Sex-Mismatched Allogeneic Bone Marrow Transplantation for Men with Metastatic Castration-Resistant Prostate Cancer

Protocol Version 5
July 2, 2020
SKCCC Protocol Number: J1608
IRB Number: IRB00086105
NCT02995330

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SYNOPSIS:

Title: *Sex-Mismatched Allogeneic Bone Marrow Transplant for Men with CRPC*

Objective*:* The primary objective of the study is to estimate the percentage of men with castrate-resistant, metastatic prostate cancer who achieve complete biochemical PSA response at 6 months post sex-mismatched haploidentical alloBMT.

Study Design**:** Men with progressive metastatic CRPC post first-line treatment with either ADT alone or ADT plus docetaxel who have an identified related female donor (mother sister, daughter, second degree relative such as granddaughter or niece) or, in those patients without a suitable related female donor, a mismatched unrelated donor who shares at least 5/10 HLA alleles will undergo BMT followed by post-transplant Cytoxan (PT/Cy) and testosterone.

Study Population*:* Men with CRPC with progressive disease (radiographically and/or biochemically) who have progressed on hormone or chemohormonal therapy.

Number of Patients: 20

Inclusion Criteria**:**

1. Performance status ≤1
2. Age ≥18 years and ≤ 75 years old
3. Histologically-confirmed adenocarcinoma of the prostate
4. Maintained on treatment with continuous androgen ablative therapy (either surgical castration or LHRH agonist/antagonist) with documented castrate level of serum testosterone (<50 ng/dl)
5. Metastatic disease radiographically documented by CT or bone scan
6. Patient must be HLA typed at high resolution using DNA based typing at the following loci: HLA-A, -B, -C, and DRB1
7. Patient must have available one or more potential first (biologic mother, sister, half-sister, or daughter) or second-degree related female donor. Mothers and daughters have a 100% chance of being haploidentical matches, sisters a 75% chance of being matched or haploidentical, and second degree relatives have a 50% chance of being haploidentical matches. In those patients without a suitable related female donor, a mismatched unrelated donor who shares at least 5/10 HLA alleles can be utilized. The donor and recipient must be HLA identical for at least one antigen at HLA-A, -B, -C and HLA-DRB1.
8. Screening PSA must be ≥ 1.0 ng/mL.
9. Prior therapy with second line hormonal therapy is allowed (i.e. bicalutamide, nilutamide, flutamide, ketoconazole, abiraterone, enzalutamide, ARN-509).
10. Prior chemotherapy with docetaxel or cabazitaxel is allowed.
11. Cardiac ejection fraction at rest must be ≥ 40%
12. Acceptable liver function: Bilirubin < 2.5 mg/dL (unless due to Gilbert's disease, AST (SGOT) and ALT (SGPT) < 5 times upper limit of normal.
13. Acceptable renal function: Serum creatinine within normal range.
14. Pulmonary function: DLCO (corrected for hemoglobin), FEV1 and FVC >50% predicted.
15. At least 4 wks since prior radiation or surgery with full recovery (no persistent toxicity ≥ Grade 1)
16. Ability to understand and willingness to sign a written informed consent document.

17. Prior therapy with Provenge or 223-Radium (Xofigo) is permitted.

Exclusion Criteria:

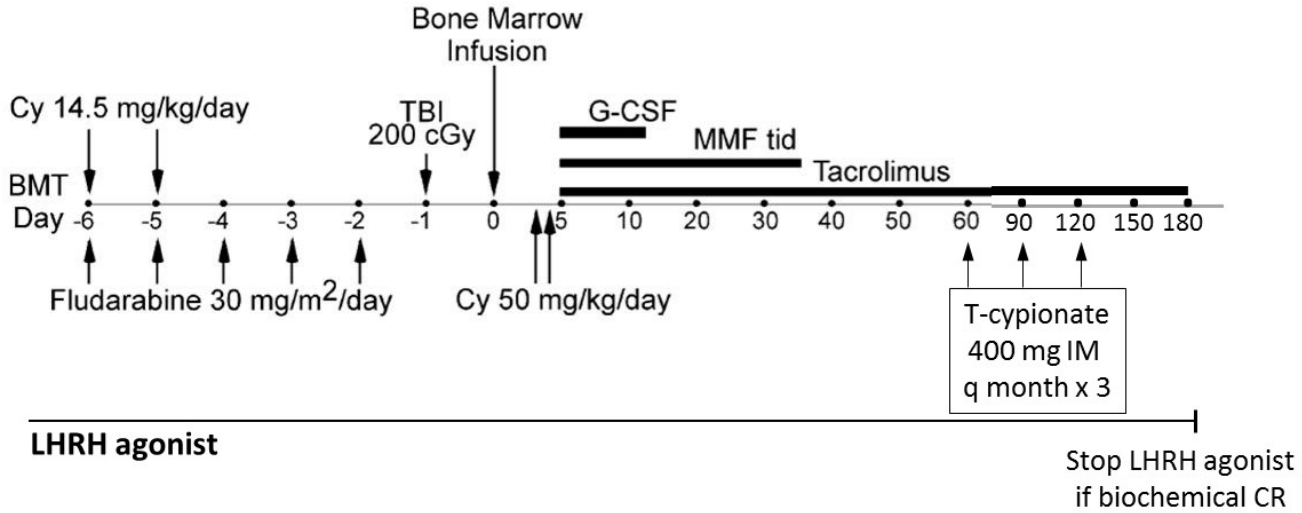
1. Evidence of serious and/or unstable pre-existing medical, psychiatric or other condition (including laboratory abnormalities) that could interfere with patient safety or provision of informed consent to participate in this study
2. Active uncontrolled infection, including known history of HIV/AIDS or hepatitis B or C.
3. Any psychological, familial, sociological, or geographical condition that could potentially interfere with compliance with the study protocol and follow-up schedule.

Treatment Plan: Eligible patients will have metastatic CRPC and will have progressed post-treatment with ADT or ADT plus docetaxel (as per CHAARTED or STAMPEDE studies). Patients are allowed to have had second line hormonal therapy post-ADT. Patients will continue on androgen deprivation therapy with LHRH agonist throughout the duration of the study to inhibit endogenous testosterone production.

Patients will undergo alloBMT according to following treatment scheme:

- The non-myeloablative preparative regimen will consist of: Fludarabine 30 mg/m^2 IV Days -6 -2; Cy 14.5 mg/kg IV Days -6. -5; Total body irradiation (TBI) 200 cGy Day -1
- Day 0 will be the day of infusion on non-T-cell depleted bone marrow
- The GVHD prophylaxis regimen will consist of: Cy 50 mg/kg IV Days 3, 4; Tacrolimus (IV or po) beginning day 5 dose adjusted to maintain a trough level of 5-15 ng/mL through day 180; Mycophenolate mofetil (MMF) 15 mg kg po tid, maximum dose 1 gram tid beginning Day 5 until Day 35
- Supportive care: Filgrastim (G-CSF) 5 mcg/kg/day beginning day 5 until ANC ≥ 1500/mm3
- Maintenance Phase Tumor Antigen stimulation: Patients will be maintained on continuous LHRH agonist to suppress endogenous testosterone production; Testosterone Cypionate 400 mg IM day 60, 90, 120 (every 30 days x 3 doses). Patients who achieve biochemical CR will stop LHRH agonist/antagonist at day 180.

Treatment Design:



Primary Endpoint:

Our ultimate research goal is to develop a therapy that will cure men of prostate cancer. We are proposing this alloBMT strategy as a first step toward this goal. Thus, we have designed this first-in-man pilot study to generate data on the feasibility, safety and efficacy of this alloBMT approach in men with castrate resistant prostate cancer who as a group have a median life expectancy of less than 3 years.

The obvious endpoint for such an approach would be long term overall survival. However, given the constraints of time and money, we will not be able to use this endpoint in this small study. As a surrogate, we have decided that our primary endpoint will be to determine the percentage of men with a complete biochemical response at 6 months post-transplant. We are aware that hitting this endpoint does not necessarily mean that the patient is cured of prostate cancer. However, we know that in men who receive local therapy with either radical prostatectomy or radiation, cure is associated with complete biochemical response and either undetectable PSA in patients post-prostatectomy and PSA <1 ng/mL in patients post-radiation. Patients who are not cured in this setting have detectable and rising PSA as their earliest sign of recurrence. Thus, while achieving undetectable PSA at 6 months post-alloBMT does not necessarily imply cure, not achieving a complete biochemical response clearly means that cure has not been achieved.

Thus, our primary objective is to estimate the biochemical complete response rate at 6 months post-BMT. Biochemical complete response is defined as PSA <0.1 ng/mL for men who are status post-radical prostatectomy and lack a normal prostate and PSA <1.0 ng/mL for men who are status post-radiation therapy who still have a prostate gland.

Secondary Endpoints:

- Incidence of transplant-related mortality (TRM) following alloBMT as described below.
- PSA response rate [Proportion of patients achieving a PSA decline ≥50% according to PCWG2 criteria)
- Objective response rate in patients with measurable disease on CT scan using RECIST criteria
- Radiographic progression-free survival (PFS) based on PCWG2 and modified RECIST.
- Time to PSA progression based on PCWG2 criteria.
- Incidence of acute GVHD grades 2-4 and grades 3-4.
- Incidence of chronic GVHD.
- Incidence of donor chimerism and graft failure.
- Assess the effects of PTCy on the immune reconstitution of T cells, B cells, and NK cells.

Study Duration: Patients will be treated over a 1.5 year period and will be followed for 3 years post BMT.

