and Tan XL, *Repurposing of Metformin and Aspirin by Targeting Ampk-Mtor Signaling and Inflammation for Pancreatic Cancer Prevention and Treatment.* Cancer prevention research, **2014**. In press.
68. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, and Andreelli F, *Cellular and Molecular Mechanisms of Metformin: An Overview.* Clin Sci (Lond), **2012**. 122(6): p. 253-70.

Appendix A

Performance Status Criteria

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.