The following protocol information is provided solely to describe how the authors conducted the research underlying the published report associated with the following article:

A Randomized Trial of Hypofractionated External Beam Radiotherapy for Prostate Cancer

Pollack, et al

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FOX CHASE CANCER CENTER

DEPARTMENT OF RADIATION ONCOLOGY

IRB# __02-602__: A PHASE III INTENSITY MODULATED RADIOTHERAPY DOSE ESCALATION TRIAL FOR PROSTATE CANCER USING HYPOFRACTIONATION

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****Chemotherapy N/A to this study****

PROTOCOL SCHEMA

1. Randomization

a. *Arm I*: Conventional Fractionated Intensity Modulated Radiotherapy (CIMRT)
A total dose of 76 Gy will be delivered in 38 fractions to the planning target volume (PTV). High Risk patients (PSA >20 ng/ml, Gleason 8-10, Stage T3 disease or ≥4 diagnostic needle biopsies containing Gleason 7 disease) will also receive 2 years of androgen ablation.

b. *Arm II*: Hypofractionated Intensity Modulated Radiotherapy (HIMRT)
A total dose of 70.2 Gy will be delivered in 26 fractions (equivalent to 84.3Gy at 2.0 Gy per fraction, assuming an α/β of 1.5) to the PTV.
High risk patients (PSA >20 ng/ml, Gleason 8-10, Stage T3 disease or ≥4 diagnostic needle biopsies containing Gleason 7 disease) will also receive 2 years of androgen ablation.

2. Stratification

a. PSA ≤10, >10, ≤20, or >20 ng/ml
b. Gleason 5-7 or 8-10.
c. Long course adjuvant androgen ablation or no adjuvant androgen ablation.

3. Patient Eligibility

a. Non-metastatic adenocarcinoma of the prostate, palpation stage T1b-T3c (AJCC 1992 staging).
b. PSA ≤80 ng/ml and Gleason score ≥5.
c. PSA >10, Gleason score >6, ≥T2b palpable disease, or ≥3 biopsy cores involved with ≥Gleason 5 disease).
d. No previous pelvic radiotherapy.
e. No previous history of radical prostatectomy.
f. Prior androgen ablation is permitted if it was started ≤4 months of protocol randomization (short term neoadjuvant androgen ablation). In intermediate-risk patients androgen ablation must be stopped at randomization.
g. No concurrent, active malignancy, other than nonmetastatic skin cancer or early stage chronic lymphocytic leukemia (well-differentiated small cell lymphocytic lymphoma). If a prior malignancy is in remission for ≥ 5 years then the patient is eligible.
h. Zubrod status <2.

4. Treatment Technique

a. Treatment Planning: Dose will be prescribed such that at least 95% of the PTV receives the prescribed dose. The gross tumor volume (GTV) will be similar to the clinical target volume (CTV), and will include the prostate ± seminal vesicles ± lymph nodes (high risk only). The amount of seminal vesicles included in the CTV will depend on T-category. The proximal seminal vesicles (≤50%) may be included for T1-T2 disease. At least 50% of the seminal vesicles will be included for T3 disease. The PTV will depend on the treatment arm. For CIMRT, the effective PTV (prescription line) will be 0.8 – 1.3 cm around the CTV in all dimensions, except posteriorly where the PTV will be 0.3 – 0.8 cm. For HIMRT, the effective PTV will be 0.5 – 1.0 cm around the CTV in all dimensions except posteriorly, where the PTV will be 0.2 – 0.6 cm.

b. IMRT: IMRT plans will be evaluated by dose-volume histogram analysis. Less than or equal to 17% and 35% of the rectum should receive ≥65 Gy and ≥40 Gy, respectively, for the conventionally fractionated patients (Arm I, 76 Gy total dose). Less than or equal to 25% and 50% of the bladder should receive ≥65 Gy and ≥40 Gy, respectively, for Arm I patients. Less than or equal to 17% and 35% of the rectum should receive ≥50 Gy and ≥31 Gy, for Arm II patients. Less than or equal to 25% and 50% of the bladder should receive ≥50 Gy and ≥31 Gy, for Arm II patients. At least 95% of the PTV should receive the prescription dose (a minor variation will be <95% to ≥90% of the prescription dose). The maximum PTV dose should not be >20% of the prescription dose.

Eligibility Checklist

_____ 1. Biopsy confirmed adenocarcinoma of the prostate.

_____ 2. All must be present:
- _____ Palpable Stage T1b-T3c (AJCC 1992 staging system).
- _____ PSA ≤80 ng/ml (before androgen ablation).
  - _____ PSA ≤4 weeks of randomization.
- _____ Gleason score ≥5.

_____ 3. PSA >10, Gleason >6, palpation stage ≥T2b, or ≥3 needle biopsy cores involved.

_____ 4. Risk Classification.

- _____ High risk (PSA >20 ng/ml, Gleason 8-10, Stage T3 or ≥4 biopsies classified as Gleason 7)
- _____ Intermediate risk (PSA ≤20 ng/ml, Gleason <8, and Stage T1-T2, unless ≥4 biopsies classified as Gleason 7).

_____ 5. Bone scan (if PSA >10 ng/ml or T3 disease) ≤4 months of randomization; negative for metastasis.

_____ 6. CT or MRI-pelvis (if Stage T3 disease) ≤4 months of randomization; negative for metastasis.

_____ 7. Serum testosterone will be drawn as a baseline study in all patients ≤4 months before randomization or after randomization but prior to the first radiation treatment.

_____ 8. Other serum hormone related tests (before androgen ablation).
If patient was started on androgen ablation prior to protocol enrollment, these tests will not be obtained.
- ____ Sex hormone binding globulin ≤4 months before randomization or after randomization but prior to first radiation treatment.
- _____ Estradiol ≤4 months before randomization or after randomization but prior to the first radiation treatment.

_____ 9. No prior pelvic radiotherapy.

_____ 10. No prior or planned radical prostatectomy.

_____ 11. No concurrent, active malignancy, other than nonmetastatic skin cancer or early stage chronic lymphocytic leukemia (well-differentiated small cell lymphocytic lymphoma). If a prior malignancy is in remission for ≥ 5 years then the patient is eligible.

_____ 12. Androgen ablation is permitted if it was started ≤4 months prior to randomization. Androgen ablation will be continued further for those in the high risk group.
- _____. Date androgen ablation started
- _____. Type of androgen ablation
- _____. Projected length of androgen ablation

_____ 13. Zubrod performance status of ≤2.

1.0 INTRODUCTION

1. Overview: The introduction of pretreatment PSA as a prognostic factor and the use of rising PSA as an endpoint have radically changed our understanding of the efficacy of radiotherapy in the treatment of prostate cancer. We now realize that radiation doses used in the 1980's were inadequate, leaving behind residual disease in the prostate. There are now several non-randomized studies showing that radiotherapy dose is an important determinant of patient outcome (1-4). In our experience, 78 Gy to the isocenter is superior to doses in the 67 – 77 Gy range, with the greatest improvement in freedom from biochemical failure seen for intermediate risk patients. A formal randomized dose escalation trial at M.D. Anderson Cancer Center (MDACC) comparing 70 to 78 Gy confirms the retrospective analyses (5, 6). With a median follow-up of 40 months, the MDACC randomized trial showed freedom from failure (FFF, mostly based on PSA) rates of 48% for the 70 Gy group versus 75% for the 78 Gy group at 5 years, when the PSA was >10 ng/ml (Figure 1). Although the trial has yet to document a benefit for increasing dose when the pretreatment PSA is <10 ng/ml (FFF of 80%), some of our data (1), and those of others (7, 8) indicate that patients with favorable features also have improved outcome from dose escalation.

   The most compelling reason for further escalating dose is that failure rates for intermediate-to-high risk patients are still unacceptable. We estimate that the intermediate risk patient population eligible for the trial will have over a 30% risk of treatment failure, and that by further escalating dose the failure rate may be reduced to 15% or less. Likewise, high risk patients have over a 30% risk of biochemical failure with the combination of androgen ablation plus external beam radiation therapy, which we hypothesize will be reduced to less than 15% with dose escalation. Our prior dose escalation experience documents that local failure is the major site of failure in such patients. Thus, further dose escalation is warranted.

   The benefit of dose escalation is apparent for patients with intermediate-to-high risk features. Other data from randomized trials show that for high risk patients early androgen ablation combined with Radiotherapy (RT) results in significantly better survival, compared to RT alone (9, 10). More recently, long-term androgen ablation was found to lengthen survival over short-term androgen ablation in Gleason 8-10 patients treated with RT (11). Since the trial described herein was designed to test the effects of dose escalation using hypofractionation in intermediate to high risk patients, risk group stratification will be performed such that the high risk patients will receive two years of androgen ablation; the intermediate risk patients will not receive adjuvant androgen ablation. All patients that have been started on neoadjuvant androgen ablation for up to four months prior to randomization will be permitted to enter the trial and will be stratified accordingly.

   The proposed study is a randomized dose escalation comparison of two dose-fractionation methods using intensity modulated radiotherapy (IMRT). Recent evidence indicates that prostate cancer behaves like a late-reacting tissue (12-15). Cells from late-reacting tissues have a broad shoulder on dose-response curves which translates into reduced cell death with conventionally fractionated doses of 1.8-2.0 Gy per fraction. Hypofractionation reduces the impact of the shoulder by going to a steeper portion of the curve. The problem with hypofractionation is that the risk of normal tissue complications increases; a balance between tumor control probability (TCP) and normal tissue complication probability (NTCP) must be considered. The α/β ratio for prostate cancer appears to be between 1.5 – 5.0 (12-15) and, as such, hypofractionation is a reasonable strategy. The premise of this dose escalation trial is that a hypofractionation dose of 70.2 Gy in 2.7 Gy daily fractions is biologically equivalent to 84.3 Gy ($\alpha/\beta = 1.5$) using 2.0 Gy conventional daily fractions. Hypofractionation affords a means to substantially increase dose, while keeping the number of fractions low. A dose of 84 Gy given in 2.0 Gy daily fractions would require about to 8.5 weeks of treatment.

The difference in dose biologically between the two regimens is 8.4 Gy, similar to the dose difference used in our prior positive trial (5). The hypofractionated intensity modulated radiotherapy scheme (HIMRT; 70.2 Gy in 26 fractions) will shorten overall treatment by 12 treatments or about 2.5 weeks from the conventional fractionation scheme (CIMRT; 76 Gy in 38 fractions). The principal hypothesis is that the higher biological dose will improve biochemical and/or disease freedom from failure for intermediate-to-high risk prostate cancer patients treated with radiotherapy. Secondarily, side effects between the arms will be examined to determine if they are similar using IMRT to minimize the amount of bladder and rectum that receives the higher dose. The premise that IMRT will provide a means for achieving this dose escalation without increasing side effects is based on work by Zelefsky et al. (4, 16) and Mohan and colleagues (17), and prior experience in treating over 300 patients with IMRT. In addition, we hypothesize that quality of life (QOL) will be similar between the HIMRT and CIMRT treatment arms. Given improved (lower) biochemical failure rates in the experimental HIMRT arm, similar side-effects and QOL would demonstrate a better risk benefit ratio for HIMRT compared to CIMRT.

The selection of patients for the protocol involves the assessment of clinical stage, Gleason score and pretreatment PSA (18). While the addition of pretreatment PSA has refined the prognostication of patient outcome after radiotherapy in absolute terms, there are still broad responses within the best models that incorporate these factors. Preliminary data indicate that Ki-67/MIB-1 immunohistochemical staining (**Figure 2**), DNA-ploidy and molecular markers of apoptosis, such as bcl-2, bax and p53 (**Figures 3 & 4**) (19-27) have potential for further delineating patient outcome. Recent data from our lab (28, 29) indicate that MDM2 may also have a key regulatory role in the apoptotic response of prostate cancer to androgen deprivation and radiotherapy. This marker will also be investigated. Another marker that shows promise is protein kinase A type I, which is involved in androgen receptor activation (30, 31), activation of the EGFR pathway (32-34) – inhibitors of this pathway have proven quite synergistic with RT (35), and hyperphosphorylation bcl-2 with bax upregulation (36, 37). In addition we have found that pretreatment serum testosterone (serum-T) has independent value in predicting distant metastasis risk (38). To date, we know of no study that has prospectively analyzed these potential markers. A prospective analysis is needed to confirm the validity of associations discovered in retrospective investigations.

1.1 IMRT: The ability to treat the prostate to high doses without increasing morbidity has been accomplished using 3-dimensional treatment planning and conformal radiotherapy (3DCRT). Reconstructions of the anatomy on a computer in 3-dimensions allows for more precise delivery of radiation to the target, sparing more of the surrounding normal tissues. The preliminary results of the MDACC randomized trial confirm that isocenter doses in the 78 Gy range are safe (39, 40) and effective (5) (**Figure 1**).