Statistical Considerations

The primary endpoint is complete biochemical response, defined as a PSA level of <0.1 ng/mL for men post-prostatectomy and <1.0 ng/mL for men post-primary radiation, at 6 months post-BMT. We will estimate the complete biochemical response rate as the proportion of subjects whose PSA level declines below these levels at 6 months post-transplant, along with a 90% confidence interval.

As a secondary endpoint, incidence of TRM will be summarized using a cumulative incidence curve based on competing risks methods. Deaths from any cause without prior disease progression are events; disease progressions are considered as competing events; and losses to follow-up are censored. We will report cause-specific incidence of TRM at 60 days, 100 days, six months, and one year, along with 90% confidence intervals. Additionally, we will estimate PSA response rate as the proportion of patients

achieving a PSA decline ≥50% according to PCWG2 criteria, and objective response rate in patients with measurable disease per RECIST criteria. Time-to-event outcomes (radiographic PFS, and time to PSA progression) will be summarized using Kaplan-Meier analysis. Cumulative incidence of each acute grade 2-4 GVHD, acute grades 3-4 GVHD, and chronic GVHD will be computed using competing risks methods, wherein graft failure, disease progression or death prior to occurrence of GVHD are considered competing risks. The incidence of graft failure following transplant will be reported using a cumulative incidence curve, wherein death prior to graft failure will be considered as a competing event.

Safety will be monitored by a stopping rule for transplant-related mortality (TRM) convincingly greater than 10%, as discussed below. Since patients with severe GVHD and engraftment failure who die will be captured in TRM, we will apply a stopping rule based on incidence of TRM. As outlined in above, the overall transplant-related mortality in multiple nonmyeloablative haploidentical BMT trials utilizing 2 doses of PTCy, MMF and Tacrolimus was approximately 6-10% at day 100.[10,11,23,24] The working hypothesis of this trial is that the overall toxicity of this non-myeloablative BMT in prostate cancer patients is not significantly greater than minihaplo BMT utilizing PTCy Cy for hematologic malignancies, and less than what has been seen with other nonmyeloablative and ablative haploidentical BMT trials that do not utilize PTCy. Consequently a TRM incidence that is convincingly greater than 10% at Day 100 would raise concerns for excessive toxicity and would trigger a referral to DSMB for evaluation. Based on these considerations, we have developed stopping rules to protect patients against toxicity that appears higher than we expect *a priori*. Our stopping rule is to halt accrual any time we feel certain that the risk of 100-day transplant-related mortality is 10% or higher. Specifically, we will apply a Bayesian toxicity monitoring rule that suspends the enrollment if the posterior probability of risk being larger than that threshold is 75% or higher. We assume *a priori* that this regimen has risk around 6% of each event and that there is about a 20% chance that these risks will be 10% or higher. This corresponds to a Beta (1, 14) prior distribution. The following table gives the corresponding stopping rules for the 20 patients. For example, if 2 patients out of the first 2 or 3 experience death prior to disease progression (e.g. death from severe GVHD or engraftment failure) prior to 100 days, we will stop accrual. If the stopping criterion is met, accrual to the trial will be temporarily halted, and the principal investigator and study team will review the toxicity data and recommend either modification or termination of the trial.

Table. Stopping rule for toxicity based on TRM

Stop if:

# patients with 100-day TRM ≥	2	3	4
Out of # patients treated	2 - 3	4 - 11	12 - 20

Sample size consideration: Our primary objective is to determine the preliminary clinical activity of BMT based on complete biochemical response. We plan to enroll a total of 20 patients. If one or more patients achieve complete biochemical responseout of 20 patients, the treatment regimen would be considered promising for further study. If the true CR is 10%, the regimen will be identified as efficacious with probability of 88%.

1. INTRODUCTION

1.1. Overview and Rationale

Every day 600 men are diagnosed with prostate cancer in the United States. The good news for these men is that 70% can be cured by local therapies such as surgery or radiation. The bad news is that for those men whose cancer has spread outside the gland, the disease remains incurable. On this basis, approximately 30,000 American men (80 every day) will die annually from prostate cancer. While prostate cancer is commonly thought of as a disease of old men, 44% of new diagnoses are made in men under age 65 and the median age at diagnosis is 66. In addition, 30% of deaths from prostate cancer occur annually in men under the age of 75.

Considerable progress over the last decade in the treatment of advanced prostate cancer has seen the approval of new hormonal agents (abiraterone, enzalutamide), chemotherapy (docetaxel, cabazitaxel, xofigo) and immunotherapy (Sipuleucel-T). However, each of these agents produces only a modest improvement in survival.(1, 2) Once the disease becomes castrate resistant, survival ranges from 1-3 years in most patients. Despite this broad palette of therapeutic options, cure of the disease has remained elusive.

In contrast to prostate cancer, hematologic malignancies such as leukemia and lymphoma are commonly thought of as diseases that afflict young people. However, SEER data demonstrate that, identical to prostate cancer, the median age of diagnosis is 66 for all types of leukemia and for Non-Hodgkin lymphoma. Like prostate cancer, about 53% of leukemia patients are diagnosed over the age of 65 with 60% of the deaths occurring in patients over 75. Remarkably, the 5-year survival rate from leukemia has almost doubled from 33% in 1975 to 61% in 2006. Similar statistics exist for Non-Hodgkin lymphoma with 55% of cases diagnosed in patients over the age of 65. 5-year survival for this disease has also substantially improved over the last 30 years from 46% in 1975 to 70% in 2006. Finally, while the cure rate for advanced prostate cancer is 0%, the cure rate for leukemia and Non-Hodgkin lymphoma ranges from 20-70% depending on the diagnosis and relevant risk factors.

	Median Age at Diagnosis	Median Age of Death	Cases <65 yo (% total)	Yearly Deaths Men	Deaths by age (% total) 45-74	Deaths by age (% total) ≥75	Cure Rate Advanced Disease
Prostate Cancer	66	80	102,520 (44%)	29,480	8872 (30%)	20608 (70%)	0%
Leukemia	66	75	24,618 (47%)	14,040	5756 (41%)	7581 (54%)	20-70%
NH-Lymphoma	66	76	32,214 (45%)	11,530	4958 (43%)	6111 (53%)	30-70%

Data source: SEER Cancer Stat Fact Sheets http://seer.cancer.gov/

Many patients with leukemia and lymphoma can be cured with intensive combination chemotherapy. For patients who are not cured and relapse after chemotherapy or autologous bone marrow transplant and those who have poor-risk factors, the only potential curative treatment is allogeneic blood or bone marrow transplantation (BMT). Initially, autologous and allogeneic BMT were developed as modalities to rescue patients after administration of high dose chemotherapy had wiped out their native bone marrow. This approach was used for hematologic malignancies and later expanded to treat solid tumors such as breast cancer. However, over time it became evident that for allogeneic BMT, the major therapeutic effect was not from the intensive chemotherapy but was instead due to the ability of the donor immune system to eliminate tumor cells in the recipient. This process, known as the Graft-vs-Tumor (GVT) effect, is primarily the result of differential expression of minor histocompatibility (H) and tumor neoantigens on malignant cells of the recipient that are recognized as foreign by donor T-cells. Since these minor H antigens are also expressed by normal tissue in the recipient, they also are responsible for Graft-vs-Host disease (GVHD) which is the major side effect of allogeneic BMT. Some of the most immunogenic of these minor H antigens are the H-Y antigens encoded by the Y-chromosome which account for increased rates GVHD and decreased risk of relapse that are observed when male recipients receive allogeneic BMT from female donors.(3)

The ability to achieve long lasting remissions/cures in patients with relapsed and poor-risk hematologic malignancies has led us to ask the question- Why not use allogeneic BMT as a treatment for prostate cancer? In the past, major arguments against this approach would include: a) the older age of prostate cancer patients that would increase risk of early transplant related death due to the high toxicity of the myeloablative conditioning regimen, b) the lack of a histocompatible donor for most patients and c) the high cost of transplant.

This last argument may be less relevant today given the high cost of new therapies for prostate cancer. Thus, the average cost of allogeneic BMT at Hopkins, $150,000, is a bargain compared to the cost of standard treatments given to a patient with recurrent metastatic prostate cancer (e.g. 5 years of Lupron therapy ($20,000), 6 mos abiraterone ($30,000), 6 mos enzalutamide ($42,000), sipuleucel-T ($93,000), Xofigo course ($80,000), 10 cycles generic docetaxel ($15,000), Cabazitaxel ($35,000), 12 mos denosumab ($12,000). Additionally, non-myeloablative conditioning regimens have been developed that allow for donor engraftment while limiting non-specific toxicity to the normal host tissues due to GVHD and high dose chemotherapy.(4) Non-myeloablative conditioning has also made it possible to safely expand use of BMT to patients up to the age of 75 and even beyond.(5) Finally, the use of high dose post-transplantation cyclophosphamide (PTCy) that was first developed by members of our team has made it possible to perform partially HLA-mismatched, or haploidentical (haplo) BMT without excessive risk GVHD or transplant-related mortality (TRM).(6) In fact, both single institution data from our group and others, as well as registry data from the CIBMTR demonstrate that haploBMT with PTCy produces similar results to those seen with matched donors. Even in patients in their 70s, non-myeloablative haplo BMT is associated with a TRM that is < 10%. The use of this strategy has now made it possible to find donors for nearly all patients.

