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12b. DISTRIBUTION CODE**13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)**

This proposed clinical trial targets men at risk for recurrence after prostatectomy by treating systemic micrometastatic disease in addition to local. Thus far, anti-angiogenesis therapy is safe in men undergoing radical prostatectomy and postulated changes in angiogenic factors as well as grade and stage have been observed.

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ANNUAL REPORT 2004 -- **REVISED**

"Combined Neoadjuvant Anti-angiogenesis & Androgen Ablation Therapy in Men Undergoing Radical Prostatectomy" (DAMD17-02-1-0231)

INTRODUCTION

High risk localized prostate cancer (preoperative PSA >10ng/ml, >50% Gleason grade 4 (or higher) on biopsy, and bilaterally bulky palpable disease) remains inadequately treated. While surgery and radiotherapy remain the treatments of choice, many men will fail despite these curative attempts. The role of neoadjuvant systemic chemotherapy has been proposed as a means of improving disease-free survival. We have successfully used a combination of castration and squalamine, a novel anti-angiogenic agent, to eradicate tumors in a preclinical model of human prostate cancer. Table 1: Specific Aims

In this investigator-initiated phase II clinical trial application, we proposed to extend these findings to the clinical setting. Our principal goal is to assess whether combined androgen ablation and anti-angiogenesis therapy can improve outcomes for

men with poor prognostic prostate cancer undergoing radical prostatectomy. The primary objectives of this proposal are to investigate if the combination of these agents results in pathologic downstaging and downgrading of the primary tumor, if tumor recurrence can be diminished, and whether anti-angiogenic agents can be given safely in the perioperative setting. The Specific Aims are outlined in the accompanying box.

Specific Aims

1. Specific Aim 1: To test the hypothesis that combined androgen ablation and anti-angiogenesis therapy will eradicate human prostate cancer.
2. Specific Aim 2: To test the hypothesis that androgen ablation therapy renders prostate tissue more susceptible to squalamine activity by inducing changes in VEGF/VEGF-receptor and integrin expression.
3. Specific Aim 3: To test the hypothesis that neoadjuvant combined androgen ablation and anti-angiogenesis therapy will diminish post-prostatectomy disease recurrence.

BODY

To test our we hypothesize that disruption of stromal-epithelial interactions by androgen ablation renders both prostate epithelial and stromal endothelial cells more susceptible to anti-angiogenic drug activity through changes in VEGF, VEGF receptor, and integrin expression, we designed a phase II clinical trial. A schematic of the trial design is included as Figure 1.