Intensity modulated radiation therapy (IMRT) goes beyond 3DCRT in both treatment planning and delivery. In IMRT, the dose pattern impinging on the patient from each beam is modulated in such a way that the total dose delivered closely matches the shape of the target volume in three dimensions and avoids normal tissues. In contrast to 3DCRT, IMRT beams can produce concave dose patterns with exceptionally sharp dose fall-off. An inverse planning process is used to compute the ideal modulation for each beam, taking into account the location and shape of the tumor and normal tissues, and the density variations in the body. Inverse planning means that the target and target dose are selected, and the computer designs the best intensity pattern to achieve the desired dose distribution, while limiting the dose to adjacent normal structures. There are two basic approaches to inverse planning. One is a quasi-random search method using simulated annealing (41), similar to optimization methods for brachytherapy and for external beam therapy with unmodulated beams. The other uses a filtered backprojection

combined with iterative reconstruction (42, 43), similar to the algorithms used for image reconstruction in CT. For the two treatment techniques now in clinical use, the target volume and each beam are subdivided into transverse sections of one to three CT cuts in thickness. A separate intensity pattern is computed for each of these beam sections. Then the beams are delivered either one section at a time using 360° rotations with specialized hardware (44) or all at once from each beam direction using sequential or dynamic multileaf collimator motions (45). Each of these technologies has its unique advantages and difficulties. The sequential multileaf system is used at Fox Chase Cancer Center.

From our previous experience with dose-escalation (2, 5) we expect 60-70% (depending on risk group) freedom from biochemical failure rate at 5 years using 3DCRT to treat the prostate to 76 Gy. IMRT allows for more precise radiation delivery, such that doses may be further escalated. The primary goal of this protocol is to determine whether biologically higher doses of 84.3 Gy will significantly improve patient outcome over 76 Gy. At the present time 76-78 Gy is being used to treat intermediate-to-high risk prostate cancer in the Department of Radiation Oncology at Fox Chase Cancer Center. Thus, the 76 Gy arm is the standard arm in this protocol.

1.2 Biomarkers: A number of laboratories have examined DNA content and/or various new biomarkers as potential prognostic factors for prostate cancer patients. Most of these efforts have been for patients undergoing radical prostatectomy, primarily because of biopsy tissue constraints from patients undergoing radiotherapy. There are three important aspects to the proposed prospective retrieval and analysis of tissue from patients enrolled in the protocol. First, immediate prospective attempts at retrieval of the pretreatment biopsy tissue should result in a much greater percentage of blocks or unstained slides recovered for this purpose. Second, prospective analysis of biomarkers has rarely been done and would add substantial credibility to the findings. Retrospective analyses typically involve a search for optimal cut-points, whereas, in a prospective study, the cut-points are established before the start. Finally, the proposed prospective analyses would determine the feasibility in applying these biomarkers clinically (e.g., establish the proportion in whom the test could not be done). The molecular biomarkers selected include p53, Ki67 (**Figure 2**), bcl-2 (**Figure 3**), bax (**Figure 4**) (19-27), MDM2 and PKA type I. The prognostic value of DNA content has been studied by the PI over the years (25) and has been a consistent correlate of patient outcome. Serum testosterone (serum-T) is a routine clinical test in our department that serves to establish baseline androgen blood levels. Serum-T has also been shown to have significant prognostic value for prostate cancer patients treated with radiotherapy alone (38). Sex hormone binding globulin and estradiol have been included because evidence suggests that the levels of these molecules may impact the prognostic significance of serum-T (46).

1.3 Proteomics and Genomics (added 7/5/04): The progress made in the separation and identification of proteins in plasma or serum has the promise of enhancing current methods of diagnosis, predicting treatment success and monitoring progression. Advances in mass spectrometry have been applied to the diagnosis of prostate cancer with encouraging results (47-52). We plan to collect plasma and serum, as well as red cells and lymphocytes (buffy coat) before treatment and at each follow-up visit to determine if the protein changes observed are useful in predicting the outcome of men treated with radiotherapy with or without androgen deprivation.

1.3.1 Proteomics of blood samples (added 7/5/04): Proteomics of blood samples: Proteomics profiles will be obtained by mass spectrometry and interrogated by two genetic algorithms to produce consensus proteomics models for prostate cancer prediction. The FCCC Proteomics Facility, under **Dr. Yeung's** direction, has substantial experience with mass spectrometry and 2D gel based proteomics, being able to routinely identify 384 protein spots each night. The Facility performs serum proteomics by two approaches. The first is MALDI-TOF (Matrix Assisted

Laser Desorption Ionization Time of Flight) on a Bruker Reflex IV mass spectrometer and/or on an Applied BioSystems Q-Star XL mass spectrometer using an oMALDI (orthogonal MALDI) source. The second is LC/MS/MS on the Q-Star and/or on a Finnegan LCQ ion-trap mass spectrometer both supported by Agilent capillary and nano HPLC systems. Serum proteomics capability here began as collaborations with three NCI-FDA serum proteomics groups, namely the laboratories of Petricoin and Liotta, Tim Veenstra, and Gordon Whiteley who directs the FDA Clinical Proteomics Reference Laboratory. Reproducibility is now optimized by careful standardization of FCCC serum sample collection and processing, by skillful use of Tecan Genesis liquid handling robot for sample fractionation, good HPLC systems, and high stability high resolution mass spectrometers. The serum samples will first be treated to release the small peptide markers that are bound to the larger and more abundant serum proteins. The condition used is dilution of the serum into a low pH polar solvent (0.1M glycine pH 2.3 in the presence of 20% acetonitrile). The treated serum is filtered by ultracentrifugation through a 30 KDa cut-off membrane (Amicon YM-30). The ultrafiltrate is then subjected to MALDI-TOF or LC/MS/MS analysis with or without further fractionation. One fractionation method for MALDI-TOF is using either zip-tips or zip-plates, both made reproducible by performing on the Tecan Genesis robot with pretesting of the tips and plates to assure proper flow properties. Another fractionation method uses complete tryptic digestion of the ultrafiltrate followed by strong cation-exchange column fractionation on a capillary HPLC system and then analyzed by nano-LC MS/MS sequencing and data base search for peptide identification on either the Q-Star or the LCQ mass spectrometer. Analysis of the MALDI-TOF data consists of pattern recognition as a continuation of a collaboration arrangement with the Correlogic group via Dr. Godwin, and with the Stony Brook Biostatistics group of Dr. Grimm collaborating through the Fox Chase Bioinformatics group of Dr. Robert Beck. Peptide peaks will be sequenced on the Q-Star in MS/MS mode to provide protein identification. For the LC/MS/MS, identified peptides of different HPLC fractions will be assembled into a 3D map (fraction number x M/Z x peak intensity) assisted by software available on the Q-Star and by the FCCC Bioinformatics group. Careful comparison of the data from normal and from cancer patient samples will allow us to identify specific marker sets that can reliable distinguish the two populations. The objectives are 1) to examine the pretreatment serum for protein patterns that predict for biochemical failure and 2) to determine if protein profiles from men treated with radiotherapy with and without androgen deprivation may be used as an adjunct to PSA in identifying failure at an earlier point in time. For the second objective, samples will be collected at each follow-up visit for 5 years as depicted in Section 17.0.

1.3.2 Genomics of urine samples (added 7/5/04): As with the proteomics project described above, the objective is to 1) determine if a certain genomic pattern observed prior to treatment predicts for an unfavorable outcome and 2) monitor the response of men with prostate cancer who are treated with radiotherapy with or without androgen deprivation to improve the detection of recurrence. This project builds on our results with the detection of hypermethylation of the glutathione S-transferase p1 (GSTP1) gene locus in urine (53). Promoter hypermethylation is a common mechanism for tumor suppressor inactivation in human cancers and is a promising target for molecular detection of prostate cancer in urine. *GSTP1* hypermethylation is well established as "early," frequent and cancer specific and can be detected by the sensitive MSP test. The hypermethylation of GSTP1 is found in >90% of primary prostate cancers, but not in normal prostatic tissue or other normal tissues. GSTP1 was detected in the urine of 79% of men with PC. *GSTP1* and *RARβ* gene hypermethylation in voided urine DNA will be assayed with the aim of 100% diagnostic coverage for molecular detection of recurrent prostate cancer. The diagnostic utility of *GSTP1* and *RARβ* hypermethylation will be determined. Further genes will be added to the detection panel to provide molecular prognostic information. The

hypothesis is that this technique will supplement PSA as a predictor of recurrence and as an early identifier of recurrence after definitive radiotherapy with or without androgen deprivation. As for the collection of serum and plasma for proteomics, urine will be collected prior to treatment and at each follow-up visit for 5 years (Section 17.0).

1.3.3 Genomics on archival diagnostic tissue (added 5/5/05): The main objectives here are 1) to determine if the pattern if the gene expression in tissue is predictive of outcome after radiotherapy ± androgen deprivation and 2) to identify novel therapeutic targets. As described in the biomarker section (section 1.2), archival formalin-fixed tissue is being collected prospectively, primarily for immunohistochemical studies. The tissue requirement for immunohistochemical analysis of gene expression at the protein level is substantial and for many of the cases only 5-6 genes will be assessed. Although there are sometimes differences between protein and RNA levels (e.g., post-transcriptional modification), the information obtained by RNA analysis will compliment the protein results and allow for many more genes to be assessed at one time. A custom low density Taqman array (Applied Biosciences, Foster City, CA) of primers for the measurement of 71 genes (including 3 housekeeping) has been constructed (Appendix J). The genes being analyzed at the protein level have been included in this array, as have some other genes with promise. The plan is to first run the immunohistochemical analyses (described in section 1.2) and to use the tissue left over for the assessment of RNA expression. Preliminary studies indicate that RNA integrity is maintained with as few as 500 cells, removed from archival tissue via laser capture microdissection. Further pilot experiments to document the reproducibility of the RNA measurements will be performed using archival tissue from prostatectomy specimens. We plan to first use prostate tumor tissue from 10 prostatectomy specimens in which snap frozen and formalin fixed and embedded tumor samples are available. RNA will be extracted and gene expression tested in these 10 nonprotocol cases initially to ensure that we are able to reproduce the gene expression profiles in formalin fixed tissue. Once the pilot experiments have been completed, we will proceed with the low density array analyses on all of the protocol cases with sufficient archival tissue remaining after the immunohistochemical biomarker analyses have been performed.

1.3.4 Single Nucleotide Polymorphisms (SNPs) in DNA from peripheral blood samples (Added 3/2/10): The most common forms of variation in the human genome are SNPs. To date, there are over 10 million human SNPs, of which 92,000 are located within protein coding sequences. Because nsSNPs alter the encoded amino acid sequence, they may have direct effects on the structure, function, and interactions of expressed proteins. We will explore the genetic predictors for RT-related adverse response with a comprehensive approach evaluating all the established genes and SNPs in the Ionizing Radiation (IR)-Induced Cell Cycle Control, Apoptosis, and DNA Repair pathways. The two main pathways for DNA double-strand breaks (DSBs) are homologous recombination (HR) and non-homologous end-joining (NHEJ). To systematically evaluate potential genes and SNPs related to RT reaction, we will test 315 SNPs in 48 genes. The candidate genes include:

- *Cell Cycle*: ATM, ATR, CDKN1A, CDKN2A, CHEK1, CHEK2, FANCD2, P21, RAD17, TGF-b, TP53;
- *Base Excision Repair (BER)*: APEX1, APEX2, LIG3, MBD4, MPG, MUTYH, NEIL1, NEIL2, NTHL1, OGG1, PARP1. PARP2. PNKP, SMUG1, TDG, UNG, XRCC1;
- *Homologous Recombination (HR)*: BRCA1, BRCA2, DMC1, MRE11A, MUS81, NBS81,RAD50, RAD51, RAD52, RAD54, XRCC3
- *Non-homologous End-joining (NHEJ)*: DCLRE1C, G22P1, Ku70, Ku80, LIG4, XRCC4, XRCC5.

The approach we are taking is to perform a targeted analysis of candidate genes related to IR-response from a larger group of genes to be analyzed. We plan to perform also a genome wide analysis, but to first look at genes related to IR-response and to then perform genome wide association studies (GWAS) secondarily. This stepped approach from IR-response targeted to GWAS is planned from the outset because our principal hypothesis is that IR-response will be most important; however, we recognize that other genes and pathways may turn out to be equally or more important.

1.4 Quality of Life: As mentioned above, IMRT, is an advanced technology that delivers the total radiation dose in a pattern that closely matches the shape of the target volume in three dimensions and avoids normal tissues. This sparing of normal tissue has the potential to decrease bladder and rectal toxicities and increase quality of life after prostate cancer therapy. Though few studies have been published documenting the effect of IMRT on quality of life, several studies have clearly documented reduced toxicity compared to conventional or conventional conformal radiotherapy (3DCRT). A recent study by Zelefsky et al (16) indicated treatment with IMRT significantly decreased the incidence of late grade 2 rectal toxicity by 12% at 3-years compared to the same dose of 81 Gy delivered by 3DCRT. Three-year actuarial incidence was 2% in the IMRT group compared with 14% in 3DCRT group ($p = 0.005$). The 5-year actuarial rate of grade 2 urinary toxicity in patients who received 75.6 Gy was 13% compared with 4% in those treated to lower doses ($p < 0.001$), but there was no difference in outcome between 3DCRT and IMRT. The authors concluded that IMRT is associated with minimal rectal and bladder toxicity and represents the treatment delivery approach with the most favorable risk-to-benefit ratio. In our experience, there is much greater sparing of the rectum from high radiation doses with IMRT, as compared to 3DCRT (54).