Allogeneic BMT historically has played a limited role in the treatment of solid tumors, largely because of limited efficacy due to poor major and minor HLA antigen expression by solid tumors.(7)However, based on the exciting developments in allogeneic BMT already discussed, we believe that it is now possible to consider the use of allogeneic BMT as a treatment for therapy resistant prostate cancer.(8, 9) Moreover, there are unique features of prostate cancer that could make it an amenable to treatment with allogeneic BMT. Clinical and animal studies have identified donor CD8+ cytotoxic T cells (CTL) and CD4+ helper T cells (Th) as the primary mediators of GVHD and GVT responses after allogeneic BMT. CD8+ and CD4+ T cells recognize antigenic peptides displayed on the surface of target cells bound to class I or class II MHC molecules, respectively. A major goal for allogeneic BMT is to develop a strategy to separate GVT from GVHD. This can be achieved by identifying peptides that are differentially expressed by malignant cancer cells and tissues that are a target of GVHD. For prostate cancer there are several broad categories of proteins that may give rise to antigens that could be targets of a selective GVT response. These include a) tumor-specific proteins resulting from chromosome translocations (e.g. TMPRSS2-ERG), alternative RNA splicing (e.g. AR-V7, PSA, etc.) or mutations (e.g. AR, BRCA1/2, SPOP, etc.) b) tissue specific proteins that are overexpressed in normal and malignant prostate cells such as PSA, KLK2, PAP, STEAP, PSCA, PSMA and c) minor H antigens that are selectively expressed in recipient cells, including prostate cancer cells, but not in donor cells. For prostate cancer the GVT effect could be further selectively enhanced through use of a female donor whose immune system should not be tolerant of antigens she lacks, such as prostate-specific peptides and H-Y minor H antigens.(10) Finally, it could be possible to administer testosterone to men post-BMT to rapidly and selectively stimulate overexpression of androgen regulated prostate cancer antigens such as PSA, STEAP, PAP, etc. whose expression is greatly decreased or turned off under castrate conditions pre-BMT. Epigenetic modifiers such as azacitidine may further upregulated prostate cancer-specific antigens as is being currently studied in an active PCF grant at Hopkins. Finally, the new non-tolerant allogeneic immune system should be the optimal allow post-transplant immunologic strategies to be optimally effective.

Therefore, our hypothesis is that sex-mismatched (female into male) allogeneic non-myeloablative haploidentical BMT could induce prolonged remission in men with castrate resistant prostate cancer that is resistant to standard hormonal and chemotherapy. Moreover, this is an ideal setting to study post-transplant maintenance approaches to further chance the anti-tumor activity of the approach. We are performing a first-in-man pilot study to test the feasibility, safety and efficacy of this treatment strategy as a first step towards developing more effective therapy that may someday lead to a cure for men with advanced prostate cancer.

1.2. Related Haplo BMT with PTCy

Although alloBMT is a potentially curative treatment for a variety of hematologic malignancies and nonmalignant hematologic disorders, the procedure has two major limitations. The first relates to the toxicity that includes the toxicity of the transplant conditioning regimen used on the patient, GVHD and post-transplant infection. The second limitation has been the lack of histocompatible donors. Unfortunately, only about a third of candidates for alloBMT have HLA-matched siblings. For patients who lack HLA-matched siblings, there are 3 alternative sources of stem cells for alloBMT: (1) volunteer matched unrelated donors, (2) umbilical cord blood, and (3) HLA-mismatched, or haploidentical donors.

Related HLA-haplo BMT refers to the situation where a donor and recipient have inherited the same set of HLA-alleles on one chromosome 6, but differ at one or more HLA loci on the unshared chromosome 6. Because any patient shares exactly one HLA haplotype with each biologic parent or child half of siblings, as well as half of second degree relatives (first cousins, nieces, nephews, grandchildren), an eligible HLA-haploidentical donor can be identified rapidly in nearly all cases. The fundamental clinical obstacle to crossing the HLA barrier in allogeneic BMT arises from intense, bi-directional responses from host and donor T cells responding to allogeneic HLA molecules resulting in unacceptably high incidences of graft rejection or GVHD. The risk of severe GVHD may be reduced in intensively conditioned recipients of grafts that have been rigorously depleted of mature T cells or selectively depleted of alloreactive T cells, but the risks of serious infection and death from prolonged immune compromise in these patients remain high; moreover, graft failure and relapse rates are also increased. Historically, the results of HLA-haplo allogeneic BMT, obtained over three decades, illustrate that T cell alloreactivity is the major barrier to success. If donor T cells are left in the graft unaltered, then morbidity and mortality from GVHD is unacceptably high with severe GVHD and mortality rates exceeding 50% (11). If donor T cells are removed from the graft, then graft failure, infections, and relapses were too frequent, similarly leading to very poor outcomes.

To solve this problem, our group at Hopkins, led by Drs. Luznik, Fuchs and Jones pioneered the use of high-dose PTCy as a highly effective approach for crossing the HLA barrier in alloBMT.(4, 5) Cy is a highly immunosuppressive antineoplastic agent that has an established role in conditioning for alloBMT. Typically, the drug is administered prior to BMT to prevent graft rejection by suppressing the host immune system. In contrast, administration of a properly timed, high dose of Cy after BMT induces inhibits both graft rejection and GVHD(12). In light of this observation, the group conducted a Phase I/II trial of high-dose PTCy after nonmyeloablative conditioning and transplantation of non-T cell-depleted, related HLA-haploidentical marrow for patients with poor risk hematologic malignancies and nonmalignant hematologic disorders.(6) That trial demonstrated that partially HLA-mismatched bone marrow can engraft rapidly and stably after nonmyeloablative conditioning and PTCy. Donor hematopoietic stem cells are resistant to even high doses of Cy because they highly express aldehyde dehydrogenase 1 (ALDH1), which is the major mechanism Cy inactivation(13). Luznik et al found that the combination of 2 doses of PTCy early after BMT (50 mg/kg x on days 3 and 4) combined with tacrolimus and MMF limited grade 3-4 acute GVHD and extensive chronic GVHD. to < 10% of patients, a striking finding that appeared even better than results seen with matched sibling donors These data suggested that PTCy would for the first time allow transplantation across HLA barriers.

More recently, McCurdy et al retrospectively evaluated 372 consecutive patients received non-myeloablative related HLA-haploidentical BMT with high dose PTCy at Johns Hopkins between 2002 and 2012.(4) The 6 month probability of TRM across all disease types was 8% which was at least comparable to that seen after HLA-matched BMT. The incidences of severe acute (4%) and any chronic GVHD (13%) were very low. In this study, 35% of the patients were over the age of 60 and age was not found to increase risk of NRM or GVHD. Survival rates across disease types with HLA-haploidentical BMT with PTCy were also similar to those seen with HLA-matched BMT. Overall, these results demonstrate the favorable safety profile associated with this BMT approach. In a second retrospective study, our group performed a retrospective analysis of 271 consecutive hematologic malignancy patients aged 50-75 years who underwent non-myeloablative related HLA-haploidentical BMT with high dose PT/Cy.(5) In this analysis, median age was 61

years with 27 patients aged 70-75. The 6 month probability of grade 3-4 acute AVHD was 3% and TRM was 8%. Patients in their 50s, 60s and 70s had 6-month TRM probabilities of 8%, 9% and 7% respectively. No statistically significant associations were found between older age (relative to ages 50-59 or as a continuous variable) and TRM, relapse, or survival. These results further document the safety of this approach and support the conclusion that haploidentical alloBMT can be considered in older patients.

1.3. Mismatched Unrelated Haplo BMT with PTCy

While the haploidentical alloBMT strategy has greatly expanded the potential pool of eligible donors for most transplant patients, there are still a subset of patient who lack HLA-matched, related haploidentical options. This becomes even more an issue when limiting donors to females as we are in this protocol. In addition to expanding the pool of related donors, the haplo BMT with PTCy can also expand the potential pool of unrelated donors. Barriers to partially HLA-mismatched, unrelated donor (mMUD) BMT include excess graft-versus-host disease (GVHD), graft failure, and death. We prospectively studied nonmyeloablative (NMA) mMUD BMT with high-dose post-transplantation cyclophosphamide (PTCy) for patients with hematologic malignancies (23). Three transplants were performed with busulfan/fludarabine conditioning, with subsequent change to our standard nonmyeloablative conditioning consisting of fludarabine/Cy/total body irradiation (flu/Cy/TBI). Twenty mMUD transplants were reported using flu/Cy/TBI, T-cell replete bone marrow grafts, and PTCy, mycophenolate mofetil, and sirolimus or tacrolimus (1 patient) for GVHD prophylaxis. The median patient age was 56. Of these unrelated grafts, 45%had ≥ 2 mismatched HLA loci, 25% had ≥3 mismatched loci, and 50%had HLA-C mismatches. No graft failure or grades 3-4 acute GVHD occurred in this study. The median times to neutrophil recovery (≥500/mL) and platelet recovery (≥20 000/mL) were 19 days and 31 days, respectively. Full-donor chimerism was achieved in 95% of evaluable patients by day 60. The 180-day probability of grades 2-4 acute GVHD (all grade 2) was 25%, and the 1-year probability of any chronic GVHD was 16% (none severe). The 2-year nonrelapse mortality probability was 6%. With 4-year median follow-up, the 1-year progression-free and overall survival probabilities were 65%and 75%, respectively. These results are essentially the same as those following nonmyeloablative related haplo BMT described above, Thus, ,nonmyeloablative, T-cell replete mMUD BMT is a viable option for patients without other suitable donors. This trial was registered at www.clinicaltrials.gov as #NCT01203722 (23).

1.4. Induction of Immunotolerance with PTCy

Along with controlling haploidentical alloreactivity, PTCy is associated with excellent immune reconstitution and a low incidence of severe opportunistic infections.(6, 14) The timing of PTCy administration appears to contribute to the drug's selectivity toward alloreactive T cells.(12) Early after allogeneic BMT, both donor and host alloreactive T cells are maximally activated and proliferative, whereas T cells specific for infectious agents are quiescent and thus less sensitive to Cy-mediated cytotoxicity. Recent data demonstrate that memory lymphocytes, including memory Tregs, like other cells with substantial proliferative capacity, also highly express ALDH1 and thus are relatively resistant to Cy;(15) these Cy-resistant cells undoubtedly also contribute to the favorable immune reconstitution seen after PTCy.