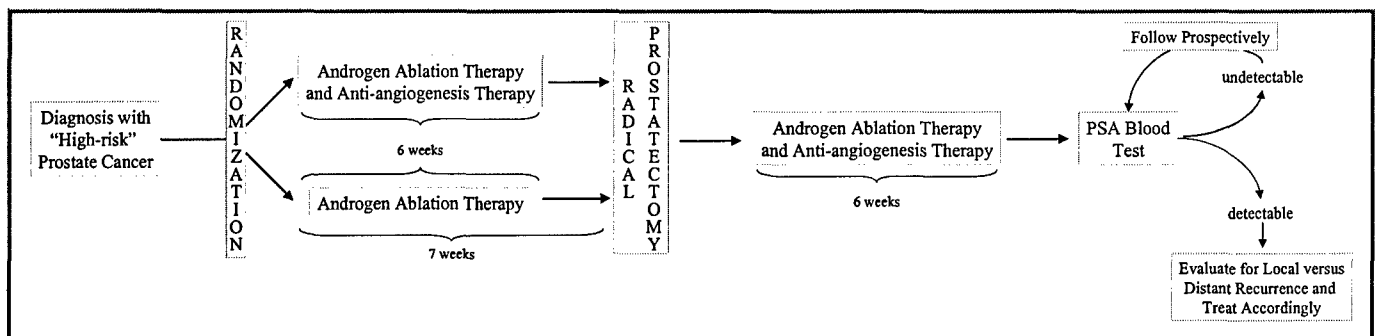


Figure 1: Schematic of clinical trial design

Preoperatively, patients will receive either androgen ablation therapy alone or combined androgen ablation and anti-angiogenesis therapy for six weeks. All patients will receive six weeks of combination androgen ablation and anti-angiogenesis therapy post-prostatectomy and will be followed prospectively for clinical evidence of disease recurrence. This randomized phase II clinical trial will investigate whether neoadjuvant combined androgen ablation and squalamine therapy is tolerable, feasible, and results in prolonged disease-free survival and pathologic downstaging and downgrading of established prostate cancers. Prostatectomy specimens will also be analyzed for changes in apoptosis, microvessel density, and VEGF, flt-1 receptor and integrin $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_6\beta_4$ expression.

This proposed clinical trial targets a population of men who are at high risk for treatment failure yet for whom there are few available therapies other than conventional surgery or radiotherapy. We will attempt to establish a role for systemic therapy for these patients, evaluate the efficacy of anti-angiogenesis therapy in prostate cancer, and validate our putative mechanisms of action which suggest that for maximum benefit, anti-angiogenesis therapy should be given in combination with androgen ablation therapy.

STATEMENT OF WORK

Specific Aim 1 : A randomized phase II clinical trial will investigate whether neoadjuvant combined androgen ablation and squalamine therapy is tolerated, feasible, and results in pathologic down-staging and downgrading of established prostate cancers.

- Task 1: Identification and enrollment of one hundred and thirty-two men with prostate cancer with characteristics denoting a "high risk" for recurrence (months 0-30)
- Task 2: Treatment of the enrolled men with either neoadjuvant androgen ablation therapy or combined neoadjuvant androgen ablation and anti-angiogenesis therapy (months 0-32)
- Task 3: Completion of radical prostatectomies on all one hundred and thirty-two men (months 2-34)
- Task 4: Evaluation of prostatectomy specimens for evidence of down-staging and down-grading (months 2-34)
- Task 5: Evaluation of safety of radical prostatectomy after treatment with an anti-angiogenic agent (months 2-36)

Specific Aim 2 : To test the hypothesis that androgen ablation therapy renders prostate tissue more susceptible to squalamine activity by inducing changes in VEGF/VEGF-receptor and integrin expression.

- Task 6: Evaluation of prostatectomy specimens and pre-prostatectomy prostate needle biopsies for changes in molecular factor expression
- Task 7: Comparison between androgen ablation alone and androgen ablation plus anti-angiogenesis treatment groups and between pretreatment (prostate needle biopsy) and post-treatment (radical prostatectomy) specimens (months 2-36)

Specific Aim 3 : In an extension study, all patients will receive eight weeks of combination therapy post-prostatectomy and will be followed prospectively for clinical evidence of disease recurrence.

- Task 8: Treatment of all enrolled men with adjuvant combined neoadjuvant androgen ablation and anti-angiogenesis therapy (months 2-36)
- Task 9: Follow men prospectively for evidence of biochemical recurrence (months 2-36, and then through standard follow-up until month 72)

Timetable

Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
First 44 patients Neoadjuvant/Surgical/Adjuvant Therapies Extension Study/Follow-up (average 2.5 years) Correlative Studies					
Second 44 patients Neoadjuvant/Surgical/Adjuvant Therapies Extension Study/Follow-up (average 1.5 years) Correlative Studies					
Third 44 patients Neoadjuvant/Surgical/Adjuvant Therapies Extension Study/Follow-up (average 0.5 years)					

Table 2: Statement of Work

Of note, Dr. Sokoloff has accepted a position as Chief of the Section of Urologic Oncology at Oregon Health and Sciences University [OHSU] and is in the process of transferring the study there. This has previously been reported to the US Army Medical Research and Material Command and more details will be forthcoming.

Progress to Date (Based on Statement of Work; completed by August 2004):

(1) Statement of Work Task 1: Identification and Enrollment

The trial official opened in January, 2003. By August 2004, well over 100 patients had been screened, from which 16 have been enrolled. All of the enrolled patients were <72 years old and had cT3N0M0 or high risk (pretreatment PSA>10ng/ml and/or $\geq 50\%$ Gleason grade 4 on biopsy) localized PC. Eight received 6 weeks of Lupron alone; eight additionally received 6 weekly doses of Squalamine [100 mg/m²IV]. Analysis to date includes safety and pathologic response.