In the study proposed here all patients will receive IMRT. The QOL assessment will provide unique data on the effects of hypofractionation with dose escalation on QOL. The EPIC and EQ5D questionnaires will be used to measure changes in QOL over time (55). In addition the EQ5D will provide a measure of utility for each arm. This will give us a measure of patient preference for each treatment arm and permit cost-utility analyses (using direct costs as determined by modeled costs). A pretreatment assessment will be done, followed by the administration of both questionnaires at 0.5, 1, 2, 3, 4, and 5 years after the completion of radiotherapy. (Modified 2/23/03)

Added at the request of the DSMB (2/23/03)
Another measure of urinary function is the American Urological Association Symptom Score or International Prostate Symptom Score (IPSS) (56) This scoring system has been established as a measure of radiation morbidity in patients treated for prostate cancer (57-60) and will be administered prior to treatment, at the end of radiotherapy and at each follow-up visit.

2.0 OBJECTIVES

2.1 The objectives of the trial are:

2.1.1. The main objective is to evaluate the efficacy of 70.2 Gy in 26 daily fractions HIMRT (equivalent biologically to 84.3 Gy at 2.0 Gy assuming an α/β of 1.5) relative to 76.0 Gy in 38 daily fractions CIMRT. The primary hypothesis is that biochemical failure free survival will be improved by HIMRT. Secondary endpoints include local control, freedom from distant metastasis, and overall survival.

2.1.2. To establish local failure by biopsy of the prostate when objective tests (PSA, ultrasound, DRE) suggest relapse. Also, to determine at 2 years after treatment the extent of disease eradication by biopsy of the prostate when no evidence of relapse is evident

2.1.3. To prospectively determine the predictive value of DNA-ploidy and selected biomarkers by immunohistochemistry (Ki-67, p53, bcl-2, and bax) using pretreatment diagnostic material.

2.1.4. To assess the impact of treatment on quality of life using The Expanded Prostate Cancer Index Composite (EPIC) at 0.5, 1, 2, 3, 4 and 5 years post-radiotherapy.

2.1.5 To assess the impact of treatment on patient preferences, utilities and cost. (added 2/23/03)

2.1.6 To investigate the association between proteomic patterns in serum (or plasma), and hypermethylated DNA in urine, and patient outcome using samples collected prior to treatment and at each scheduled follow-up visit for 5 years. (added 7/5/04)

3.0 PATIENT ELIGIBILITY CRITERIA

3.1 Patients referred for radiotherapy with histologically proven adenocarcinoma of the prostate with clinical stage T1b-T3c (1992 AJCC palpation staging system) disease, and without clinical-radiographic evidence of metastasis are potentially eligible.

3.2 Eligibility Criteria:

- 3.2.1 Biopsy proof of adenocarcinoma of the prostate.
- 3.2.2 Bone scan ≤4 months of randomization if PSA>10 ng/ml or T3 disease.
- 3.2.3 CT or MRI-scan of pelvis ≤4 months of randomization if T3 disease.
- 3.2.4 Suitable medical condition; Zubrod <2.
- 3.2.5 Pretreatment PSA <80 ng/ml done ≤4 weeks of randomization. If neoadjuvant androgen ablation has been given, then the pre-androgen ablation PSA should be used for stratification.
- 3.2.6 Clinical (palpation) Stage T1b – T3c disease (1992 AJCC staging system). While a transrectal ultrasound is typically obtained at prostate biopsy, and endorectal coil MRI is often obtained as part of the workup, staging will not be based on these findings.
- 3.2.7 Gleason score ≥5.
- 3.2.8 Serum testosterone, estradiol and sex hormone binding globulin are not required for randomization.
- 3.2.9 One must be present: PSA >10, Gleason >6, category ≥T2b palpable disease, or ≥3 biopsy cores involved with Gleason score ≥5.
- 3.2.10 Informed consent must be obtained.

4.0 PATIENT INELIGIBILITY CRITERIA:

- 4.1.1 Prior pelvic radiotherapy.
- 4.1.2 Greater than 4 months of prior androgen ablation therapy.
- 4.1.3 Prior or planned radical prostate surgery.
- 4.1.4 Clinical, radiographic or pathologic evidence of nodal or distant metastatic disease.
- 4.1.5 Concurrent, active malignancy, other than nonmetastatic skin cancer or early stage chronic lymphocytic leukemia (well-differentiated small cell lymphocytic

lymphoma). If a prior malignancy is in remission for ≥ 5 years then the patient is eligible.

4.1.6 Zubrod status ≥2.

4.1.7 Pretreatment PSA >80 ng/ml or Gleason score <5.

4.1.8 PSA <10, Gleason ≤6, and Stage T1b-T2a, unless ≥3 biopsy cores are positive with Gleason score ≥5.

4.1.9 Stage T4 disease.

5.0 PRETREATMENT EVALUATION

5.1.1 History and physical. Include Zubrod status.

5.1.2 Pathologic review of prostate biopsy at Fox Chase Cancer Center

5.1.3 Serum PSA ≤4 weeks of protocol randomization

5.1.4 Bone scan if PSA > 10 ng/ml or T3 disease ≤4 months of randomization.

5.1.5 CT or MRI of pelvis if T3 disease ≤4 months of randomization.

5.1.6 Serum testosterone will be drawn ≤4 months before randomization or after randomization but prior to receiving the first radiation treatment.

5.1.7 Other serum hormone related tests (before androgen ablation).

If a patient was started on androgen ablation prior to protocol enrollment, these tests will not be obtained.

5.1.7.1 Sex hormone binding globulin will be drawn ≤4 months before randomization or after randomization but prior to receiving the first radiation treatment.

5.1.7.2 Serum estradiol will be drawn ≤4 months before randomization or after randomization but prior to receiving the first radiation treatment..

5.1.8 Quality of Life Questionnaires: The EPIC questionnaire (61) will be administered prior to treatment and at 0.5, 1, 2, 3, 4, and 5 years after completion of radiotherapy.

5.1.9 Blood and urine collection for proteomic and genomic studies (added 7/5/04): Six tubes of blood (~10 cc each) will be collected prior to treatment and at each scheduled follow-up visit for 5 years (see Sections 15.5 & 17.0).

6.0 TREATMENT PLAN

6.1 Randomization

6.1.1 Patients entered into the study will be stratified according to:
Pre-treatment PSA ≤10 vs > 10 – 20 vs >20 ng/ml.
Gleason score 5-7 vs 8-10.
High risk (2 years of androgen ablation, mainly adjuvant) vs intermediate risk (no adjuvant androgen ablation).

6.1.2 Randomization will be one of the following two treatments:
4.1.2.1 *Arm I*: CIMRT (76 Gy in 38 fractions).
4.1.2.2 *Arm II*: HIMRT (70.2 Gy in 26 fractions).

6.1.3 High risk patients are so classified if pretreatment PSA is >20 ng/ml, Gleason score is 8-10, T-catgeory is T3 or ≥4 diagnostic needle biopsy specimens contain Gleason 7 disease. The latter criterion is based on recent evidence that these patients have about a 45% risk of lymph node metastasis (62). High risk patients will receive 2 years of androgen ablation. The two years of androgen ablation would include any neoadjuvant androgen ablation given prior to protocol entry.

6.1.4 Intermediate risk patients include all other eligible patients (PSA ≤20 ng/ml, Gleason score 7, and T-category <T3; including Gleason score 5-6 if ≥3 biopsies are positive; excluding ≥4 diagnostic needle biopsy specimens containing Gleason 7 disease).

6.1.5 Randomization will be done by the Data Management Section in the Department of Radiation Oncology by Teri White ext. 2994 (Pager #306-6757)

6.2 Request for prostate diagnostic biopsy tissue, blood products and urine

6.2.1 Prostate diagnostic biopsy tissue: Archival tissue will be requested for analysis of biomarkers at FCCC. The research nurse will send a copy of the release form, the FCCC pathology report and a cover letter to the outside Pathology department. The requests for, and receipt of, the biopsy material will be coordinated by the study chairman, Dr. Pollack in collaboration with Dr. Al-Saleem. They will oversee the immunohistochemical analyses of Ki-67, p53, bcl-2, and bax, and the DNA content analysis. Initially, department funds will be used for these tests, with the goal of acquiring funds from an extramural source.

6.2.2 Blood and urine: Pretreatment serum PSA and testosterone will be determined as it is routinely in our clinic. Serum sex hormone binding globulin and estradiol levels will also be obtained through FCCC.

6.2.3. Pretreatment and follow-up serum, plasma and urine for proteomics and genomics studies will be obtained as outlined in Sections 15.5 & 17.0, and Appendix I (added 7/5/04). Charges for these tests will be referred to the Department of Radiation Oncology until an outside funding source is identified. Directions for collaborating institutions are in Section 16.0

7.0 CHEMOTHERAPY

Chemotherapy does not apply to this study.

8.0 RADIATION THERAPY

8.1 IMRT planning and treatment

8.1.1 Planning CT-Scan Simulation: The patient will be instructed not to void before the scan, to mimic bladder position during treatment. An enema will be administered within 2.0 hr of simulation to empty the bowel. The CT-scan images will be taken at 3 mm intervals from the top of the sacrum to 1 cm below the ischial tuberosities (to include the entire bladder and rectum). All patients will have tattoos placed at the anterior, right lateral, and left lateral isocenter skin points. An MR scan simulation may be used secondarily to aid in defining prostate anatomy.

IMRT Planning: The CT-Scan is loaded into a planning computer. At each slice level, the pelvic bones, bladder, rectum, prostate, and seminal vesicles are outlined. The rectum will be outlined from the anterior flexion of the rectosigmoid superiorly to the ischial tuberosities inferiorly. The entire bladder will be outlined. The femoral heads should be outlined down to the region between the greater and lesser trochanters. The gross tumor volume (GTV) will be similar to the clinical target volume (CTV1), and will include the prostate and a portion of the seminal vesicles. The CTV may include an extra 1-2 mm beyond the GTV in regions of known bulky disease in high risk patients, as determined by the diagnostic biopsy information and/or imaging. The CTV1 for intermediate risk patients (there is no other CTV for intermediate risk patients) is the prostate and proximal seminal vesicles (usually about 9 mm and <50%). The CTV1 for high risk patients includes the prostate and at least 50% of the seminal vesicles, including any grossly involved regions. At the abutment of the prostate and seminal vesicles, it is sometimes unclear where the prostate ends and the seminal vesicles begin; this region should be outlined as prostate. In high risk patients, the uninvolved portions of the seminal vesicles (CTV2) should be treated to 56 Gy in the CIMRT arm and 50-52 Gy in the HIMRT arm. The superior aspects of the seminal vesicles should not be outlined if they extend around more than 50% of the lateral width of the rectum on the lateral projection. For high risk patients, the CTV3 should be comprised of the peri-prostatic, peri-seminal vesicle, external iliac, obturator and internal iliac lymph nodes (Appendix K).

The PTV1, PTV2 and PTV3 margins should be consistent within each arm, but are different for the two treatment groups. For CIMRT, the desired PTVs are 0.8 cm in all dimensions except posteriorly (the prostate-rectal interface for PTV1), where the margin should be 0.5 cm. For HIMRT, the desired PTVs are 0.7 cm in all dimensions except posteriorly, where the margin should be 0.3 cm. The PTV margins are smaller for the HIMRT arm to reduce the potential increased complication risk from hypofractionation. For CIMRT, the effective PTV (where the prescription line falls relative to the CTV on a slice-by-slice basis) will be 0.8 – 1.3 cm around the CTV in all dimensions, except posteriorly where the PTV will be 0.3 – 0.8 cm. For HIMRT, the effective PTV will be 0.5 – 1.0 cm around the CTV in all dimensions, except posteriorly where the PTV will be 0.2 – 0.6 cm. The prescription line for the HIMRT plans may be within 2 mm of the CTV posteriorly, instead of 3 mm with CIMRT.

The maximum dose heterogeneity allowable in the PTV will be 20%, a variation will be >20% and a violation >25%. Since the dose is prescribed to the minimum isodose line of the PTV, the dose variability is seen in portions of the target volume receiving higher than the specified dose.

8.1.2 Evaluation and acceptance of the plan: A series of dose-volume histograms will be generated and analyzed to determine the adequacy of the plan. At least 95% of the PTV

should receive the prescribed dose; a variation will be noted if <95% to 90% of the PTV receives the prescribed dose and a protocol violation will be noted if <90% of the PTV receives the prescribed dose. The dose marker levels for bladder and rectum will be set, based on prior studies (40). The plan will be deemed acceptable under the following conditions. Less than or equal to 17% and 35% of the rectum should receive ≥65 Gy and ≥40 Gy, respectively, for the conventionally fractionated patients (Arm I, 76 Gy total dose). Less than or equal to 25% and 50% of the bladder should ≥65 Gy and ≥40 Gy, respectively, for the conventionally fractionated patients (Arm I, 76 Gy total dose). Less than or equal to 17% and 35% of the rectum should receive ≥50 Gy and ≥31 Gy, respectively, for the hypofractionated patients (Arm II, 70.2 Gy total dose). Less than or equal to 25% and 50% of the bladder should receive ≥50 Gy and ≥31 Gy, respectively, for the hypofractionated patients (Arm II, 70.2 Gy total dose). The criteria for the bladder have been relaxed because a clear cut-point has never been defined. A variation will be noted if up to an additional 7.5% of the rectal and bladder volumes receive above the target doses specified. The inclusion of rectal volumes beyond these constraints will be considered a protocol violation. The inclusion of bladder volumes beyond these constraints will be considered a secondary protocol variation; it will not be considered a protocol violation since a distinct bladder dose volume histogram relationship has not been defined previously.