1.5. Sex-Mismatched Donor and role of H-Y Minor Histocompatibility Antigens in Graft vs Tumor Effect

It has long been recognized in allogeneic BMT that grafts from female donors into male recipients lead to increased rates of GVHD.(16, 17) GVHD is associated with alloimmunity that occurs when donor lymphocytes target H antigens on recipient tissue.(18, 19) Since GVHD occurs even with matched donors,

minor H antigens (mHAs) are clearly a target of alloreactivity. mHA are peptides which, when presented in HLA class I and class II proteins, are able to elicit an adaptive immune response. Perhaps the best characterize class of mHAs are the H-Y antigens encoded on the Y-chromosome. H-Y proteins are highly expressed throughout the body. H-Y proteins that have been identified as mHAs include those expressed by DBY, UTY, ZFY SMCY and DFFRY.(20) Some of the H-Y proteins have considerable similarity with homologous H-X proteins on the X-chromosome while others are specific to the Y chromosome. There are distinct regions of these H-Y antigens that are highly immunogenic and these antigens appear to be the important mediators of GVHD in sex-mismatched transplant. However, H-Y antigens are also present on malignant cells leading to a beneficial GVT effect leading to a lower relapse in male recipients of female allografts.

While H-Y antigens were first described as T-cell-specific targets, H-Y antigens have been shown to elicit a coordinated B-cell and T-cell response. Miklos et al studied 150 patients using H-Y ELISA and found that H-Y antibodies are ten times more frequent (50%) in male recipients of female allografts compared to male recipients of male allografts (5 %).(3) Subsequent studies demonstrated that H-Y antibodies to one of five H-Y proteins (DBY, UTY, ZFY, RPS4Y, and EIF1AY) detected a year post-transplant was associated with both chronic GVHD and long-term disease remission. In this study, none of H-Y seropositive patients relapsed compared to 48% relapse in H-Y seronegative patients. Thus, the development of H-Y seropositivity could serve as a biomarker to identify patients who might have long term response.

While H-Y antigens are expressed on many normal tissues, H-Y expression on prostate cancer cells could be critical to induction of a graft-vs-prostate (GVP) effect. Previously Lau et al evaluated Y chromosome genes identified by positional cloning for expression in normal and malignant prostate samples.(10)Results from this expression analysis of 31 of the 33 genes, isolated so far from the Y chromosome, revealed three types of expression patterns: i) specific expression in other tissues (e.g., AMELY, BPY1, BPY2, CDY, and RBM); ii) ubiquitous expression among prostate and control testis samples, similar to those of house-keeping genes [e.g., ANT3, XE7,ASMTL, IL3RA, SYBL1, TRAMP, MIC2, DBY (DDX3Y), RPS4Y, and SMCY (KDM5D)]; iii) differential expression in prostate compared to testis samples. The last group included X-Y homologous [e.g., ZFY, PRKY, DFFRY (USP9Y), TB4Y, EIF1AY, and UTY] and Y-specific genes (e.g., SRY, TSPY, PRY, and XKRY). All of the known H-Y antigen mHA were expressed by prostate cancer samples in this study including three of the differentially expressed genes [ZFY, DFFRY (USP9Y) and UTY]. Additionally, this group determined that expression of a subset of these genes was under androgen regulation in LNCaP cells. These results suggest the potential for an enhanced GVP effect following sex-mismatched alloBMT in men with prostate cancer. This GVP effect could potentially be augmented by administration of testosterone to upregulate H-Y antigen and androgen regulated prostate cancer antigens post-transplant in the castrated patient.(21, 22)

2. STUDY OBJECTIVES

2.1. Primary Objectives

Estimate the percentage of patients with complete biochemical response at 6 months post-transplant (PSA <0.1 ng/mL for patients post-prostatectomy and PSA < 1 ng/mL for patients post-radiation therapy)

2.2. Secondary Objectives

- Estimate the incidence of transplant-related mortality (TRM) following alloBMT

- Estimate PSA response rate [Proportion of patients achieving a PSA decline ≥ 50% according to Prostate Cancer Working Group (PCWG2) criteria]
- Estimate Objective response rate in patients with measurable disease on CT scan using RECIST criteria
- Estimate Time to PSA progression based on PCWG2 criteria
- Estimate Time to clinical/radiographic progression based upon PCWG2 and RECIST criteria
- Assess incidence of acute graft versus host disease grades 2-4 and grades 3-4
- Assess incidence of chronic GVHD
- Assess incidence of donor chimerism and graft failure
- Assess the effects of post-transplantation cyclophosphamide on the immune reconstitution of T cells, B cells, and NK cells.
- Evaluate incidence of HLA specific antibodies developed after HLA mismatched, allogeneic partially HLA-mismatched bone marrow transplantation

3. PATIENT POPULATION AND SELECTION

Eligible patients will have with metastatic castration-resistant prostate cancer with progression after androgen deprivation therapy and second line hormonal therapy. Prior docetaxel for castration-sensitive disease is allowed.Patients will continue on ADT with LHRH agonist (i.e. Zoladex, Trelstar, Eligard or Lupron) or LHRH antagonist (Degarelix) if not surgically castrated throughout the duration of the study. A total of 20 patients will be recruited.

3.1. Inclusion Criteria.

3.1.1 Performance status ≤1

3.1.2 Age ≥18 years and ≤ 75 years old

3.1.3 Histologically-confirmed adenocarcinoma of the prostate

3.1.4 Treated with continuous androgen ablative therapy (either surgical castration or LHRH agonist/antagonist)with documented castrate level of serum testosterone (<50 ng/dl)

3.1.5 Metastatic disease radiographically documented by CT or bone scan

3.1.6 Patient must be HLA typed at high resolution using DNA based typing at the following loci: HLA-A, -B, -C, and DRB1

3.1.7 Patient must have available one or more potential first (biologic mother, sister, half-sister, or daughter) or second-degree related female donor. Mothers and daughters have a 100% chance of being haploidentical matches, sisters a 75% chance of being matched or haploidentical, and second degree relatives have a 50% chance of being haploidentical matches. In those patients without a suitable related female donor, a matched or mismatched unrelated donor who shares at least 5/10 HLA alleles can be utilized. The donor and recipient must be HLA identical for at least one antigen at HLA-A, -B, -C and HLA-DRB1.

3.1.8 Screening PSA must be ≥ 1.0 ng/mL.

3.1.9 Prior therapy with second line hormonal therapy is allowed (i.e. bicalutamide, nilutamide, flutamide, ketoconazole, abiraterone, enzalutamide, ARN-509).

3.1.10 Prior chemotherapy with docetaxel or cabazitaxel is allowed.

3.1.11 Cardiac ejection fraction at rest must be ≥ 40%

3.1.12 Acceptable liver function: Bilirubin < 2.5 mg/dL (unless due to Gilbert's disease, AST (SGOT) and ALT (SGPT) < 5 times ULN.

3.1.13 Acceptable renal function:Serum creatinine within normal range.

3.1.14 Pulmonary function: DLCO (corrected for hemoglobin), FEV1 and FVC >50% predicted.

3.1.15 At least 4 wks since prior radiation or surgery with full recovery (no persistent toxicity ≥ Grade 1)

3.1.16 Ability to understand and willingness to sign a written informed consent document.

3.2. Exclusion Criteria

3.2.1. Evidence of serious and/or unstable pre-existing medical, psychiatric or other condition (including laboratory abnormalities) that could interfere with patient safety or provision of informed consent to participate in this study

3.2.2. Active uncontrolled infection, including known history of HIV/AIDS or hepatitis B or C.

3.2.3. Any psychological, familial, sociological, or geographical condition that could potentially interfere with compliance with the study protocol and follow-up schedule.

3.3 Inclusion of women and minorities

This study is focused on prostate cancer, therefore is applicable to men only. Women and children will not be included on this study as patients. Men from all ethnic and race groups are eligible for this study.Women are included as bone marrow donors based on the study design.