Many interested patients were excluded due to a common practice among community urologists to give their patients androgen ablation therapy upon diagnosis with high risk prostate cancer, thus excluding them from this trial. This practice is widespread and has clearly hindered enrollment. It will be addressed in a pending amendment (in preparation). Of those patients screened who did qualify for the study, the most common reason for not enrolling was an aversion to clinical trials, quite common in the community surrounding the University of Chicago. To improve accrual, we began an outreach program in which community urologists and oncologists are educated on the clinical trial and encouraged to refer patients for consideration. Consequently, we saw a steady and progressive increase in interested patients.

Due to concerns regarding regulatory issues by the manufacturer of Squalamine, the study was placed on "hold" from August through November 2003. In addition, due to the relocation of the P.I. from the University of Chicago to Oregon Health and Science University (henceforth OHSU), accrual has been on hold since August 2004. Hence, accrual is at 31% of target. We hope to open the study at OHSU soon.

(2) Task 2: Treatment

To date, 16 patients have been enrolled. All of the enrolled patients were <72 years old and had cT3N0M0 or high risk (pretreatment PSA>10ng/ml and/or $\geq 50\%$ Gleason grade 4 on biopsy) localized PC. Eight received 6 weeks of Lupron alone; eight additionally received 6 weekly doses of Squalamine [100 mg/m²IV].

The treatments have been tolerated well. One patient had a superficial thrombophlebitis at the infusion site and several had transient mild elevations in liver function tests, which resolved. Early in the study, many patients experienced temporary irritation (pain) at the infusion site. By increasing the volume of infusion (while maintaining the same dose) and slowing the rate, these complaints resolved. (NOTE: Safety Data is included under Task 5, below.)

(3) Task 3: Radical Prostatectomies

To date, 16 patients have been enrolled. All were taken to surgery. One patient had grossly positive lymph nodes at time of surgery, and the prostatectomy was aborted, as per standard of care (as outlined in the protocol). Two of the surgeries were performed in a laparoscopic fashion. One patient (with locally advanced disease and a PSA>170ng/ml) developed a rectourethral fistula. Three patients have had lymphoceles: two in the androgen ablation alone group and 1 in the combined therapy group. One patient in the combined therapy group has had a bladder neck contracture. (NOTE: Safety Data is included under Task 5, below.)

(4) Task 4: Preliminary Pathologic Findings

Of the 16 enrolled patients:

- **PSA:** the average pretreatment PSA was 32ng/ml (range 6.2-187)
- **pathologic stage** (see Table 3):
 - on pre-operative exam: 6% had cT1c disease, 13% had cT2b disease, 31% had cT2c disease, and 50% had stage cT3a disease
 - at surgery: 50% had pathologic stage pT2N0 disease, 6% had pT2N1 disease, 31% had pT3N0 disease, 6% had pT3bN1 and 6% had pTxN1
 - of the seven patients who completed prostatectomy after receiving androgen ablation alone: 2 had pathologic downstaging, 2 had the same stage, and 3 had pathologic upstaging
 - of the eight patients who completed prostatectomy after receiving combined therapy: 5 had pathologic downstaging, 2 had the same grade, and 1 had pathologic upstaging
- **pathologic grade** (see Table 3):
 - on biopsy: 37% had Gleason score [GS] 6, 31% had GS 7, 19% had GS 8, and 13% had GS 9
 - at prostatectomy: 33% had Gleason score [GS] 6, 40% had GS 7, 13% had GS 8, and 13% had GS 9;
 - of the seven patients who completed surgery after receiving androgen ablation alone: 2 had pathologic downgrading, 1 had the same grade, and 4 had pathologic upgrading
 - of the eight patients who completed surgery after receiving combined therapy: 4 had pathologic downgrading, 2 had the same grade, and 2 had pathologic upgrading

Table 3: Preliminary Pathologic Changes

Group	n	Stage			Grade		
		↓	↔	↑	↓	↔	↑
AA alone	7	2	2	3	2	1	4
Combo	8	5	2	1	4	2	2

(5) Task 5: Safety

To date, 16 patients have been enrolled. The intravenous treatments have been tolerated well. One patient had a superficial thrombophlebitis at the infusion site and several had transient mild elevations in liver function tests, which resolved. Early in the study, many patients experienced temporary irritation (pain) at the infusion site. By increasing the volume of infusion (while maintaining the same dose) and slowing the rate, these complaints resolved.