8.1.3 Image Guidance (added 7/5/04): Prostate position varies day-today and some type of correction for this interfraction motion must be considered. At FCCC, transabdominal BAT ultrasound images are acquired and adjustments in the isocenter made as appropriate. Collaborating institutions must either use a similar method of correcting for interfraction prostate motion on a daily basis using ultrasound guidance or fiducial marker visualization via film or electronic portal imaging.

9.0 HORMONE THERAPY

9.1 Patients with high risk features will be treated for two years with androgen ablation. Androgen ablation may begin up to four months prior to enrollment in the trial. Hormone therapy will consist of Lupron,, Zoladex or similar LHRH agonist (e.g., Eligard) given in either 3 or 4 month Depo injections, as available. The anti-androgens Casodex or Eulexin are recommended during the first month of treatment with the LHRH agonist, but, should not be used continuously thereafter.

9.2 Many patients are started on androgen ablation prior to referral and consideration for protocol entry. Such patients will still be eligible if they are randomized ≤4 months of starting androgen ablation. For patients with intermediate risk features, androgen ablation will not be continued beyond the time in which the patient is randomized. For patients with high risk features, androgen ablation will be continued as outlined above. The start date of androgen ablation will be recorded in all cases. For the high risk patients, the androgen ablation start date will be used for the calculation of the two year duration of androgen ablation required for this group.

10.0DURATION OF THERAPY

10.1 RT will be for either 38 fractions over 7.5 weeks (76 Gy, CIMRT) or 26 fractions over close to 5 weeks (70.2 Gy, HIMRT).

10.2 Androgen ablation will be for a total of 2 years for patients with high risk disease. Androgen ablation may be begun up to 4 months prior to randomization in the trial, but the intent is for androgen ablation to start at the beginning of RT (adjuvant androgen ablation).

10.3 Neoadjuvant androgen ablation is permitted for up to four months prior to protocol entry in both intermediate and high risk patients, but the intent is for intermediate risk patients to be treated with radiation alone.

10.4 Treatment will be stopped for grade 4 acute toxicity, but may be resumed if the treatment break is less than 8 working days. If grade 4 toxicity returns, the treatment will be discontinued. The patient will then be removed from study, but not from intent-to-treat analysis.

10.5 Treatment will be stopped if metastasis is detected and the patient will be removed from the study, but not from intent-to-treat analysis.

11.0 MEASUREMENT OF EFFECT

11.1 All patients will be evaluated for clinical or biochemical evidence of relapse as defined below. If any of these tests suggest relapse, then the prostate will be biopsied. In the case of a partial response (question of palpable residual disease) and a stable PSA, biopsy will be delayed to 2 years after treatment.

11.2 At 2 years after completion of treatment (radiotherapy or androgen deprivation – whichever is longer) all patients without documented failure will undergo needle biopsy of the prostate, unless clinically contraindicated or the patient refuses. A minimum of 10 core biopsies will be taken and additional biopsies will be taken from any suspicious areas (ultrasound or palpation) and/or the original site of biopsy confirmation of prostate cancer at diagnosis. The 10 biopsy sites include sextant, bilateral anterior horns, and bilateral transition zone. These data will enable us to evaluate the extent of disease eradication, as well as the prognostic significance of positive biopsies in otherwise palpably normal prostate glands after treatment.

11.3 Clinical primary tumor response will be measured by palpation and recorded in the following ways:

(a) Pretreatment: A representative drawing of the pretreatment tumor status, if palpable, will be recorded in the radiotherapy chart.

(b) Post-treatment: The change in palpable tumor volume will be recorded qualitatively using these criteria:

(i) Complete response: no palpable tumor.

(ii) Partial response: at least 50% decrease in the tumor mass (in case of more than one nodule, each must have decreased by at least 50%).

(iii) Stable disease: changes too small to qualify for partial response or progression.

(iv) Progression: at least a 25% increase in the size of the tumor relative to the smallest volume recorded, or new extension of tumor beyond the capsule, or re-extension of tumor beyond the capsule after initial regression, or urinary obstructive symptoms with carcinoma found at TURP. In all cases of clinically suspected local failure, prostate biopsy confirmation of carcinoma will be requested, unless clinically contraindicated.

11.4 PSA response: In 98% of patients treated with definitive radiotherapy there is a drop in PSA within 3 months. Those patients that have not responded should be investigated to define the site of progression (local-regional vs distant metastases). In patients that have responded, a rising PSA later heralds relapse. Biochemical failure will be modeled after the ASTRO consensus guidelines of three rises in PSA (63), although backdating of failure will be to the PSA prior to the first rise rather than the nadir PSA and consecutive PSA rises will not be required (stepwise rises will be counted). Evaluation of patients with a rising PSA profile will include a bone scan, CT-pelvis, and prostate biopsy. ProstaScint scan has not been shown to be consistent for defining relapse pattern and is not recommended.

11.5 Nodal relapse will be scored as having occurred when appropriate clinical-radiographic evidence (CT or MRI evidence) of this becomes evident (biopsy proof not required in the presence of a rising PSA).

11.6 Hematogenous relapse will be scored as having occurred when appropriate clinical-radiographic evidence, shows this to be so (biopsy proof not required).

11.7 Quality of Life: The Expanded Prostate Cancer Index Composite (EPIC) (61) is a contemporary QOL questionnaire that measures patient function and bother, emphasizing urinary, bowel, sexual, and hormonal aspects. Test-retest reliability and internal consistency are high. The EPIC is considered to be a robust QOL instrument. A baseline QOL assessment will be done prior to treatment and then QOL assessments will be done at 0.5, 1, 2, 3, 4, and 5 years after radiotherapy.

11.8 Treatment costs: Treatment cost will be collected prospectively for each arm of the study using CPT codes, Medicare Resource Based Relative Value Units (RBRVUs), HCPC codes, DRGs, and IDC-9 codes. The collection of these measures will allow for economic comparisons using direct treatment costs, as well as costs for symptom management, office visits, and hospitalizations associated with treatment.

11.9 Following relapse the patient will be managed with all appropriate therapy and will continue to be followed as part of this study in order to document the full course of his disease until death.

12.0 MEASUREMENT OF TOXICITY

12.1 Acute proctitis and cystitis lasting for up to 4 months after completion of radiotherapy are accompaniments of radiotherapy for carcinoma of the prostate. The severity of these reactions is routinely evaluated during treatment and will be scored according to the criteria outlined in Appendix D. In our extensive experience, grade 3 or 4 acute toxicities are rare.

12.2 Delayed toxicities are usually related to urinary, rectal, and sexual function. The anticipated urinary and rectal toxicities and severity criteria are those shown in Appendix E. Other untoward clinical events will, however, also be documented.

13.0 STATISTICAL CONSIDERATIONS

13.1 Primary Endpoint: The primary hypothesis of the trial is that dose escalation using hypofractionation will significantly increase freedom from biochemical and/or disease failure rates, as compared to conventional dose-fractionation. Biochemical failure will be modeled after the ASTRO consensus guidelines of three rises in PSA (63), although backdating of failure will be to the PSA prior to the first rise rather than the nadir PSA and consecutive PSA rises will not be required (stepwise rises will be counted).Previous experience shows that approximately 70% of intermediate to high risk prostate cancer patients treated to doses equivalent to 76 Gy using conventional fractionation (CIMRT, Arm I) will remain biochemically free of disease at four years post-treatment. Eighty percent of patients treated in a similar fashion to a median dose of 82 Gy remain biochemically free of disease at four years. Based on these percentages and taking into account the shape of the dose response curve, we expect that the risk of biochemical failure for patients treated to a dose biologically equivalent to 84.3 Gy at 2.0 Gy per fraction (HIMRT, Arm II) to be approximately 15%. A total sample size of 300 patients split equally between the two arms, or 68 biochemical failures, achieves 90% power to detect a hazard ratio of 0.46 when the proportions free of failure for the two arms at 4 years after the last patient is entered (2 years after the completion of androgen deprivation) are 70% and 85% at a significance level of 0.05 using a two-sided log-rank test (64). An interim analysis is also planned at two years after the last patient entered completes treatment, which is at the time the last prostate biopsies will be obtained for

those treated with RT alone. These calculations assume that two sequential tests are made, the interim at two years and the final at four years post-enrollment of the last patient. The calculations were made using the O'Brien-Fleming spending function to determine the test boundaries and assume that the survival times are exponential. The assumptions made here are reasonable considering that the Principal Investigator's prior randomized dose escalation trial was also positive with an 8 Gy dose difference in 300 patients.

Prior experience indicates that about 65% of the 150 eligible intermediate to high risk patients referred for prostate cancer radiotherapy each year will participate in this study. Thus, approximately 100 patients will be accrued yearly over a period of 3 years. Patients will be randomized equally between CIMRT and HIMRT arms, using a permuted block design and stratification levels previously specified. The following table of intermediate/high risk prostate patients treated per annum with radiation therapy illustrates the conservative nature of our accrual estimates.

Year	N	%	Cumulative N	Cumulative %
1992	151	8.29	262	14.39
1993	177	9.72	439	24.11
1994	160	8.79	599	32.89
1995	180	9.88	779	42.78
1996	173	9.50	952	52.28
1997	178	9.77	1130	62.05
1998	226	12.41	1356	74.46
1999	275	15.10	1631	89.57
2000	190	10.43	1821	100.00

13.2 Secondary Clinical Endpoints: Local control, freedom from distant metastasis, and overall survival.

13.3 Stopping rule for survival: To permit the possibility of stopping the trial early in the event of a significant disparity between the treatment arms in terms of overall survival, survival differences will be tested in a group sequential manner. Specifically, a maximum of three sequential two-sided log-rank tests (64) will be conducted with the trial terminating after any test if a significant difference in overall survival has been established. The overall group sequential test will be based on the O'Brien-Fleming spending function to determine the test boundaries and on the assumption that the survival times are exponential. The test boundaries will be constructed so that the test has 90% overall power, at the overall 5% significance level, to detect a hazard rate of 0.49 when the proportions surviving in the two arms are 76% and 90%. If there is no difference between the arms in terms of overall survival, the probability is less than 0.01 that the trial will be incorrectly stopped early after either 100 or 200 patients become evaluable for survival.

13.4 Stopping rule for late toxicity: The following table provides a stopping rule for groups of 30 patients in the experimental arm with respect to late (beginning at one-year follow-up) grade 3 or 4 toxicity. The boundaries are based upon Fleming's (65) one-sample multiple testing procedure which employs the standard single-stage test procedure at the last test, while allowing for early termination and preserving

No. of Pts with Unacceptable Toxicities	Total No. of Evaluable Pts.
8	30
9	60
11	90
13	120
15	150

the size, power and simplicity of the single-stage procedure. The computations are based upon a total of 150 patients, an unacceptable grade 3 or 4 late toxicity rate of 6% (66), and an overall type I error rate of 5%.

13.5 Stopping rule for poor patient accrual: Accrual to the trial will be examined yearly with the goal of at least one-third the accrual target. The timing of the accrual target analysis will begin on the date of the first patient entered (not when the trial is first approved). The expected accrual per year is 100; if less than a third of this number is reached, the trial will be considered for closure.

13.6 Tissue Biomarker Analyses: Pretreatment biopsy material will be stained for DNA content analysis, Ki-67, p53, bcl-2, bax and MDM2 prospectively as the samples are received. Information on pretreatment sex hormone binding globulin and estradiol will be recorded as patients are accrued.

At the time of interim analysis, the following will be accomplished: the distributional form of each marker, along with previously determined predictors of biochemical control (stage, grade, pretreatment PSA) will be assessed and necessary transformations will be applied prior to inferential analysis. Since patients will be randomly allocated to the two arms, significant differences in prognostic factors across arms are not expected. Contingency table analysis and t-tests will be used to determine whether significant differences exist between the two groups with respect to markers and prognostic factors. The effect of potential confounders will be controlled for in multivariate analysis of outcome.

At the time of interim and final analysis, univariate analysis of biochemical control will involve estimation of rates over time using Kaplan-Meier (67) and cumulative incidence methodology (68); comparisons will be made using the log rank test and Gray's test (69). Estimates will be computed for the entire patient population as well as by markers, prognostic factors, and treatment group. A multivariate Cox regression model will be used to determine the influence of predictor variables, including treatment group and markers, on biochemical control using the hazard function (70). Stepwise modeling will be used to establish the optimal model, taking into account the interpretation of variables and possible interactions. The validity of the proportional hazards assumption will be tested using a graphical display of Schoenfeld residuals plotted against time (71). The validity of the linearity assumption imposed on the log relative hazard will be tested using plots of Martingale residuals against the covariate or the linear predictor (72). Influential cases, or cases having a disproportionate effect, on the result of the regression will be evaluated using DFBETA statistics (72). In the event that assumptions on the hazard function are not met, parametric regression models will be constructed and evaluated to assess the independent predictive capability of covariates.