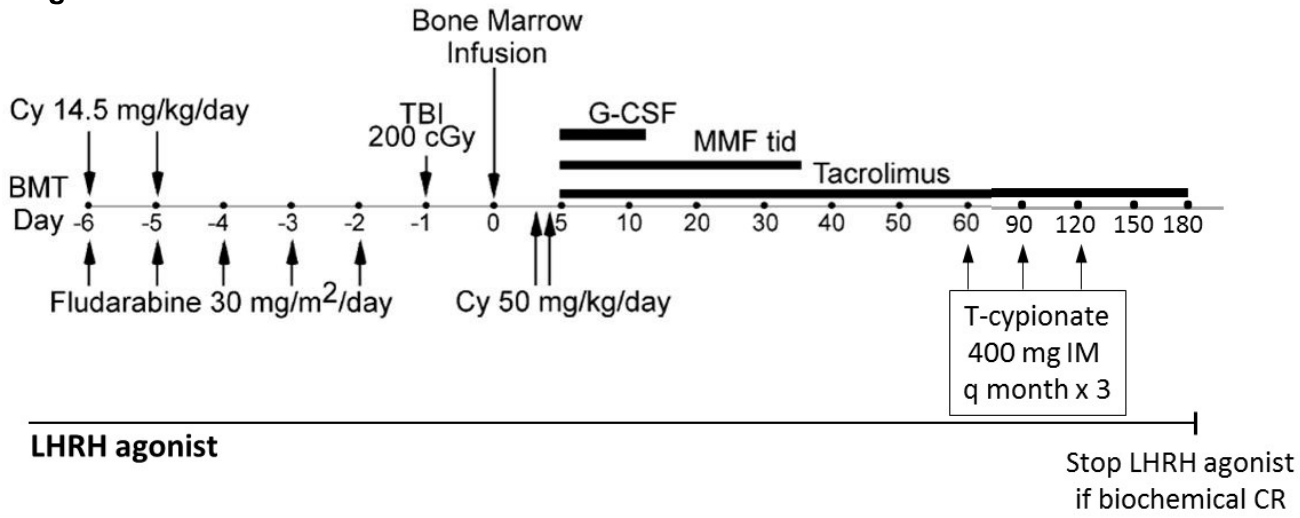
4. TREATMENT PLAN

4.1. Study Design

Men will undergo pre-transplant screening evaluation and be enrolled in the study. Subjects will be treated with a standard non-myeloablative conditioning regimen consisting of Fludarabine 30 mg/m^2 IV Days -6 to -2; Cy 14.5 mg/kg IV Days -6 and -5; Total body irradiation (TBI) 200 cGy Day -1. On Day 0, patients will be infused with non-T-cell depleted bone marrow from a related female donor. Patients will receive GVHD prophylaxis consisting of: Cy 50mg/kg IV on Days +3 and +4; tacrolimus (IV or PO) beginning on Day +5 [dose adjusted to maintain trough level of 5-15 ng/mL] through day+180; Mycophenolate mofetil (MMF) 15 mg/kg PO TID, with a maximum dose of 1g TID beginning on Day +5 through Day +35. Patients will receive filgrastim (G-CSF) 5 mcg/kg/day beginning on Day +5 and continued until ANC ≥ 1500/mm3. Lastly, to produce maintenance tumor antigen stimulation, patients will be maintained on continuous LHRH agonist/antagonist therapy (if not previously surgically castrated) to suppress endogenous testosterone

production throughout the treatment period; testosterone cypionate 400 mg IM will be administered on Day +60, +90, and +120 (every 30 days x 3 doses). Patients who achieve biochemical CR will stop LHRH agonist/antagonist treatmentat day 180. Patients will be followed for 3 years post-BMT.

Study Design Schematic



4.2. Study Treatments

4.2.1. Indwelling central venous catheter.

Placement of a double lumen central venous catheter will be required for administration of IV medications and transfusion of blood products.

4.2.2. Pre-treatment Evaluation

All patients will require documentation of a detailed history and physical examination and standard evaluation of cardiac, pulmonary, liver and renal function.

4.2.3. Preparative regimen

Fludarabine: administered as an IV infusion over 30 minutes on D-6 to D-2. The dose will be 30 mg/m2/dose (adjusted for renal function). The body surface area (BSA) for fludarabine dosing is based on actual body weight.

Pre-transplantation Cyclophosphamide: Cy 14.5mg/kg/day is administered as an IV infusion over 1- 2 hours, (dependingon volume) on Day -7 and -6. Note: Hydration and Mesna will be utilized for the Day 3 and Day 4 post BMT cyclophosphamide doses, not for the pre-BMT cyclophosphamide doses as per institutional standards.

Total body irradiation: 200 cGy AP/PA with 4MV or 6MV photons at 8-12 cGy/min at the point of prescription (average separation of measurements at mediastinum, abdomen, and hips) will be administered in a single fraction on day -1.

Day of rest: A day of rest, i.e. after preparative regimen completion and prior to bone marrow infusion, is not routinely scheduled. Up to one day of rest in-between TBI and the infusion of bone

marrow may be added in this window based on logistical considerations or clinically as indicated.

4.2.4. Bone marrow transplantation

Bone Marrow will be harvested and infused on day 0. Institutional guidelines for the infusion of bone marrow (i.e. major or minor ABO incompatible bone marrow, etc.) will be followed. The marrow infusion will be done by designated members of the BMT team. The bone marrow graft will not be manipulated to deplete T cells. The donor will be harvested with a target yield of 4 x 10^8nucleated cells/kg recipient IBW. The lowest acceptable yield is 1.5 x 10^8nucleated cells/kg. The CD 34_+, $CD4_+$, $CD8_+$, and $CD3_+$ cell count in the marrow will be quantified by flow cytometry.

4.2.5. GVHD Prophylaxis

Post-transplantation Cyclophosphamide: Cyclophosphamide 50mg/kg will be given on Day +3 post-transplant (within 48-72 hr of marrow infusion) and on Day +4 post-transplant. Cyclophosphamide will be given as an IV infusion over 1- 2 hours (depending on volume). Patients will be instructed to increase fluids overnight before cyclophosphamide administration. Hydration with normal saline at 3 mL/kg/hr IV will be started 8 hr prior to cyclophosphamide, then the rate will be reduced to 2 mL/kg/hr for 1 hr pre-cyclophosphamide and continued for at least 8 hr post-cyclophosphamide or administered per institutional standards. Mesna will be given in divided doses IV 30 min pre- and at 3, 6, and 8 hours post-cyclophosphamide or administered per institutional standards. Mesna dose will be based on the cyclophosphamide dose being given. The total daily dose of mesna is equal to 80% of the total daily dose of cyclophosphamide. **It is crucial that no immunosuppressive agents are given until 24 hours after the completion of the post-transplant Cy. This includes steroids as anti-emetics**.

Mycophenolic acid mofetil (MMF): MMF will be given at a dose of 15 mg/kg PO TID (based upon actual body weight) with the maximum total daily dose not to exceed 3 grams (1 g PO TID). MMF prophylaxis will be discontinued after the last dose on day +35.

Tacrolimus: Tacrolimus will be given at a starting dose of 2mg PO q12hr. The starting dose of tacrolimus may be increased with PI or co-PI permission should institutional practice guidelines change. Tacrolimus can be changed to a starting dose of 1.6 mg IV daily if the patient cannot tolerate oral medications. The dose is adjusted to maintain a serum trough level of 10 – 15 ng/Ml. If there is tacrolimus toxicity at these troughs, lower troughs will be permitted to allow for tolerance of the drug after consultation with the PI. If tacrolimus is completely impossible for the patient to tolerate, alternative immunosuppression may be chosen after consultation with the PI or Co-PI. Tacrolimus prophylaxis will be discontinued after the last dose on Day +180.

4.2.6. Infectious Prophylaxis and therapy.

Infection prophylaxis and therapy All infection prophylaxis and therapy will be administered and discontinued as per institutional requirements. The following are recommendations only.

i) During pre-transplant evaluation patients will be screened for respiratory syncitial virus, influenza A, B and parainfluenza viruses if symptomatic. Assays of these viruses must be negative for symptomatic patients to be admitted for transplant. Strong consideration should

be given to institution of ribavirin therapy if positive for adenovirus or nalidixic acid if positive for BK virus.

ii) Oral hygiene will be maintained according to institutional standards.

iii) Prophylactic anti-microbial therapy will be started during the preparative regimen, per institutional guidelines.

iv) Empiric therapy with broad-spectrum antibiotics will be instituted for the first neutropenic fever (specific agents as per current practice).

4.2.7. Growth Factor Support

Patients will receive G-CSF 5µg/kg/day SC or IV starting at Day 5 and continuing until the ANC>1000/mm^3 x 3days or two consecutive measurements over a three day period. For use in the case of fungal infections or subsequent neutropenia (ANC<500/mm3), G-CSF should be continued until the WBC>10,000-15,000.

4.2.8. Transfusion support

Platelet and packed red cell transfusions will be given per current institutional recommendations.

4.2.9. Post-BMT evaluation.

Patients will be followed for 3 years post-BMT, in conjunction with referring physician.

4.2.10. Post-BMT Testosterone Therapy

Patients will receive intramuscular testosterone cypionate or testosterone enanthate at the FDA approved dose of 400mg on Day +60, +90, and +120. With ongoing PSA and clinical response, GnRH agonists/antagonists will be stopped.

4.3 Concomitant Therapy

Enrolled patients will be followed through closely through engraftment. In the event that patients experience symptomatic progression of prostate cancer after Day 90 post-transplant, patients may pursue further standard of care or experimental therapies per referring physician preference.

4.5 Prohibited Concomitant Medications

Concomitant therapy during the initial phase of the study (from receipt of preparative regimen to Day +90 post-transplant) with any of the following listed is prohibited:

- Chemotherapy
- Immunotherapy
- Bicalutamide, nilutamide, flutamide, enzalutamide, abiraterone
- Systemic ketoconazole (or other azole drugs such as fluconazole and itraconazole)
- Diethylstilbestrol, PC-SPES, and other preparations such as saw palmetto thought to have endocrine effects on prostate cancer
- Radiopharmaceuticals such as radium-223, strontium (89Sr), or samarium (153Sm)

- Other experimental drugs or treatments

5 PATIENT MONITORING

5.1 Screening Period

All patients must sign a written informed consent form before study specific screening procedures are performed. Prior to enrollment, patient must have documented insurance coverage demonstrating ability to pay for standard BMT-related care. All required screening assessments must be completed within 28 days of the study start date. All treatment and post-treatment study procedures and assessments must be done within 5 days (+/-) of the specified study visit date.

Pre-BMT Screening Evaluation

These represent the basic baseline studies required on all patients prior to starting their preparative regimen. Additional investigations may be clinically indicated in certain individuals. Other baseline studies may be required for the purposes of non-preparative regimen protocols on which the patient is enrolled. In this case, such requirements will be stipulated in the pertinent protocols.

Complete medical history which should include particular attention to the following details:

a) previous treatment and response
b) allergies
c) current medications
d) assessment of performance status

Whenever possible, previous transfusions and transfusion reactions and previous serious infections should be documented.