All enrolled patients have been taken to surgery. One patient had grossly positive lymph nodes at time of surgery and the prostatectomy was aborted, as per standard of care (as outlined in the protocol). Two of the surgeries were performed in a laparoscopic fashion. No differences in difficulty or length of surgery were noted. The mean blood loss was 800 (group 1) and 494ml (group 2). The mean hospital stay was 2.2 (group 1) and 2.5 days (group 2). One patient (with locally advanced disease and a PSA>170ng/ml) developed a rectourethral fistula. Three patients have had lymphoceles: two in the androgen ablation alone group and 1 in the combined therapy group. One patient in the combined therapy group has had a bladder neck contracture. The operative data is summarized in Table 4. This study has been monitored by a Data Safety Committee.

<u>Operative Data:</u>	<u>Group 1 (AA alone)</u>	<u>Group 2 (combined therapy)</u>
Mean blood loss (ml):	800	494
Mean hospital stay (days):	2.2	2.5
Lymphocele (n):	2	1
BN Contracture (n):	0	1
Thrombophlebitis (n) :	0	1
Rectourethral fistula (n) :	1	0

Table 4: Operative Data

(6) Tasks 6 and 7: Molecular Factors

The numbers of enrolled patients and the minimal time of follow-up have limited evaluation of our end-point objectives. Evaluation of expression of molecular factors has not been performed on the vast majority of specimens. Pilot evaluations to establish technique have been performed on prostatectomy tissue. These demonstrated diminished microvessel density and increased apoptosis in patients who underwent neoadjuvant treatment. We have also noted decreased integrin and receptor expression.

In a medical student-initiated project, we evaluated a subpopulation of African-American men enrolled in the trial. Of the 16 men treated prior to prostatectomy to date, 2 are of African-American decent (for purposes of clarification these patients will be labeled as "group 1"). Both received combination therapy. We compared their results to Caucasian men enrolled in the study (for purposes of clarification these patients will be labeled as "group 2") and African-American ("group 3") and Caucasian ("group 4") men with high risk prostate cancer treated with prostatectomy alone. Biopsy specimens were recovered for all men in group 1 and three men in group 3 and were compared to their corresponding prostatectomy specimens.

In this medical student-initiated project, all men treated with combined hormonal ablation and Squalamine therapy had lower microvessel density [MVD] at prostatectomy: 64.6 (groups 1 and 2) versus 78.2 (groups 3 and 4; $p=0.15$). Average MVD at prostatectomy for African-American and Caucasian men (all groups) were similar ($p=NS$). Average MVD at biopsy of all African-American was 82. At prostatectomy, MVD decreased by 57% in African-American treated with combined hormonal ablation and Squalamine (group 1); MVD increased by 55% in African-American treated with prostatectomy alone (group 3; sample sizes too small for statistical analysis). Time between biopsy and prostatectomy averaged 8 weeks and was similar for both groups.

(7) Tasks 8 and 9: Follow-up

The numbers of enrolled patients and the minimal time of follow-up have limited evaluation of our end-point objectives. It is clearly premature to investigate benefits in disease-free survival. However, biochemical-free recurrence data is available for the first twelve patients. With the exception of the patient with grossly positive lymph nodes (and an aborted prostatectomy), all had undetectable PSA serologies at the 6 week follow-up. Subsequently, however, 2 of the 12 patients (17%) have had detectable PSA levels. Clearly, more patients and longer follow-up is needed to determine efficacy of treatment with anti-angiogenesis therapy prior to radical prostatectomy.