13.7 Proteomic and Genomic Analyses (added 7/5/04, modified 5/5/05, modified 3/2/10): The objectives are 1) to examine protein in pretreatment serum, hypermethylated DNA in urine and RNA in archival diagnostic tissue (the leftover tissue from section 13.6) for patterns that predict for biochemical failure and 2) to determine if the profiles from serum and urine in men treated with radiotherapy with and without androgen deprivation may be used as an adjunct to PSA in identifying failure at an earlier point in time. For the second objective, serum and urine samples will be collected at each follow-up visit for 5 years as depicted in Section 17.0. Analysis of the MALDI-TOF protein data consists of pattern recognition. This type of analysis is in a state of development, as are the methods for protein analysis. Of key importance is to have such samples collected prospectively on a well-defined group of patients.

We anticipate that pretreatment serum protein, urine DNA and tissue RNA will be obtained from over 100 cases (50 from each treatment group). While these are exploratory studies, the goal is to relate the results to biochemical failure. The RNA gene expression analyses will provide results on 71 genes (including 3 housekeeping) using a custom made low density Taqman® array (see section 1.3.3). Standard descriptive statistics (means, medians, standard deviations) will be used to characterize the expression data. Next, we will use Wilcoxon signed-rank tests to analyze the expression data from 25-50 matched pairs to identify genes that are differentially expressed between patients who fail treatment and those who do not. These two sided tests will use a type I error of 0.01, to control the false positive rate. Power computations were performed for a less powerful, 2-sided, sign test. We arbitrarily define "success" as observing higher or lower expression in a sample from an individual who failed treatment, as compared to the paired patient who did not fail. Given data from at least 25 matched-pairs, we will have greater than 80% power to detect any gene that has a probability of "success" that is either greater than 84% or less than 16%. We will also perform exploratory analyses of these data using the methods of Bittner {Bittner, 2000 #2217} to determine whether, using un-supervised learning techniques (e.g., hierarchical clustering), we can identify clusters based on expression profiles that are associated with clinical characteristics.

Similar types of analyses will be performed for the serum protein and urine DNA analyses. If it is possible to dichotomize the resultant pretreatment patterns for serum protein and urine DNA, then the approach will be similar to that described for the biomarkers (Section 13.6). If the patterns are found to be best translated into a continuous variable, which is more likely for the objective related to the samples acquired during follow-up, optimal models would be established by comparing categorical covariates with the predictive value of the continuous covariates n multivariate analyses. Recursive partitioning could be used to dichotomize or categorize the continuous measures. These analyses would be similar in principle to statistical strategy used above for the biomarkers.

SNP analysis: Briefly, we would apply a series of filters to determine which SNPs are considered for the final model. Then, LDA (linear discriminant analysis), combined with a dose-volume-risk model will be used in a logistic regression framework to select and weight SNPs. The number of SNPs selected will be minimized under further reductions cause a significant drop in model predictive power on cross-validation. In addition to the testing of strategically selected SNPs in IR-Response Genes with functional significance, genome wide associations will also be examined. From the statistical modeling point of view, the goal of this project is primarily to produce clinical predictive risk models. A secondary, but equally important, approach is to identify key biological pathways that affect normal tissue response to radiation therapy. A more effective clinical risk model for the individual toxicity endpoints would help physicians identify patients who, regarding normal tissue endpoints, are either relatively sensitive to radiation (allowing effective selection of patients for dose de-escalation or modality modifications) or are resistant to radiation damage (allowing for safe dose escalation). Correlations with key biological pathways (as represented by the presence of SNPs in the risk models) could identify the importance of known and unknown genes and pathways in processing radiation damage.

13.8 QOL Analysis: Patient self-assessment of symptoms will be performed using EPIC domains (bowel, sexual, urinary, vitality/hormonal) and subscales for Summary, Function, and Bother. Late QOL effects will be evaluated on the basis of assessments at 0.5, 1, 2, 3, 4, and 5 years after completion of radiotherapy. If more than 20% of the items that comprise a domain

summary score or subscale score are missing a response, the corresponding domain summary or subscale score will be excluded from analysis (61). Mixed effects modeling will be used to evaluate changes over time as a function of covariates, particularly treatment.

13.9 Economic Analysis: Direct treatment costs for a 26 fraction and a 38 fraction schema will be summarized using descriptive statistics based on CPT codes and total RBRVUs and associated 2004 cost equivalents. An incremental cost-effectiveness analysis will be conducted and translated into societal over-expenditure on the basis of prostate cancer prevalence.

13.10 Utilities and Health Related Quality of Life (QOL) Using EQ5D (added 2/23/03): The EQ5D is a method for obtaining valuations of health-related quality of life (HRQOL) to be used as an adjustment to survival and in the cost-utility analysis. It is a two-part questionnaire that takes approximately 5 minutes to complete (55). The first part consists of 5-items covering 5 dimensions including: mobility, self care, usual activities, pain/discomfort, and anxiety/depression. Each dimension can be graded on 3 levels including: 1-no problems, 2-moderate problems and 3-extreme problems. Health states are defined by the combination of the leveled responses to the 5 dimensions, generating 243 (3^5) health states to which unconsciousness and death are added (73). The second part is a visual analogue scale (VAS) valuing current health state, measured on a 20 cm 10 point-interval scale. Worst imaginable health state is scored as 0 at the bottom of the scale and best imaginable health state is scored as 100 at the top. Both the 5-item index score and the VAS score are transformed into a utility score between 0 "Worst health state" and 1 "Best health state". Either the index score or the VAS score can be used in the quality adjusted survival analysis, or enter the cost-utility equation, depending on the health state(s) of interest (74).

Quality adjusted survival can be defined by the weighted sum of different time episodes added up to a total quality-adjusted survival time [**U**= sum of quality ($\mathbf{q_i}$) of health states **K** times the duration ($\mathbf{s_i}$) spent in each health state (75).

13.11 Cost Utility (added 2/23/03): We plan to collect cost data on patients who consent to participate. Taking a Medicare payer's perspective we will collect direct costs using CPT and APC codes. We will perform an incremental cost-utility analysis with the standard fractionation arm being the standard arm and the hypofractionated radiation arm being the experimental arm. An incremental cost-utility analysis will be performed with the following equation:
Cost of the Experimental treatment-Cost of the Standard Treatment/QALY of the Experimental Treatment-QALY of the Standard treatment. A sensitivity analysis will be performed on the effect of travel cost on the analysis. The cost of travel is not paid by Medicare but could be an important cost driver. Distance from the patient's home will be calculated and multiplied by $0.36/mile.

14.0 PHARMACOLOGIC INFORMATION

14.1 The drugs outlined below are used in routine management of prostate cancer and will not be supplied.

14.2 Lupron (leuprolide acetate) is a synthetic nanopeptide analog of naturally occurring gonadotropin-releasing hormone *(GnRH or LH-RH)*. The analog possesses greater potency than the natural hormone. Leuprolide acetate, a LH-RH agonist, acts as a potent inhibitor of gonadotropin secretion when given continuously and in therapeutic doses. Human studies indicate that following an initial stimulation, chronic administration of leuprolide acetate results

in suppression of ovarian and testicular steroidogenesis. This effect is reversible upon discontinuation of drug therapy. Administration of leuprolide acetate has resulted in inhibition of the growth of certain hormone dependent tumors *(prostatic tumors in Noble and Dunning male rats and DMBA-induced mammary tumors in female rats)* as well as atrophy of the reproductive organs. In humans, administration of leuprolide acetate results in an initial increase in circulating levels of luteinizing hormone *(LH)* and follicle stimulating hormone *(FSH)*, leading to a transient increase in levels of the gonadal steroids *(testosterone and dihydrotestosterone in males, and estrone and estradiol in premenopausal females)*. However, continuous administration of leuprolide acetate results in decreased levels of LH and FSH. In males, testosterone is reduced to castrate levels. These decreases occur within two to four weeks after initiation of treatment, and castrate levels of testosterone in prostatic cancer patients have been demonstrated for more than five years with continuous drug administration. Leuprolide is recommended as either 22.5 mg *(three month)* or 30 mg *(four month)* depots for intramuscular injection when used to suppress androgen levels for 2 years (8 or 6 injections). Each kit contains a vial of sterile lyophilized microspheres, which is leuprolide incorporated in a biodegradable polymer of polylactic acid. Any formulation may be used. The vial of leuprolide and the ampule of diluent may be stored at room temperature. Product does not contain preservative, discard if not used immediately.

Toxicity

In the majority of patients testosterone levels increased above baseline during the first week, declining thereafter to baseline levels or below by the end of the second week of treatment. The most common side effect of Leuprolide is vasomotor hot flashes; edema, gynecomastia, bone pain, thrombosis, and GI disturbances have occurred. Potential exacerbations of signs and symptoms during the first few weeks of treatment is a concern in patients with vertebral metastases and/or urinary obstruction or hematuria which, if aggravated, may lead to neurological problems such as temporary weakness and/or paresthesia of the lower limbs or worsening of urinary symptoms.

14.3 Zoladex (goserelin) is a LHRH analog with substitutions for the L-amino acid Glycine in positions 6 and 10. These substitutions produce analog with 50-100 times the potency and longer duration of action than the naturally occurring peptide when assessed in acute animal tests. Zoladex is commercially available. The 10.8 mg, 3-month formulation is recommended when Zoladex is used to suppress androgen levels for 2 years (8 injections).

The Zoladex 10.8-mg depot is supplied with a 14-gauge needle. The unit is sterile and comes in a sealed, light- and moisture-proof package. The pack should be stored at approximately 25° C (room temperature). Before being opened, each package must be inspected for damage in which case the syringe must not be used. Being sterile, the syringe should be removed from its package only by the physician/nurse immediately before use.

Zoladex will be injected subcutaneously using an aseptic technique. Insert the needle to its full length, pull it back 1cm, then inject. The manufacturer recommends inserting the needle into the subcutaneous fat then changing the direction of the needle so that it parallels the abdominal wall before inserting the needle to its full length. This will create a little pocket for the Zoladex plug so that it does not extend when the needle is withdrawn. After rechecking to ensure that the depot has been discharged, the used syringe will be discarded in a safe manner. One can ensure that the depot has been discharged by ensuring the tip of the plunger is visible within the tip of the needle.

Toxicity

During routine screening of Zoladex, no significant pharmacological activity was apparent in the cardiovascular, respiratory, central nervous, renal, metabolic, coagulation or gastric acid secretory systems. Studies have shown that serum levels of testosterone can be reduced and maintained within the castrate range resulting in objective evidence of tumor regression. Other than the occasional transient worsening of cancer symptoms (tumor flare) due to an initial temporary rise in testosterone serum levels on initiating therapy, no significant toxicity apart from that attributed to castration (hot flashes, decreased erections, impotence) has been reported. In general, allergic reactions have been extremely uncommon with Zoladex therapy. There have been isolated reports of urethral obstruction, urticaria, or spinal cord compression.

14.4. Casodex (bicalutamide) is a nonsteroidal antiandrogen which has no androgenic or progestational properties. The chemical name is Propanamide, N-[4-cyano- 3(trifluoromethyl)phenyl]- 3- [(4-fluorophenyl)sulphonyl]- 2-hydroxy- 2- methyl, (+,-). Casodex is a racemic mixture with the antiandrogen activity residing exclusively in the (-) or (R) enantiomer. Casodex 50 mg has the status of an approved new drug. Casodex has a long half-life compatible with once-daily dosing. Casodex is well tolerated and has good response rates in phase II trials. Nonpharmacological adverse events, reported in the trial using bicalutamide 50 mg as monotherapy include asthenia, pelvic pain, peripheral edema, pruritus, rash, constipation, impotence, dyspnea, nausea, and pain (76). There has been no observed change in cardiac parameters during long-term administration of bicalutamide 50 mg daily. When bicalutamide 50 mg was given in combination with an LHRH analogue, the LHRH analogue adverse event profile predominated with a high incidence of hot flashes (49%) and relatively low incidences of gynecomastia (4.7%) and breast pain (3.2%), the associated pharmacological effects of bicalutamide monotherapy (76). Bicalutamide or flutamide is recommended during the first month of LHRH agonist treatment.

14.5. Eulexin. (flutamide) is a substituted anilide. It is a fine, light, yellow powder, insoluble in water but soluble in common organic solvents such as aromatic or halogenated hydrocarbons. Its concentration in plasma can be determined by gas chromatography. Flutamide is a non-steroid anti-androgen that is metabolized into a hydroxylated derivative, which effectively competes with the hydrotestosterone for androgen receptor sites. Flutamide is supplied as 125 mg capsules and is commercially available. Flutamide should be stored at temperatures ranging from 20-30° C *(36°-86° F)* and protected from excessive moisture. The drug is administered orally at a dose of two 125 mg capsules three times a day for a total daily dose of 750 mg *(six capsules).* Flutamide or bicalutamide is recommended during the first month of LHRH agonist treatment.