Thorough general medical evaluation which should include:

a) Physical examination
b) Baseline investigations including:
    - Hematologic
        - i. CBC with platelets, differential, reticulocyte count
        - ii. PT, PTT
        - iii. ABO and Rh typing
    - Chemistries
        - i. Comprehensive chemistry panel including electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium, and LDH
        - ii. Routine and microscopic urinalysis with culture and sensitivity
    - Cardiac
        - i. EKG
        - ii. Echocardiogram with Left Ventricular Ejection Fraction (LVEF)
    - Pulmonary
        - i. Sinus CT scan
        - ii. Pulmonary function tests including at least FEV1 and FVC
    - Renal
        - i. GFR or Cr clearance
    - Immunologic / Infections
        - i. HBsAg, anti-HBC, anti-HCV

ii. RPR
iii. HIV antibody
iv. Serology for CMV, HSV, and VZV IgG
v. HLA typing/lymphocytotoxic antibody screen

HLA Antibody Testing: Routinely done by the immunogenetics lab on all pre-transplant patients.

c) Prostate Cancer Staging studies:
PSA level
Testosterone level
CT scan chest, abdomen pelvis
Bone Scan

5.2 Post BMT Evaluation

Day 0 through Day 60 (+/- 5 days) evaluation. These represent the minimum required. More frequent determinations and additional investigations may be indicated by the clinical condition of the patient.

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

1. CBC daily with a WBC differential once the total WBC is greater than 100 until ANC > 500 for three days or two consecutive measurements over a three day period. Then, CBC weekly with differential.

2. Comprehensive metabolic panel (electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium) once a week.

3. Patients will have evaluations for infectious complications as clinically indicated. Surveillance cultures according to institutional protocol

4. Evaluations by history and physical examination for GVHD will be performed as per BMT unit standards.

Evaluations on day 30 (+/-3 days)

1. History and physical examination, performance status, assessment of toxicity.

2. T cell engraftment follow-up for donor chimerism on peripheral blood

3. CBC and differential and comprehensive panel (electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium.

4. PSA, Testosterone

Evaluations on day 60 (+/-5 days)

1. History and physical examination, performance status, assessment of toxicity.

2. T cell engraftment f/u for donor chimerism on peripheral blood

3. CBC and differential and comprehensive panel (electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium.

4. PSA, Testosterone

Evaluations on Day 90 (+/- 7 days)

1. History and physical examination, performance status, assessment of toxicity.

2. Prostate Cancer Disease Restaging [PSA, Testosterone, CT C/A/P, NM Bone Scan]

Evaluations on Day 120 (+/- 7 days)

1. History and physical examination, performance status, assessment of toxicity.

2. PSA, Testosterone

Evaluations on day 180 (+/-10 days)

1. History and physical examination, performance status, assessment of toxicity.

2. T cell engraftment f/u for donor chimerism on peripheral blood

3. Prostate Cancer Disease Restaging [PSA, Testosterone, CT C/A/P, NM Bone Scan]

4. CBC and differential and comprehensive panel (electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium.

5. EKG/Echo

6. PFTs (Spirometry and DLCO)

Evaluations every 90 days ongoing through 3 years

1. History and physical examination, performance status, assessment of toxicity.
2. Prostate Cancer Disease Restaging (PSA, Testosterone)
3. CBC and differential and comprehensive panel

Evaluations every 180 days ongoing through 3 years

1. CT scan chest abdomen and pelvis
2. Bone Scan

6 Safety Assessment

Definition of Adverse Event (AE)

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered

causally related to the product. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). In clinical trials, from the time of signing an informed consent, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no trial treatment has been administered.

Definition of Serious Adverse Event (SAE)

A serious adverse event is an AE occurring during any trial phase (i.e., run-in, treatment, washout, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above

Adverse events and SAEs will be documented on case report forms and submitted to the Coordinating Center. Adverse and severe adverse events will be collected through the time of discharge from the transplant center, but no longer than 180 days after transplantation.

The agents being used in this study are commercially available and used extensively in the BMT setting and have well-defined toxicity profiles. In addition, there are many expected toxicities of allogeneic BMT. The following are examples of toxicities that are serious but not unexpected: Grade 4 cytopenias; neutropenic fever and sepsis; bacterial, fungal, or viral (including CMV, BK virus) infection; severe mucositis; severe GVHD; hepatic veno-occlusive disease; pulmonary toxicities; hemorrhagic cystitis; bleeding without hemodynamic compromise.

For study purposes, the following serious adverse events and adverse events will be recorded and reported in accordance with IRB requirements:

a. any non-relapse mortality within the first 180 days after BMT, and any later death which is potentially transplant-related

b. any graft failures (defined as <5% donor chimerism) associated with failure of neutrophil recovery to >500/mm^3 by day ~60 after transplantation

c. Any unexpected grade 3 event with hospitalization or prolongation of hospitalization that is possibly, probably, or definitely related to the therapy within first 180 days as deemed by the PI

d. Any unexpected grade 4 or 5 events as deemed by the PI within 6 months post-transplant.

In addition, the following toxicities will be tracked for study purposes:

a. Clinically significant infections during the first year of transplant, with the exception of uncomplicated, culture-negative neutropenic fever. This includes CMV disease, other clinically significant documented viral infections, bacterial infections, and documented or suspected fungal infections.

b. CMV reactivation (including asymptomatic reactivation)

c. Hepatic veno-occlusive disease

d. Grade 3 or greater pulmonary toxicity during the first year of transplant that is possibly, probably, or definitely transplant-related

e. Hospitalizations deemed related to the transplant by the PI after the initial discharge from BMT and its reason in the first year after transplant.

Additional therapies, complications and toxicities may be tracked. This is in addition to evaluating hematologic parameters, GVHD, and disease and survival endpoints.

6.1 Toxicity monitoring

Toxicities are graded using the NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)

In those cases where the NCI criteria do not apply, intensity will be defined as:

- Mild: awareness of symptom or sign, but easily tolerated
- Moderate: discomfort is enough to cause interference with normal activities
- Severe: inability to perform normal daily activities
- Life threatening: immediate risk of death from the reaction as it occurred

6.2 Risks and Toxicities

Testosterone Cypionate or Testosterone Enanthate (DEPO-Testosterone Injection)
Testosterone esters are less polar than free testosterone. Testosterone esters in oil injected intramuscularly are absorbed slowly from the lipid phase; thus, Testosterone Cypionate and Testosterone Enanthate can be given at intervals of two to four weeks.

Testosterone in plasma is 98 percent bound to a specific testosterone-estradiol binding globulin, and about 2 percent is free. Generally, the amount of this sex-hormone binding globulin in the plasma will determine the distribution of testosterone between free and bound forms, and the free testosterone concentration will determine its half-life.

About 90 percent of a dose of testosterone is excreted in the urine as glucuronic and sulfuric acid conjugates of testosterone and its metabolites; about 6 percent of a dose is excreted in the feces, mostly in the unconjugated form. Inactivation of testosterone occurs primarily in the liver. Testosterone is metabolized to various 17-keto steroids through two different pathways. The half-life of Testosterone Cypionate and Testosterone Enanthate when injected intramuscularly is approximately eight days. The two forms of the drug demonstrate identical pharmacokinetic properties.

Precautions:
1. Patients with benign prostatic hypertrophy may develop acute urethral obstruction. Priapism or excessive sexual stimulation may develop.
2. Oligospermia may occur after prolonged administration or excessive dosage. If any of these effects appear, the androgen should be stopped and if restarted, a lower dosage should be utilized.
3. Testosterone Cypionate or Enanthate should not be used interchangeably with testosterone propionate because of differences in duration of action.
4. Testosterone Cypionate and Testosterone Enanthate are not for intravenous use.
5. Patients should be instructed to report any of the following: nausea, vomiting, changes in skin color, ankle swelling, too frequent or persistent erections of the penis.
6. Hemoglobin and hematocrit levels (to detect polycythemia) should be checked periodically in patients receiving long-term androgen administration.
7. Serum cholesterol may increase during androgen therapy.
8. Androgens may increase sensitivity to oral anticoagulants. Dosage of the anticoagulant may require reduction in order to maintain satisfactory therapeutic hypoprothrombinemia.
9. Concurrent administration of oxyphenbutazone and androgens may result in elevated serum levels of oxyphenbutazone.
10. In diabetic patients, the metabolic effects of androgens may decrease blood glucose and, therefore, insulin requirements.
11. Androgens may decrease levels of thyroxine-binding globulin, resulting in decreased total T4 serum levels and increased resin uptake of T3 and T4. Free thyroid hormone levels remain unchanged, however,

and there is no clinical evidence of thyroid dysfunction.

Adverse Reactions
The following adverse reactions in the male have occurred with some androgens:
1. Endocrine and urogenital: Gynecomastia and excessive frequency and duration of penile erections (priapism). Oligospermia may occur at high dosages.
2. Skin and appendages: Hirsutism, male pattern of baldness, seborrhea, and acne.
3. Fluid and electrolyte disturbances: Retention of sodium, chloride, water, potassium, calcium, and inorganic phosphates.
4. Gastrointestinal: Nausea, cholestatic jaundice, alterations in liver function tests, rarely hepatocellular neoplasms and peliosis hepatis.
5. Hematologic: Suppression of clotting factors II, V, VII, and X, bleeding in patients on concomitant anticoagulant therapy, polycythemia, thrombosis.
6. Nervous system: Increased or decreased libido, headache, anxiety, depression, and generalized paresthesia.
7. Allergic: Hypersensitivity, including skin manifestations and anaphylactoid reactions.
8. Miscellaneous: Inflammation and pain at the site of intramuscular injection.
Testosterone is a controlled substance under the Anabolic Steroids Control Act, and Testosterone Cypionate and Testosterone Enanthate Injection has been assigned to Schedule III.