KEY RESEARCH ACCOMPLISHMENTS

The numbers of enrolled patients and the minimal time of follow-up have limited evaluation of our end-point objectives. It is clearly premature to investigate benefits in disease-free survival. However, we have begun preliminary investigations into expression of angiogenic factors, and this data has already been presented. Surgery has been completed safely and successfully in all men treated with the experimental agent and there have been few surgically-related adverse events attributable to the experimental agent. In fact, the more significant complications have occurred in the control group, including a recto-urethral fistula as well as a persistent lymphocele and abscess.

Our conclusions to date:

- Combination therapy is well-tolerated and safe
- Treatment with combined therapy has resulted in down-grading and down-staging in some patients
- Expression of cellular and molecular factors has changed (predictably) after therapy

We have been able to refine and expand our administrative infrastructure and our recruitment methods. Although we are only at 31% of projected enrollment, this was the result of a slow start and a temporary "hold" placed by the drug manufacturer. We have seen a steady and progressive increase in interested patients.

Most importantly, we are in the process of transferring the study to OHSU, which has a proven track-record of successful enrollment of patients into neoadjuvant anti-prostate cancer clinical trials due to a sophisticated infrastructure, dedicated medical and surgical urologic oncology faculty, a wide patient referral and encatchment area, and collaborations with other research institutions in the Pacific Northwest and California. **An extension without funds will be requested.**

REPORTABLE OUTCOMES

1. Abstracts and presentations:

- Brendler, CB, Charette, J, O'Laughlin, R, Landini, C, Shalhav, AL, Stadler, WM, Zagaja, GP, Desai, A, Holroyd, K, Sokoloff, MH. Neoadjuvant combination anti-angiogenesis and androgen ablation therapy in men with high grade and locally-advanced prostate cancer undergoing radical prostatectomy changes tumor histopathology. AACR Annual Meeting (Orlando; March 2004).
 - Starks, C, and Sokoloff, MH. Anti-angiogenesis therapy in African American men with prostate cancer. Summer Student Research Program, the University of Chicago (to be presented Chicago; August 2004).
 - Sokoloff, MH, Charette, J, O'Laughlin, R, Landini, C, Brendler, CB, Zagaja, GP, Shalhav, AL. Neoadjuvant combination androgen ablation and anti-angiogenesis therapy in men with high grade and locally-advanced prostate cancer [PC] undergoing prostatectomy. 27th Congress of the Société Internationale d'Urologie (to be presented Honolulu, October, 2004).
 - Sokoloff, MH. Anti-angiogenesis therapy for high risk prostate cancer. Society for Urologic Oncology Annual Meeting (to be presented San Antonio; April 2005)
2. The successful creation of tumor repository of prostatectomy specimens as well as several prostate needle biopsies.
 3. A University of Chicago Medical Student Summer Research project successfully evaluated the effects of treatment in the African-American patients and this student received an award for being one of the top 10 (of 72) projects (and presentations). A manuscript from this project is in preparation.
 - As a direct result of participation in this study, this student became very enthusiastic about clinical research in African American men and future research with him is in development designed to evaluate the role of clinical trials and treatment in African-American men with prostate cancer.
 4. Partially through experience and expertise gained with this trial, the Principal Investigator was recruited to head the Section of Urologic Oncology at OHSU, where plans are to continue this study and to invigorate enrollment and evaluation. Strategies for the latter are in place and speaking engagements throughout the Pacific Northwest are planned and being scheduled so that referrals to the trial will be facilitated.

CONCLUSIONS

This proposed clinical trial targets a population of men who are at high risk for treatment failure yet for whom there are few available therapies other than conventional surgery or radiotherapy. After a delay in initiation, a slow start on accrual, and a temporary "hold," our enrollment numbers are increasing and we are confident that we will ultimately meet all of our accruals. **An extension without funds will be requested.**

Data analyzed thus far indicates safety of neoadjuvant anti-angiogenesis therapy as well as postulated changes in angiogenic and pathologic factors.

We believe that we will be able to establish a role for systemic therapy in "high risk" prostate cancer patients, conclusively evaluate the efficacy of anti-angiogenesis therapy in prostate cancer, and validate our putative mechanisms of action which suggest that for maximum benefit, anti-angiogenesis therapy should be given in combination with androgen ablation therapy.

REFERENCES

None