Toxicity

The reported side effects of treatment include diarrhea and anemia. A high percentage of patients treated with flutamide alone developed gynecomastia within 2-8 months. There have been post-marketing reports of hospitalization, and, rarely, death due to liver failure in patients taking flutamide. Evidence of hepatic injury included elevated serum transaminase levels, jaundice, hepatic encephalopathy, and death related to acute hepatic failure. The hepatic injury was reversible after prompt discontinuation of therapy in some patients. Approximately half of the reported cases occurred within the initial 3 months of treatment with flutamide.

15.0 PATHOLOGY/SAMPLE COLLECTION (Modified 7/5/04)

15.1 Pathologic review: The Fox Chase Cancer Center Pathology Department will review all diagnostic tumor biopsies to corroborate reported histology and Gleason score. For patients treated at an outside collaborating institution, the randomization may be made using the outside pathologic review. However, ultimately the slides must be sent to FCCC for confirmation. If a discrepancy is found that would have resulted in a change in treatment, it will be noted. Such errors should be few and should even out over time.

15.2 Serum Estradiol and Sex Hormone Binding Globulin: These serum analyses are collected and sent out for analysis at FCCC for patients who are enrolled there. Billing is made to an account for this purpose, so that the patient's insurance company is not charged. For collaborating institutions, an account will be set up to pay for these tests. The Department of Radiation Oncology will reimburse for these tests through existing grants.

15.3 Archival tissue collection and staining: Drs. Pollack and Al Saleem will coordinate and implement the DNA content (image analysis) and immunohistochemical analyses of Ki-67/MIB-1, p53, bcl-2, bax, MDM2, and PKA on pretreatment diagnostic material. Requests for pretreatment diagnostic archival material will be coordinated by the Department of Radiation Oncology at FCCC through Dr Pollack's office (Lorraine Medoro, 215-728-2940) and laboratory (Paul Hachem, 215-214-1479).

15.4 Post-treatment biopsies: All post-treatment biopsies done at 2 years after treatment for those free of failure (preferably done at FCCC because the biopsies are performed at cost here and reimbursed through an NCI grant) or otherwise for a rising PSA (done outside of FCCC for collaborating institutions as part of standard practice) will be reviewed at FCCC as well. There should be at least 10 regions biopsied, including the standard sextants, the bilateral anterior horns, the bilateral transition zones and at least one central biopsy. Attention should be given to the region of original biopsy positivity. Slides and blocks of samples obtained outside of FCCC should be sent to Dr. Pollack's laboratory at FCCC for review.

15.5 Plasma, serum and urine collection for proteomics and genomics studies (added 7/5/04): Blood will be collected prior to treatment, at 3 months after treatment, at 6 month intervals for 2 years and then yearly for a total of 5 years, using the same schedule as for follow-up as outlined in Section 17.0. Six tubes of approximately 10 cc each will be drawn and processed in accordance with the procedure outlined by the Biosample Repository at FCCC (headed by Dr Andrew Godwin, see Appendix I). Blood will be drawn into two "purple top" EDTA-containing tubes for collecting plasma, two "yellow top" tubes containing 1.5 mls of Acid Citrate Dextrose (ACD) for plasma, erythrocytes, and lymphocytes, and two "red top" tubes for collecting serum. The tubes should be processed within one to four hour following draw (kept at room temperature during this time). Plasma and serum samples will be aliquotted into tubes at 0.5 ml per tube and frozen at -70^{o} C. Lymphocytes ("buffy coats") and erythrocytes will be aliquoted as described in appendix A and stored at –70˚C. Shipping to Fox Chase on dry ice will be coordinated by JoEllen Weaver (215-214-1633; Email: Joellen.weaver@fccc.edu), Biosample Repository (P2011), Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia, PA 19111. The date and time of the blood draw and the time that the aliquotted material is frozen should be recorded. The main purpose of the blood collection is for proteomic studies, but erythrocytes and lymphocytes will also be stored per the biosample repository current practice.

Approximately 50 mL of urine will be collected in a 250 mL widemouth container at the same times as the blood for genomic studies. The amount of the sample, the time it is obtained and the time it is frozen should be recorded. The entire urine specimen will be frozen at -70^{o} C.

16.0 PROCEDURE FOR COLLABORATING INSTITUTIONS (added 7/5/04)

16.1 Radiotherapy planning QA: Submission of sample plans for a case with intermediate and high high risk prostate cancer, planned for treatment with 76 and 70.2 Gy is required. Thus, there are four plans that should be submitted to Dr Price and Dr Pollack for review. If any of the plans are considered unacceptable, then additional plans may be requested, at the discretion of the Principal Investigator.

16.2 After the site has approval for patient accrual, the plans should be electronically submitted to FCCC within 7 days of treatment start. The details of this transfer will be decided by Dr Robert Price).

16.3 Once a patient has signed consent, a call will be placed to a research nurse at FCCC (Teri White, R.N. oversees the protocol) at 215-728-2994 who will coordinate the faxing of the pertinent data (Eligibility Checklist, lab test results, imaging studies) needed for randomization.

16.4 PSA and testosterone are standard prostate cancer tests and are reimbursed through normal mechanisms. However, serum estradiol and sex hormone binding globulin are not standard. An account should be set up at the collaborating institution for the initial payment of these tests; the Department of Radiation Oncology at Fox Chase will then reimburse the institution through an NCI grant.

16.5 The collection of serum, plasma and urine will be performed as described in section 15. Blood products will be shipped overnight on dry ice to JoEllen Weaver (215-214-1633; Email: Joellen.Weaver@fccc.edu; see Appendix I), Biosample Repository (P201), 333 Cottman Ave, Philadlephia, PA 19111 (Marked: RUSH-PERISHABLE BIOLOGIC MATERIAL). Blood should not be drawn on a Friday. The Department of Radiation Oncology will pay for the processing and mailing of these samples. If the collaborating institution is a stand alone radiation oncology facility and does not have the resources for processing this material within 4 hr of collection, the Principal Investigator may waive this requirement.

16.6 Copies of the completed consent and any questionnaires should be sent to Teri White, R.N., Department of Radiation Oncology, 333 Cottman Ave, Philadelphia, PA 19111 within 7 days of receipt.

16.7 Prostate biopsy at 2 years after completion of therapy should be done at FCCC for reimbursement reasons. This is an optional procedure, but is a very important early endpoint. The biopsies are being funded through a grant. It may be possible to contract the biopsies to another institution using the reimbursement rate identified in the Principal Investigator's grant.

16.8 Collaboration between FCCC, Washington University and the University of Miami (Added 3/2/10). Whole blood (200 µl) or buffy coat, stored at -80°C in the Biosample Repository at FCCC will be processed to isolate DNA using the QIAamp Blood Kit (QIAGEN Inc., Chatsworth, CA). UM will supply coded vials and the isolated DNA will be shipped overnight on dry ice to Jennifer Hu, 1511 Clinical Research Bldg, Miami, FL 33136 (305-243-7796; Email: JHu@med.miami.edu), Marked: RUSH-PERISHABLE BIOLOGIC MATERIAL) Dr. Hu was funded with an Instrumentation Grant from the State of Florida to purchase the BeadArray System (Illumina Inc., San Diego, CA). The instrument is currently housed at the UM/Sylvester Oncogenomics Shared Resource, which will provide high-throughput genotyping service. Quality control/assurance will include (1) 4 internal controls in each plate, and (2) the Hardy-Weinberg Equilibrium (HWE) test will be used to identify potentially problematic SNPs. The dosimetry and acute toxicity data of the patients treated on protocol 02-602 will be transferred to Washington University. Full dose-volume histogram dosimetric data, acquired for the clinical target volume (CTV), planning target volume (PTV), bladder, rectum. and femoral heads will be anonymized and formatted. In addition, dosimetric data for the penile bulb and corporal bodies will be made available. Dosimetry data will be combined with tumor-related parameters and SNP analysis results to create a model or models of tumor side effects and health-related quality of life.

17.0 PROTOCOL SCHEDULE

Modified at the request of the DSMB 2/23/03.

			Follow-up			
	Prior to XRT	During XRT	F/U Q 3 Mo	F/U Q 6 Mo	F/U Q Yearly	Other
History & Physical Exam*	X	Wkly	x1	x 2 yr	After 2.25 yr	
PSA[†]	X		x1	Thereafter		
Bone Scan	X[a]					As needed
CT or MRI-Pelvis	X[b]					As needed
Serum-T[c]	X					
SHBG[d]	X					
Serum estradiol[e]	X					
Prostate Biopsy[f]	X					At 2 yrs
EPIC, [g]	X					At 6 mos and 1-5 yrs
IPSS[h]	X		x1	x 2 yr	After 2.25 yr	
Plasma, serum and urine collection[i]	X		x1	x 2 yr	After 2.25 yr	

*Toxicity will be assessed at each scheduled history and physical exam visit. Interval history will be obtained during and following XRT at these visits.

[†]Obtained ≤ 4 weeks of randomization.

[a]Obtained ≤ 4 months of randomization if PSA >10 ng/ml or T3 disease.

[b]Obtained ≤ 4 months of randomization if T3 disease.

[c]Serum testosterone will be drawn as part of routine workup ≤4 months before randomization or after randomization but prior to the first radiation treatment.

[d]Sex hormone binding globulin will be drawn ≤4 months before randomization or after randomization but prior to the first radiation treatment. If the patient was started on androgen ablation prior to protocol enrollment, these tests will not be obtained.

[e]Serum estradiol will be drawn ≤4 months before randomization or after randomization but prior to the first radiation treatment. If the patient was started on androgen ablation prior to protocol enrollment, these tests will not be obtained.

[f]A letter will be sent to retrieve the pretreatment biopsy blocks from the referring institution. In follow-up, prostate biopsy will be obtained at first sign of local failure or a rising PSA, or at 2 years after treatment (after radiotherapy or androgen deprivation – whichever is longer) if no evidence of failure.

[g]At 0.5, 1, 2, 3, 4, and 5 years after the completion of radiotherapy.

[h]International Prostate Symptom Score (Appendix H) will be administered pretreatment, at the end of treatment and at each follow-up visit.

[i] Six tubes of blood and 50 mL of urine will be collected, as described in Sections 15.5 and 17.0, for proteomics and genomics studies.

18.0 GENDER AND MINORITY CONSIDERATIONS

The National Institute of Health Revitalization Act of 1993 specifies that women and minorities be included in clinical research. The goal is for accrual targets to resemble the gender, racial, and

ethnic composition of the U.S. population. Since this is a prostate cancer study, women will not be included. Based on our extensive radiation oncology prostate cancer database at FCCC, we expect 5% of patients on study to be African American (n=15). Clearly, if this is the case there will not be enough minorities for subgroup analysis of the results in minorities. There are mechanisms available that may help to enrich the minority population entered into the trial. The Prostate cancer Risk Assessment Program (PRAP) has been very successful at attracting minorities for screening and advice. Patients in this program will have access to this trial. Also, we are developing a joint research effort with Temple University, which has a substantial minority population. At the present time, Temple University does not have the radiotherapy equipment needed for this trial; however, they plan to have such equipment in 1-2 years. At that point Temple University will be added as a collaborating institution. Thirdly, we plan to promote the trial to minorities in Philadelphia through radio spots, which have been used successfully for the PRAP program.

19.0 DATA AND SAFETY MONITORING BOARD

19.1 Dr Gary Hudes has agreed to chair the data and safety monitoring board (DSMB). As specified in the Fox Chase institutional plan, the board will consist of three clinicians with expertise in genitourinary oncology, a biostatistician and a lay representative. The DSMB will meet at least twice per year to evaluate patient recruitment, randomization, intervention, subject safety, data management, plans for auditing primary subject records, quality control and analysis, and to identify needed modifications.

19.2 Prior to trial initiation the DSMB will review the protocol and consent, and must give approval or recommend changes before beginning patient accrual.

19.3 During the trial patient charts will be reviewed and coded by an individual with experience in this work, who will work independently of any trial collaborators. A database will be formulated, which will allow for patient identification, but which will be kept secure. These data will be available to the study statistician and the statistician on the DSMB. The DSMB will examine the results, with particular emphasis initially on toxicity and early stopping as outlined in section 13.

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21.0 FIGURES

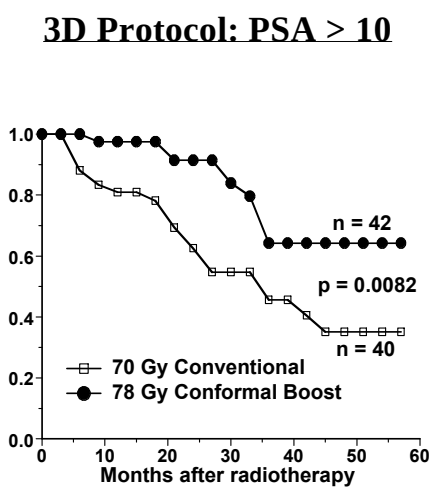


Figure 1. Preliminary results of the MDACC randomized dose escalation trial using freedom from a rising PSA as the endpoint. Minimum follow-up is 1 year (5).