TBI
Early side effects (< 1 month): Most patients experience some degree of nausea, vomiting, and diarrhea either during or immediately after treatment. Fever that develops soon after TBI is not uncommon. Other side effects include skin erythema, parotid gland swelling, diminished salivary gland function, stomatitis, mouth ulcers, sore throat, generalized weakness and fatigue and alopecia. Myelosuppression occurs promptly following TBI and doses of 1000cGy or more are assumed to cause permanent bone marrow aplasia and would be lethal without BMT.
Intermediate side effects (1-4 months): Interstitial pneumonia can be seen with a fatal incidence of up to 5-10%. Other side effects may include graft versus host disease and infection from prolonged immunoincompetence.
Late effects (>4months): An increased risk of sterility and cataracts is known; the risk of developing a second malignancy may be increased. Additional complications for long term survivors may include cardiac, pulmonary, liver, and kidney damage as well as hearing loss. Changes in hormone levels may also occur.

Fludarabine
Fludarabine is a fluorinated nucleoside analog. After phosphorylation to fluoro-ara-ATP the drug appears to incorporate into DNA and inhibit DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. Excretion of fludarabine is impaired in patients with impaired renal function.
Fludarabine toxicities:
a) Hematologic: pancytopenia, immunosuppression, autoimmune hemolytic anemia.
b)Gastrointestinal: nausea, vomiting, anorexia, and weakness.
c) Neurologic: agitation, visual disturbances, confusion, coma, peripheral neuropathies have been reported. With high doses, confusion, blindness, coma and death have been reported (when used in doses of at least 120 mg/m2 daily for 4-5 days or a total dose of ≈ 500 mg/m2).

Cyclophosphamide
Cyclophosphamide Toxicities:
a) Hematologic: Leukopenia, anemia
b) Dermatologic: Alopecia
c) Gastrointestinal: Nausea, vomiting, increased AST, ALT, mucositis, diarrhea

d) Neurologic: Headache, dizziness
e) Cardiovascular: Cardiac necrosis rarely with high dose cyclophosphamide
f) Renal: Hemorrhagic cystitis, SIADH
g) Other: teratogenic, may cause secondary neoplasms, anaphylaxis (rare)
h) Fluid retention. Cy has anti-diuretic effect usually counteracted by furosemide administration. Careful physical examination should be made and accurate weights should be determined to detect fluid overload early.
i) Cardiomyopathy. At doses greater than 200mg/kg, Cy can cause fatal myocardial necrosis with clinical heart failure. Non-specific ST changes on EKG are not unusual but a decrease in voltage is significant.
j) Hemorrhagic cystitis. Hematuria is not uncommon at this dose level, but is usually not symptomatic or severe unless there is inadequate diuresis. An occasional patient will get severe cystitis despite prophylactic measures.

Mesna (sodium -2-mercapto ethane sulphonate)
Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxazophosphorines (cyclophosphamide and ifosfamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxazophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxazophosphorines.
At the doses used for uroprotection, mesna is virtually non-toxic. However, adverse effects which may be attributable to mesna include nausea and vomiting, diarrhea,abdominal pain, altered taste, rash, urticaria, headache, joint or limb pain, hypotension and fatigue.

Mycophenolate mofetil (MMF)
Mycophenolate mofetil (MMF) is an ester prodrug of the active immunosuppressant mycophenolic acid. This active metabolite is a noncompetitive, reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH). There are no pharmacokinetic interactions with gancyclovir, cotrimoxazole, oral contraceptives and cyclosporine. The side effect profile includes diarrhea, leukopenia, sepsis, allergic reactions, and vomiting. There is also an increase in certain types of infection mainly from the herpes virus family.

Tacrolimus (FK 506)
Tacrolimus, also known as FK-506, is a macrolide immunosuppressant. It inhibits lymphocytes by forming a complex with FKBP-12, calcium, and calmodulin, leading to the decrease in the phosphatase activity of calcineurin. This drug is used with corticosteroids for prophylaxis of organ rejection in patients receiving allogeneic liver transplants. Its use is also currently being investigated in kidney, bone marrow, cardiac, pancreas, pancreatic islet cell and small bowel transplantation. This drug is well-absorbed orally. It is metabolized in the liver by unknown mechanisms, but demethylation and hydroxylation have been proposed based on in vitro studies. The metabolized products are excreted in the urine. Nephrotoxic drugs, antifungals, calcium channel blockers, cimetidine, danazol, erythromycin, methylprednisone and metoclopramide increase the bioavailabilty of FK-506. In contrast, phenobarbital, phenytoin, rifamycins and carbamazepine decrease FK-506 levels. Adverse reactions include tremor, headache, diarrhea, hypertension, nausea, and renal dysfunction.

Acute and Chronic GVHD
The major toxicity of using bone marrow from HLA-mismatched, related donors is GVHD. Using nonmyeloablative conditioning regimens and haploidnetical bone marrow for hematologic malignancies, we have shown GVHD rates of 30% (Grades 2-4) and severe GVHD (Grades 3-4) of 10%. Acute graft-versus-host disease (GVHD) shall be graded clinically according to the criteria developed by the consensus conference on acute GVHD[85] (Appendix 3). *All suspected cases of acute GVHD must be confirmed histologically by biopsy of an affected organ (skin, liver, or gastrointestinal tract).* For purposes of reporting,

a pathologist at SKCCC will be ultimately responsible for determining whether a patient does or does not have histologic evidence of GVHD. Diarrhea and/or hyperbilirubinemia in a patient with histologically documented skin GVHD may beassumed to be a manifestation of visceral GVHD and will be graded as such. All patients with histologically documented, clinical grade >2 acute GVHD should receive initial treatment with corticosteroids according to institutional preference. If skin GVHD resolves with treatment but suspected visceral GVHD does not, biopsy of the affected organ (liver or gastrointestinal tract) should be obtained to rule out other causes of hyperbilirubinemia and/or diarrhea. Steroid refractory acute GVHD will be treated according to institutional preferences.

The following information shall be collected on all patients with acute GVHD:
Date of onset (defined as the date of first biopsy confirming GVHD)
GVHD evaluation form at the time of onset, weekly until GVHD resolves, and Day 100
Initial overall clinical grade
Maximum overall clinical grade
Date of onset of grade III-IV acute GVHD, if any

Engraftment Failure
Another significant risk is failure-to-engraft due to rejection by host lymphocytes. However, because of the nonmyeloablative nature of the conditioning regimen we would expect patients to have full autologous, hematologic recovery.

Prostate Cancer Disease Progression
Progression of the underlying prostate cancer may occur during the BMT, which would limit possible treatment options if still undergoing recovery, engraftment or other treatment of complications.

Infection
Infection is a major cause of morbidity and mortality in the peri-transplant period (100 d post-BMT). However, given current supportive care and the intensive infection prophylaxis of this protocol, we expect the risk to be acceptable. Prolonged neutropenia may increase this risk in the case of graft rejection, however.

Transplant-related mortality (TRM)
Causes of TRM, i.e., death in the absence of relapse, will be documented as important indicators of procedure-associated toxicity, particularly as these causes relate directly or indirectly to GVHD. Analysis will stratify mortality with respect to the peri-transplant period (<100 d post-BMT) or later times post-BMT.

7 STUDY PARAMETERS

7.1. Donor chimerism

Donor chimerism will be measured in the peripheral blood around day 30 and again in the peripheral blood around day 60. Patients with any amount of donor chimerism around day 60 will be considered as having engrafted. Chimerism determinations will be made on peripheral blood by a number of different methods depending on the specific patient. Methods may include (i) the usual standard of restriction fragment length polymorphism (RFLP) if the donor and recipient RFLPs are informative, (ii) fluorescence in-situ hybridization (FISH) for Y-chromosome markers on PBMC if the donor is male, (iii) cytogenetic analysis, (iv) flow cytometric analysis of HLA-A, B or DR on lymphocytes in the peripheral blood if haploidentical and suitable reagents exist or (v) PCR analysis of variable nucleotide tandem repeats (VNTR) in PBMC if informative. Mixed donor chimerism will be defined as >0%, but <95%. Complete donor chimerism will be defined as >95%. Patients

who have relapsed or died prior to day 60 will not be evaluable for full donor chimerism, as these are competing risk factors.

7.2. GVHD

Patients will be followed for development of acute and chronic GVHD using standard criteria. Chronic GVHD usually develops beyond the high-risk, peri-transplant period (i.e., >100 d post-BMT, but can occur earlier) and is assessed according to standard criteria.

7.3. Transplant-related mortality

Transplant-related mortality, which is defined as death in the absence of relapse or progression, will be characterized at 100 days and at one year after BMT.

7.4. Response Criteria

All tumors will be assessed for response by CT based RECIST criteria and bone lesions by NM Bone Scan per PCWG-2 Criteria.

8 STATISTICAL METHODS

8.1 Accrual

We will accrue a total of 7 subjects per year, for a total of 20 subjects. Hopkins is a national referral center for prostate cancer as well as BMT.