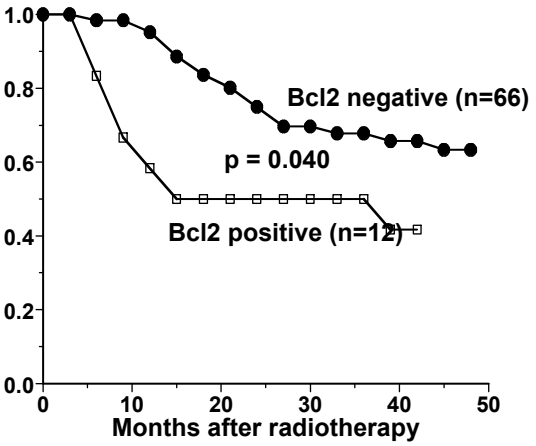


Figure 3. Relationship of bcl-2 staining to freedom from a rising PSA.

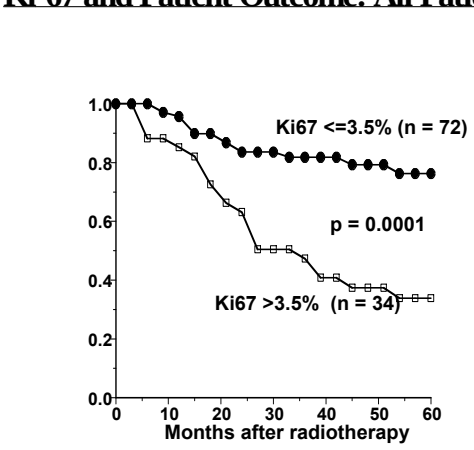


Figure 2. Relationship of Ki-67 staining to freedom from a rising PSA (77).

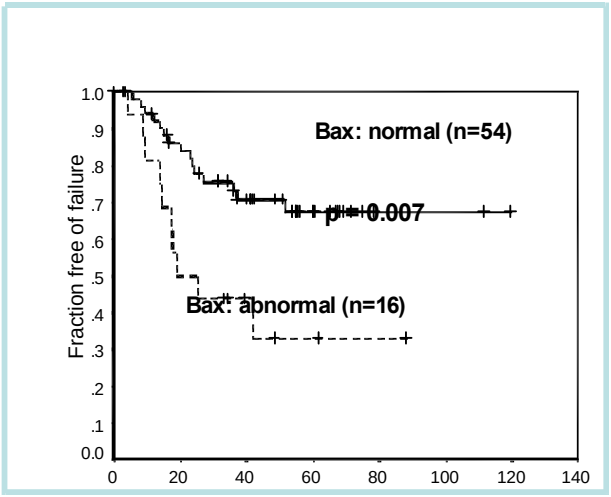


Figure 4. Relationship of Bax staining to freedom from a rising PSA.

Appendix A
EPIC Questionnaire
(Pages 26-39)

Medical Record #_____
Name (optional) _____


EPIC 8.2000.pdf


EPIC Scoring 8-2000.pdf


epicvalrevfinal.pdf

Appendix B

PERFORMANCE STATUS

Zubrod Scale*	
0	Normal activity
1	Symptoms but nearly fully ambulatory
2	Some bed time but needs to be in bed less than 50% of normal daytime.
3	Needs to be in bed more than 50% of normal daytime.
4	Unable to get out of bed.

*Zubrod et al: J. Chronic Dis. 11:7, 1960.

Appendix C

Disease Staging

1992 AJCC Palpable Clinical Staging System

T Stage	Description
T1	**Non-palpable tumor**
T1a	Nonpalpable, 5% or less of TURP-resected tissue with cancer
T1b	Nonpalpable, more than 5% of TURP-resected tissue with cancer
T1c	Nonpalpable, needle-biopsy positive, no TURP
T2	**Tumor palpably confined within the prostate**
T2a	Palpable, size $\leq$ 1/2 lobe
T2b	Palpable, size > 1/2 lobe but $\leq$ 1 lobe
T2c	Palpable, size > 1 lobe
T3	**Tumor palpably extends through the prostatic capsule**
T3a	Palpable, unilateral capsule penetration
T3b	Palpable, bilateral capsule penetration
T3c	Palpable, invading seminal vesicles
T4	**Tumor is fixed or invades adjacent structures other than the seminal vesicles**
T4a	Invasion of bladder neck, external sphincter, or rectum
T4b	Invasion of levator muscles and/or fixation to the pelvic wall

TURP: transurethral resection of prostate.

Appendix D

Acute Radiation Toxicity Grading

	Grade I	Grade II	Grade III	Grade IV
Lower Gastro-intestinal	**Increased frequency or change in quality of bowel habits not needing ≤2 anti-diarrheals/wk medication. Rectal discomfort not requiring analgesics. Mild rectal bleeding not needing medication.**	**Diarrhea needing more than 2 anti-diarrheals/wk. Mucous discharge requiring ≤one sanitary pad per day. Rectal pain needing analgesics or occasional narcotics (=1 pill/day).. Rectal bleeding needing Anusol HC or other medication. Rectal bleeding or other GI symptoms requiring a treatment break ≤1 week.**	**Diarrhea needing >2 anti-diarrheals/day or parenteral support. Severe mucous discharge requiring >1 sanitary pad/day. Rectal pain requiring frequent narcotics (≥2 pill/day) for more than a week. GI bleeding requiring one transfusion. Rectal bleeding or other GI symptoms requiring a treatment break of >1 week.**	**Acute or subacute obstruction. Fistula or perforation. GI bleeding requiring more than one transfusion. Abdominal pain or tenesmus requiring bowel diversion.**
Urinary	**Frequency or nocturia twice pretreatment habit or non-narcotic medication (e.g., alpha blocker) once/day over baseline. Dysuria not needing medication. Microscopic or infrequent gross hematuria not needing medication.**	**Frequency or nocturia less frequent than hourly. Dysuria and/or bladder spasm needing an anesthetic (Pyridium) or occasional narcotics (<=1 pill/day). Hematuria or GU symptoms requiring medication and/or a treatment break ≤1 week. Urinary obstruction requiring temporary catheterization (including foley or self-cath) for ≤1week..**	**Frequency or nocturia hourly or more. Dysuria, pain or spasm needing narcotics >1 dose/day for >1 week. Hematuria or GU symptoms requiring a treatment break of >1 week. Gross hematuria requiring one transfusion. Urinary obstruction requiring catheterization (including foley, self-cath or suprapubic) for >1 week.**	**Hematuria needing more than one transfusion. Hospitalization for sepsis due to obstruction, ulceration, and/or necrosis of the bladder.**

Appendix E

Delayed Radiation Toxicity Grading

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Lower Gastro-intestinal	**Excess bowel movements twice baseline or need for ≤2 anti-diarrheals/wk. Slight rectal discharge or bleeding not requiring pads or medication.**	**More than 2 antidiarrheals/week. Two or fewer coagulations for bleeding. Temporary steroids per suppositories or enema for symptoms/ulceration of ≤1 month. Two or fewer dilatations. Mucous discharge requiring sanitary pads <2/day. Infrequent use of sanitary pads. Non-narcotic or narcotic medication for pain once /day for less than a month. Regular non-narcotic or occasional narcotic for pain.**	**More than two antidiarrheals/day for more than a month. One blood transfusion or more than two coagulations for bleeding. Steroids per suppositories or enema for >1 month. Hyperbaric oxygen treatment for ulceration or bleeding. More than 2 dilations. Sanitary pads of ≥2/day for more than a month. Narcotic use of >once/day for more than a month.**	**Fistula or obstruction requiring surgery. More than one blood transfusion.**	**Fatal toxicity**
Urinary	**Nocturia twice baseline or non-narcotic med (e.g., alpha blocker) once per day increase over baseline. Microscopic hematuria. Light mucosal atrophy and minor telangiectasia. Dysuria not requiring medication. Incontinence or dribbling not requiring sanitary pad (over baseline).**	**Frequency <= once every hour, requiring alpha blocker >once per day increase over baseline. Nocturia >2x baseline Generalized telangiectasias. Macroscopic hematuria requiring two or fewer cauterizations. Dysuria requiring medication: Non-narcotic >1/day or narcotic for pain ≤1/day over baseline. Two or fewer dilations. Foley or Self-cath for ≤2 weeks. Incontinence requiring <=2 sanitary pads (over baseline).**	**Frequency more than once every hour or dysuria requiring narcotics >1 per day. Nocturia more frequent than once every hour. Reduction in bladder capacity (150cc). At least one blood transfusion or >2 cauterizations for bleeding. Narcotic use of >1/day. Hyperbaric oxygen, Foley or self- cath for >2 weeks. Urethrotomy, TURP or more than 2 dilatations. Incontinence requiring >2 sanitary pads (over baseline)**	**Gross hematuria requiring >1 blood transfusion. Severe hemorrhagic cystitis or ulceration requiring urinary diversion and/or cystectomy.**	

APPENDIX F

GUIDELINES FOR REPORTING OF ADVERSE DRUG REACTIONS (ADRs)

Radiation Toxicity Guidelines

1. Phase II and III Studies: Unknown Reaction

   Grades 2-3: Submit a written report within 10 working days to the chairman of the study.

   Grades 4 and 5: Submit a written report within 10 working days to the chairman of the study.

2. Phase II and III Studies: Known Reactions

   Grades 1-3: No report is required, except as part of study results.

   Grades 4 and 5: Submit a written report within 10 working days to the chairman of the study.

   Exception: Grade 4 myelosuppression need only be submitted as part of the study results.

3. All fatal toxicities (Grade 5) resulting from protocol treatment must be reported by telephone to the Study Chairman within 24 hours of discovery.

4. All life-threatening (Grade 4) toxicities results from protocol treatment using non-standard fractionated treatment or pharmaceuticals must be reported by telephone to the Study Chairman within 24 hours of discovery.

5. The IRB will be notified by the Study Chairman of all Grade 4 reactions within 5 days of reporting and all Grade 5 reactions within1 day of reporting.

APPENDIX G
URINARY SYMPTOM SCORE

The American Urologic Association has developed the questionnaire below. Please take a few minutes to fill out the questionnaire as it will aid your physicians in providing the best possible care.

(Please circle your response)	Not at all	Less than 1 in 5 times	Less than half the time	About half the time	More than half the time	Almost always
1. Incomplete Emptying: Over the past month, how often have you had the sensation of not emptying your bladder completely, after you have urinated?	0	1	2	3	4	5
2. Frequency: Over the past month, how often have you had to urinate again in less than two hours after you finished urinating?	0	1	2	3	4	5
3. Intermittency: Over the past month, how often have you found you stopped and started again several times when you urinate?	0	1	2	3	4	5
4. Urgency: Over the past month, how often have you found it difficult to postpone urination?	0	1	2	3	4	5
5. Weak Stream: Over the past month, how often have you had a weak urinary stream?	0	1	2	3	4	5
6. Straining: Over the past month, how often have you had to push to begin urination?	0	1	2	3	4	5
Note: Question 7 is scored differently						
7. Nocturia: Over the past month, **how many times** did you most typically get up to urinate from the time you went to bed at night until the time you got up in the morning?	0	1	2	3	4	5

ADD YOUR TOTAL SCORE: _____

	Delighted	Pleased	Satisfied	Mixed	Dissatisfied	Unhappy	Terrible
Quality of life due to urinary symptoms: If you were to spend the rest of your life with your urinary condition, just the way it is now, how would you feel about that?	0	1	2	3	4	5	6

Patient Signature

Date

Physician Signature

Date

APPENDIX H

Health Questionnaire

English version for the US

From EQ5D group 10-30-02

By placing a checkmark in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

Self-Care

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

Pain/Discomfort

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

Anxiety/Depression

I am not anxious or depressed ❑

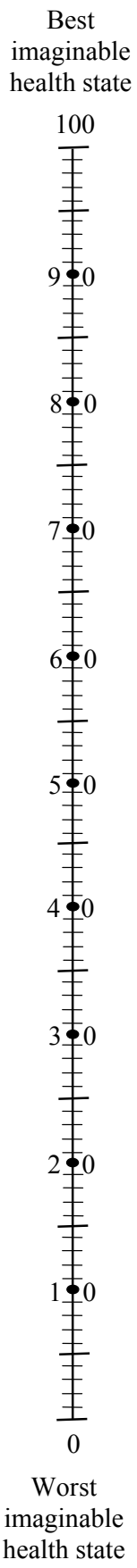
I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

Your own health state today



Because all replies are confidential, it will help us to understand your answers better if we have a little background data from everyone, as covered in the following questions.

1. Have you experienced serious illness?

	Yes	No
in you yourself	❑	❑
in your family	❑	❑
in caring for others	❑	❑

PLEASE CHECK APPROPRIATE BOXES

2. What is your age in years ? ☐

3. Are you:

Male	Female
❑	❑

PLEASE CHECK APPROPRIATE BOX

4. Are you:

a current smoker	❑
an ex-smoker	❑
a never smoker	❑

PLEASE CHECK APPROPRIATE BOX

5. Do you now, or did you ever, work in health or social services?

Yes	No
❑	❑

PLEASE CHECK APPROPRIATE BOX

If so, in what capacity? .....

6. Which of the following best describes your main activity?

employed (including self employment)	❑
retired	❑
keeping house	❑
student	❑
seeking work	❑
other (please specify)	❑ .....

PLEASE CHECK APPROPRIATE BOX

7. What is the highest level of education **you have completed?**

some high school or less	❑
high school graduate or GED	❑
vocational college or some college	❑
college degree	❑
professional or graduate degree	❑

PLEASE CHECK APPROPRIATE BOX

8. If you know your zip code, please write it here ☐

APPENDIX I
Biosample Repository Procedure For Specimen Processing And Shipping

Specimen Processing

Please try to complete all sample processing and freeze samples within 4 hours of obtaining the samples. Use the Specimen Collection Form to document specimen ids associated with each specimen.

Red Top Tubes Processing for Serum:

- Allow the blood to clot by leaving at room temperature for at least 30 minutes.
- Centrifuge tubes at 1,200 x g for 7 – 10 minutes in a clinical centrifuge at room temperature.
- Use a transfer pipet to remove serum, aliquoting 0.5 ml of serum into up to 10 to 15 microcentrifuge tubes.
- Label each microcentrifuge tube with a specimen-specific id.
- Freeze at -70ºC.
- Samples may be batch shipped as discussed subsequently.

Yellow Top (Citrate) Processing:

- Mix the yellow top tube gently and let settle for at least 10 minutes.
- Centrifuge tubes at 1,200 x g for 7 – 10 minutes in a clinical centrifuge at room temperature. After centrifugation, the blood will be separated into three distinct layers: the plasma at the top of the tube, the erythrocytes (red blood cells) at the bottom of the tube, and the lymphocytes or “buffy coat" in a thin white layer between the plasma and red cells.
- The plasma is pulled off using a 5 ml pipette, divided into 4 x 1 ml aliquots (1.8 ml cryovials; Simport, T311-2), and frozen at –70˚C.
- Transfer the buffy coat (the whitish layer of peripheral blood mononuclear cells, lying between the remaining plasma and the top of the red blood cells) to a 15 cc tube.
- Add an equal volume of the freezing medium (recipe below) to the Buffy coat. For example, if the buffy coat volume is 1.5 ml, add 1.5 ml of freezing medium to the tube. Mix gently to resuspend cells in the medium.
- Quickly aliquot the cell suspension into 2 labeled 1.8 ml cryovials, about 1.0 to 1.5 ml per vial. Each vial should be labeled with a specimen-specific id.
- Place the cryovials in a –70°C freezer. Leave at –70°C at least overnight if sample is to be stored in a colder storage site such as LN2.
- Finally the erythrocytes are divided into 2 x 1 ml aliquots and frozen at –70˚C.

Freezing Medium

	For 100 mls of medium:
60% RPMI Medium	60 mls
20% Fetal Bovine Serum	20 mls
20% DMSO (Sigma Catalog # D-5879)	20 mls

Lavender top (EDTA) Processing for Plasma:

- Centrifuge tubes at 1,200 x g for 7 – 10 minutes in a clinical centrifuge at room temperature.
- Taking care not to disturb the buffy coat, use a transfer pipet to remove plasma, aliquoting 1 ml of plasma into up to 10 microcentrifuge tubes.
- Label each microcentrifuge tube with a specimen-specific id.
- Freeze at -70ºC.

Specimen Packing and Shipping Instructions

For shipping use a standard shipping container with 8 pounds of dry ice. Do not permit specimens to thaw. Specimens can be batched shipped (maximum 15 to 30 patients per shipment depending on number of tubes). Group up to 10 tubes in a Ziploc baggie labeled with a biohazard tag. Inside each baggie should be an absorbent pad capable of absorbing the whole volume of fluid in that baggie. These absorbent pads are available from Saf-T-Pak. Specimens should not be shipped until shipping personnel are educated in shipping regulations for biological specimens.

For absorbent material plus any needed education on new regulations concerning shipping human specimens, contact 1-800-SAFTPAK or www.saftpak.com.

Before sending frozen shipments to FCCC, please contact:

JoEllen Weaver, B.S., MT.
Biosample Repository
P2011
Fox Chase Cancer Center
333 Cottman Ave.
Philadelphia, PA 19111
Phone: 215-214-1633
Email: Joellen.Weaver@fccc.edu

Secondary Contact:

Charlette McRoy
Biosample Repository
P2011
Fox Chase Cancer Center
333 Cottman Ave.
Philadelphia, PA 19111
Phone: 215-214-1633
Email: Charlette.McRoy@fccc.edu

APPENDIX J

Gene Expression Profile On Taqman Low Density Array Cards

Num ber	Gene	Assays	Number	Gene	Assays
1	18S		36	IGFBP2	
2	ACTB		37	IGFBP3	
3	AKT1		38	IGFBP4	
4	APAF1		39	IKIP	
5	AR		40	IL2	
6	ATF1		41	IL6	
7	BAD		42	ITGB1	2
8	BAX	2	43	JUN	
9	BBC3		44	MAD2L1	
10	BCL2	3	45	MAP2K2	
11	BCL2L1	2	46	MAPK1	
12	CASP1	3	47	MDM2	3
13	CASP3	3	48	MMP10	
14	CASP8	3	49	MMP7	
15	CASP9	4	50	MYC	
16	CCNA1		51	NFKB1	
17	CCNB1		52	PCNA	
18	CCND1		53	PTEN	
19	CDKN1A		54	PTGS2	
20	CDKN1B		55	RAF1	
21	CDKN2A		56	RAP1A	2
22	E2F1		57	RB1	
23	EGFR		58	SOD2	
24	EGR1		59	STAT1	
25	ERBB2		60	STAT3	2
26	EZH2	2	61	STK6	
27	FOS		62	TIMP3	
28	FRAP1		63	TNF	
29	GAPD		64	TNFRSF6	3
30	HIF1A		65	TNFSF10	2
31	HSPCA		66	TP53	2
32	ICAM1	2	67	TP73	
33	IGF1		68	TPR	
34	IGF1R		69	UBE2C	
35	IGFBP1		70	VCAM1	2
			71	VEGF	
TOTAL			**71 genes**		**96 assays**

APPENDIX K

Example Of Clinical Target Volumes In A High Risk Patient

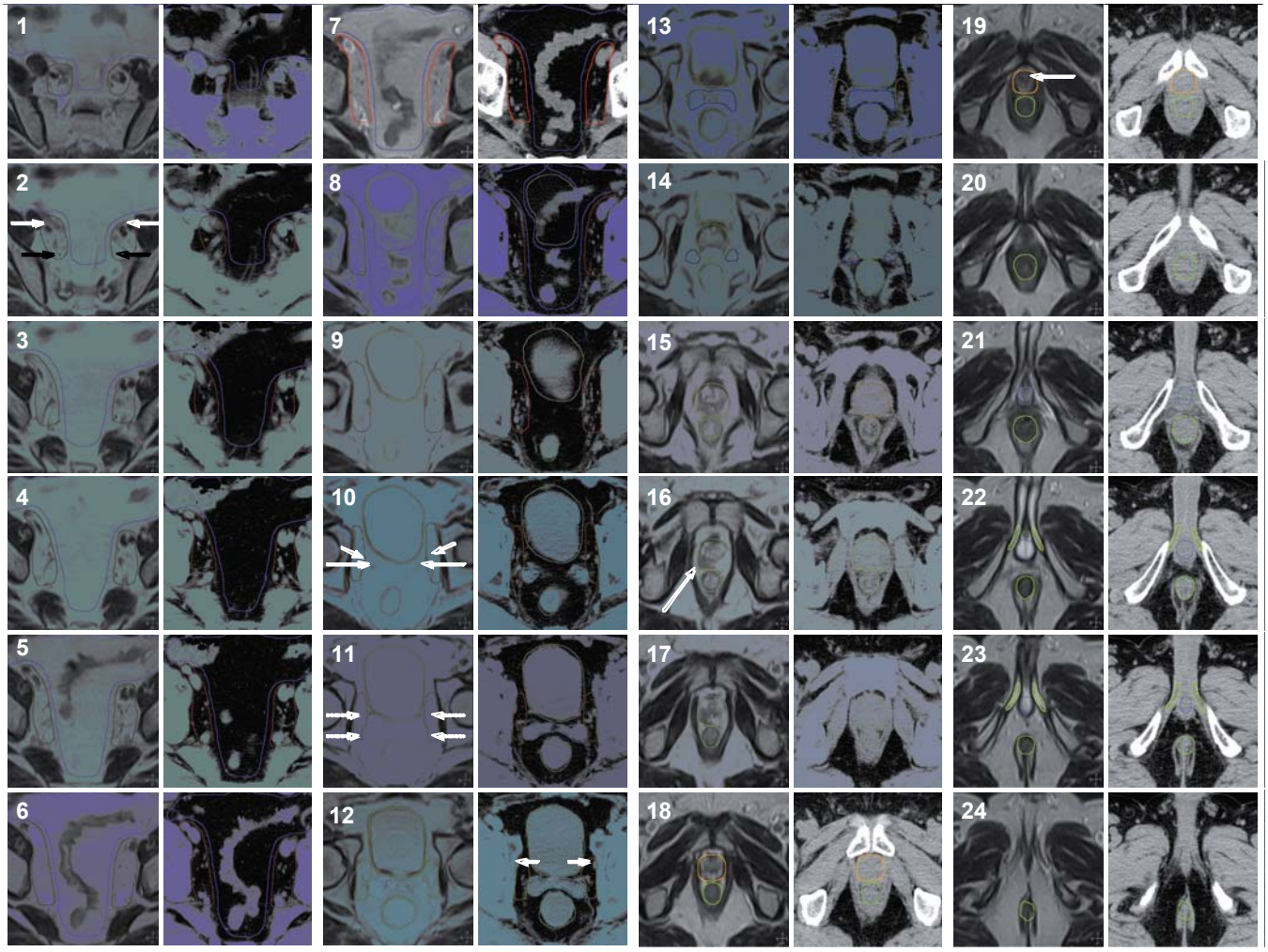


Illustration of the target and normal tissue volumes. MRI and CT images were obtained at 3 mm intervals and fused. Every other image slice (every 6 mm) is displayed. The structures outlined are displayed as follows: urinary bladder, yellow; rectum, dark green; prostate, orange; proximal seminal vesicles, dark blue; distal seminal vesicles, light blue; periprostate lymph nodes, mustard; pelvic lymph nodes, red; bowel (area of potential small bowel and distal colon/proximal sigmoid), purple; penile bulb, royal blue; corporal bodies, light green. The following structures are labeled: external iliac vessels (panel 2, white arrow); internal iliac vessels (panel 2, black arrow); ureter (panel 10, short white arrow); vas deferens (panel 10, long white arrow); vesicoprostatic venous plexus (panel 11, dashed white arrow); obturator vessel (panel 12, short white arrow); intraprostatic mass (panel 16, long white arrow); prostatic apex (panel 19, arrow).

APPENDIX L
DATA SUBMISSION SCHEDULE

FORM TITLE	SUBMISSION SCHEDULE
ON STUDY REGISTRATION	AT TIME OF REGISTRATION/RANDOMIZATION THESE FORMS MUST BE FAXED TO (215-214-3713) ATTN: TERI WHITE/ELAINE CALLAHAN. AFTER RANDOMIZATION, A CONFIRMATION WILL BE FAXED BACK TO YOU WITH AN ASSIGNED PATIENT SEQUENCE NUMBER AND TREATMENT ASSIGNMENT..
ELIGIBILITY CHECKLIST	
ELIGIBILITY VERIFICATION SUPPLEMENTAL SOURCE DOC	
SIGNED PATIENT CONSENT FORM	
ON STUDY - PAST MEDICAL HISTORY ON STUDY - PAST TREATMENT	WITHIN 1 WEEK OF PATIENT REGISTRATION
	TO BE COMPLETED WEEKLY DURING RADIATION THERAPY AND TO BE SUBMITTED AT 1 MONTH FOLLOWING COMPLETION OF RADIATION THERAPY.
FLOW SHEET(S)-TOXICITIES/MEDICAL PROBLEMS	
END OF TREATMENT FORM	SUBMITTED AT 3 MONTHS AFTER COMPLETION OF TREATMENT WITH THE 3 MONTH FOLLOW-UP FORM
FOLLOW-UP FORM	SUBMITTED AT 1 MONTH AFTER EACH FOLLOW-UP: AT 3 MONTHS, THEN EVERY 6 MONTHS X 2 YEARS, THEN ANNUALLY
PROSTATE DATABASE FORM (INITIAL AND FOLLOW-UP)	INITIAL: SUBMITTED AT 1 MONTH OF COMPLETION OF RADIATION THERAPY. FOLLOW-UP: SUBMITTED 1 MONTH AFTER EACH FOLLOW-UP: AT 3 MONTHS, THEN EVERY 6 MONTHS X2 YEARS, THEN ANNUALLY.