8.2 Analysis

The primary efficacy endpoint is complete biochemical response, defined as a PSA level of <0.1 ng/mL for post-prostatectomy patients and <1 ng/mL for post-radiation patients, at 6 months post-transplant. We will estimate the complete biochemical response rate as the proportion of subjects whose PSA level declines below these levels at 6 months post-transplant, along with a 90% confidence interval. Patients who are treated with second line hormonal therapy within the 6 month period post-transplant and achieve biochemical CR will not be considered responders for this trial. As a secondary endpoint, incidence of TRM will be summarized using a cumulative incidence curve based on competing risks methods. Deaths from any cause without prior disease progression are events; disease progressions are considered as competing events; and losses to follow-up are censored. We will report cause-specific incidence of TRM at 60 days, 100 days, six months, and one year, along with 90% confidence intervals. Additionally, we will estimate PSA response rate as the proportion of patients achieving a PSA decline ≥50% according to PCWG2 criteria, and objective response rate in patients with measurable disease per RECIST criteria. Time-to-event outcomes (radiographic PFS, and time to PSA progression) will be summarized using Kaplan-Meier analysis. Cumulative incidence of each acute grade 2-4 GVHD, acute grades 3-4 GVHD, and chronic GVHD will be computed using competing risks methods, wherein graft failure, disease progression or death prior to occurrence of GVHD are considered competing risks. The incidence of graft failure following transplant will be reported using a cumulative incidence curve, wherein death prior to graft failure will be considered as a competing event.

For correlative studies, we will summarize the markers at each time point as well as the change (or percent change) from baseline, and evaluate their effect on the clinical outcomes.

Safety will be monitored by a stopping rule for transplant-related mortality (TRM) convincingly greater than 10%, as discussed below. Since patients with severe GVHD and engraftment failure who die will be captured in TRM, we will apply a stopping rule based on incidence of TRM. As outlined in above, the overall transplant-related mortality in multiple nonmyeloablative haploidentical BMT trials utilizing 2 doses of PTCy, MMF and Tacrolimus was approximately 6-10% at day 100.[10,11,23,24] The working hypothesis of this trial is that the overall toxicity of this minihaplo BMT in prostate cancer patients is not significantly greater than minihaplo BMT utilizing PTCy Cy for hematologic malignancies, and less than what has been seen with other nonmyeloablative and ablative haploidentical BMT trials that do not utilize PTCy. Consequently a TRM incidence that is convincingly greater than 10% at Day 100 would raise concerns for excessive toxicity and would trigger a referral to DSMB for evaluation. Based on these considerations, we have developed stopping rules to protect patients against toxicity that appears higher than we expect *a priori*. Our stopping rule is to halt accrual any time we feel certain that the risk of 100-day transplant-related mortality is 10% or higher. Specifically, we will apply a Bayesian toxicity monitoring rule that suspends the enrollment if the posterior probability of risk being larger than that threshold is 75% or higher. We assume *a priori* that this regimen has risk around 6% of each event and that there is about a 20% chance that these risks will be 10% or higher. This corresponds to a Beta (1, 14) prior distribution. The following table gives the corresponding stopping rules for the 20 patients. For example, if 2 patients out of the first 2 or 3 experience death prior to disease progression (e.g. death from severe GVHD or engraftment failure) prior to 100 days, we will stop accrual. If the stopping criterion is met, accrual to the trial will be temporarily halted, and the principal investigator and study team will review the toxicity data and recommend either modification or termination of the trial.

Table 2. Stopping rule for toxicity based on TRM

Stop if:

# patients with 100-day TRM ≥	2	3	4
Out of # patients treated	2 - 3	4 - 11	12 - 20

8.3 Sample size consideration

Our primary objective is to determine the preliminary clinical activity of BMT based on complete biochemical response. We plan to enroll a total of 20 patients. If one or more patients achieve complete biochemical response out of 20 patients, the treatment regimen would be considered promising for further study. If the true CR is 10%, the regimen will be identified as efficacious with probability of 88%.

9 DATA MONITORING AND REPORTING REQUIREMENTS

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring. The Clinical Research Office will perform an audit after the first subject has been treated and then periodically depending on the rate of accrual and prior audit results. All trial monitoring and reporting will be reviewed annually by the SKCCC Safety Monitoring Committee. The PI is responsible for internally monitoring the study. Data must be reviewed to assure the validity of data, as well as, the safety of the subjects. The PI will also monitor the progress of the trial, review safety reports, and clinical trial efficacy endpoints and to confirm that the safety outcomes favor continuation of the study.

Additionally, scheduled meetings will take place monthly and will include the protocol principal investigator, research nurse, data manager, and, when appropriate, the collaborators, subinvestigators, and biostatistician

involved with the conduct of the protocol.

During these meetings the investigators will discuss matters related to: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for secondary objectives.

Appendix I

Response Evaluation Criteria in Solid Tumors (RECIST) Quick Reference

Eligibility
Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint.

Measurable disease –
The presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable lesions –

Lesions that can be accurately measured in at least one dimension with longest diameter ≥20 mm using conventional techniques or ≥10 mm with spiral CT scan.

Non-measurable lesions –

All other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques.

All measurements should be taken and recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Methods of Measurement
CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen and pelvis. Head and neck tumors and those of extremities usually require specific protocols.
Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.
When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.
The utilization of endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely

validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.
Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response when all lesions have disappeared.
Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

Baseline Documentation of "Target" and "Non-Target" Lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as ***target lesions*** and recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).

A sum of the longest diameter (LD) for *all target lesions* will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.

All other lesions (or sites of disease) should be identified as ***non-target lesions*** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Response Criteria

Evaluation of Target Lesions

Complete Response (CR):	Disappearance of all target lesions
Partial Response (PR):	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

Evaluation of Non-Target Lesions

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level
Incomplete Response / Stable Disease (SD):	Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Although a clear progression of “non-target” lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

Evaluation of Best Overall Response

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having “symptomatic deterioration”. Every effort should be made to document the objective progression even after discontinuation of treatment.
In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Confirmation

The main goal of confirmation of objective response is to avoid over-estimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.
To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.
In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol

Duration of Overall Response

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

Duration of Stable Disease

SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.
The clinical relevance of the duration of SD varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

Response Review

For trials where response rate is the primary endpoint it is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of patients' files and radiological images is the best approach.

Reporting of Results

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).

All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients.

Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

The 95% confidence intervals should be provided.

Appendix 2

Prostate Cancer Working group 2 (PCWG2) Criteria

Pathology response criteria

When evaluating measurable soft-tissue target lesions, the RECIST definitions will apply. The first assessment must show an increase in the sum longest diameter (LD) of both preexisting and new lesions of ≥ 20% when compared with the smallest sum LD recorded since treatment started.
In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated further (*e.g.* by aspirate/biopsy) before confirming the complete response status.

Evaluating non-target lesions

When assessing non-target lesions, the following RECIST definitions will apply:

Complete response:	Disappearance of all non-target lesions, and normalization of tumor marker levels.
Progressive disease:	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.
Stable disease:	Persistence of one or more non-target lesions and/or maintenance of tumor marker above the normal limits.

Evaluating best overall response

The best overall response is the best response recorded from the start of treatment until disease progression/recurrence. The investigator's determination of best overall response will be based on the response criteria and will not require confirmation scans. For defining disease progression, confirmation scans will only be required in the case where progression is seen on the first follow-up bone scan, but not if progression is shown on CT.
Patients with global deterioration of health status who require discontinuation of treatment without objective evidence of disease progression should be classified as having symptomatic deterioration. Every effort should be made to document their objective progression, even after discontinuation of treatment.
The table below summarized the recommendations of the PCWG2 for measuring response/progression outcomes in phase II clinical trials of prostate cancer. Although these guidelines have been largely followed in the design of the present trial, this table should not be used as a substitute for the clinical protocol.

Prostate Cancer Clinical Trials Working Group (PCWG2) Outcome Measures

Variable	Control/Relieve/Eliminate	Prevent/Delay
PSA	Record the percent change from baseline (rise or fall) at 12 weeks, and separately, the	**Decline from baseline:** Record time from start of therapy to first PSA

	maximal change (rise or fall) at any time using a waterfall plot	increase that is ≥ 25% and ≥ 2 ng/mL above the nadir, and which is confirmed by a second value ≥ 3 weeks later (*i.e.* a confirmed rising trend). Recording the duration of PSA decline of little value **No decline from baseline:** PSA progression ≥ 25% and ≥ 2 ng/mL after 12 weeks
Soft-tissue lesions	Use RECIST with caveats Only report changes in lymph nodes that were ≥ 2 cm in diameter at baseline Record changes in nodal and visceral soft tissue sites separately Record complete elimination of disease at any site separately Confirm favorable change with second scan Record changes using waterfall plot	Use RECIST criteria for progression, with additional requirement that progression at first assessment be confirmed by a second scan 6 or more weeks later (particularly important for biologic therapies) Note that for some treatments, a lesion may first increase in size before it decreases
Bone	Record outcome as new lesions or no new lesions *First scheduled reassessment:* No new lesions: continue therapy New lesions: perform a confirmatory scan 6 or more weeks later Confirmatory scan: No new lesions: continue therapy Additional new lesions: progression *Subsequent re-assessments:* No new lesions: continue New lesions: progression	The appearance of ≥ 2 new lesions, and, for the first reassessment only, a confirmatory scan performed 6 or more weeks later that shows a minimum of 2 or more additional new lesions The date of progression is the date of the first scan that shows the change
Symptoms	Consider independently of other outcome measures Document pain and analgesia at entry with a lead in period and measure repeatedly at 3- to 4-week intervals Perform serial assessments of global changes in HRQOL, urinary or bowel compromise, pain management, additional anticancer therapy Ignore early changes (< 12 weeks) in pain or HRQOL in	

	absence of compelling evidence of disease progression Confirm response or progression of pain or HRQOL end points > 3 weeks later

Abbreviations: PSA, prostate-specific antigen; HRQOL, health-related quality of life

Appendix 3

NCI COMMON TOXICITY CRITERIA, VERSION 4.0

Version 4.0 of the NCI CTC, dated May 28, 2009, may be viewed and/or downloaded by accessing the following websiteshttp://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae_4_with_lay_terms.pdf

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

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