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13. ABSTRACT (Maximum 200 Words)

This research attempts to develop the infrastructure for comparative studies of prostate cancer involving West Africa, the Caribbean and the United States (Ibadan, Nigeria, Kingston, Jamaica and Chicago, Illinois). Six essential areas are addressed: case recruitment, case characterization, tissue collection and storage, integrated database development, targeted laboratory expertise and pilot research. *Key Research Accomplishments During Year 1:* 1) established reliable recruitment and data collection strategies in Chicago and Kingston; 2) established a centralized data repository in Chicago consisting of demographic and clinico-pathologic histories and tissue (serum/plasma, leukocytes, erythrocytes and prostate tissue) for biochemical and molecular studies; 3) met solicitation and recruitment target in Chicago and Kingston; 4) successfully applied secure web-based technology to solve the problem of pathologists determining histologic grade of cases enrolled by consensus; 5) positioned to conduct preliminary biologic and molecular comparison of cases in from Chicago and Kingston, pending approval of the latter's assurances with OHRP; and 6) 2 manuscripts have been submitted, and 1 is in preparation from our pathology working group. *Problems & Remedies:* Results in Ibadan were disappointing. We plan to transfer the West African limb of the study to another site by February, 2002. Ghana (Accra) is the most promising.

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► INTRODUCTION

The mortality rate from prostate cancer among U.S. blacks is 2-times that of whites (1). An understanding of the factors responsible could provide insight into fundamental etiologic pathways (genes vs. the environment) of the disease. Unfortunately etiologic studies based on within-U.S. black vs. white contrasts are limited since race and social class, both of which influence environmental exposures, are highly confounded in the U.S. Black of the African Diaspora share a common genetic background, yet reside in widely different environmental settings/ Comparisons of prostate cancer across these populations could shed light on potential causative factors in the environment as well as inheritable aspects of the disease. However, such studies are complex.

The purpose of this research is to develop the infrastructure for comparative studies of prostate cancer involving West Africa, the Caribbean and the United States. This collaborative effort addresses six essential infrastructure areas: case recruitment, case characterization, tissue collection and storage, integrated database development, targeted laboratory expertise and pilot research.

► BODY

Approved Statement of Work

Task 1. Provide reliable recruitment of incident cases in region (Month 1-24)

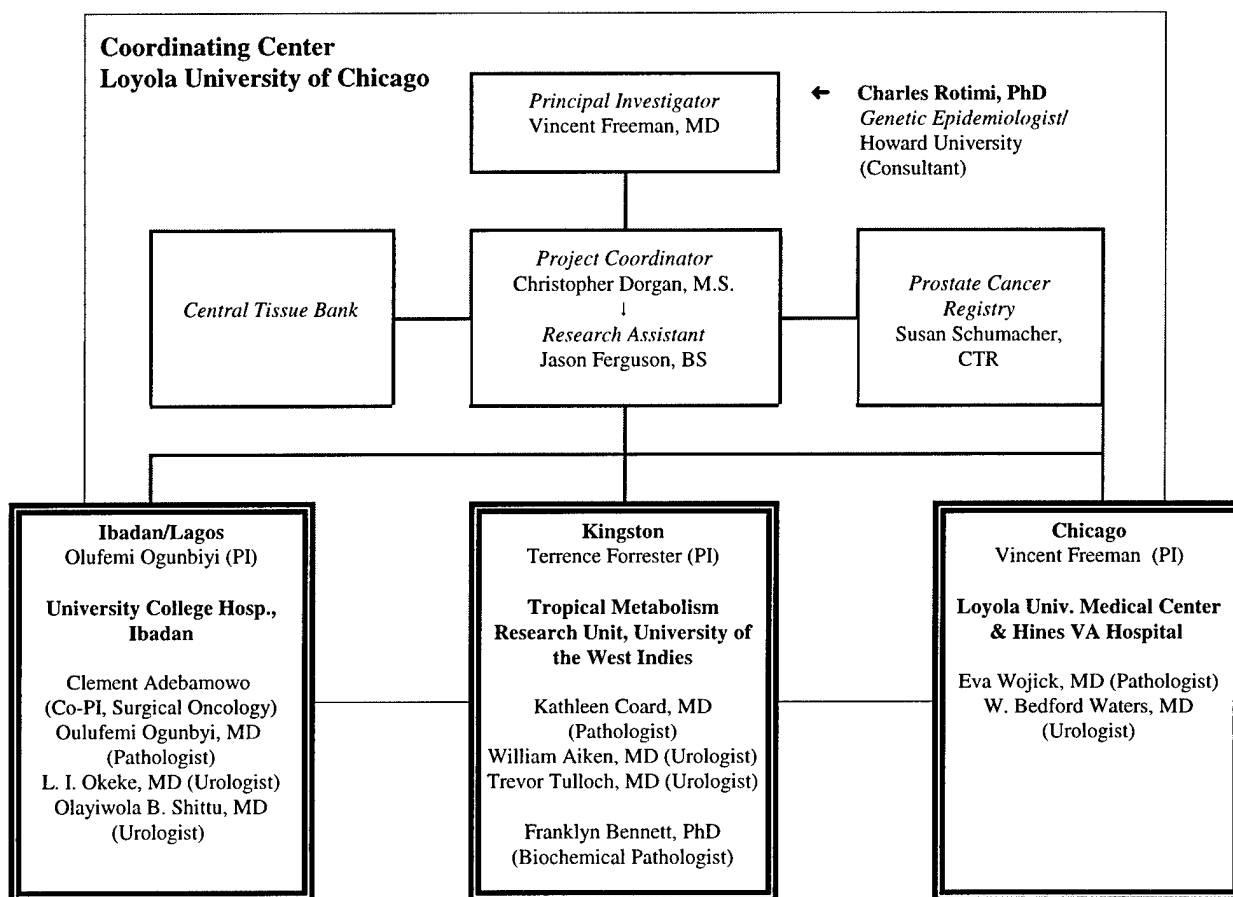
- a. Create consortia of urologist and pathologists in each region: southwest Nigeria (including Ibadan and Lagos), the island of Jamaica and Chicago, IL.
- b. Develop incident case recruitment strategies appropriate for each research site, with the goal of *soliciting participation* of 75% of newly diagnosed cases per site (25-50 cases per region) per year.

ACCOMPLISHMENTS

a. Consortia of urologists and pathologists created in each region. Although we had originally planned to activate the study at Lagos and Ibadan, we decided to focus our effort at the University of Ibadan first. This was done for three reasons. By the time we were notified of the award, 1) roads between Lagos and Ibadan had become increasingly in disrepair, increasing commute times to about 4 hours by motorcoach, 2) the University Hospital in Lagos had become more susceptible to strike and electrical outages, making recruitment less certain and specimen storage in the absence of backup generators hazardous, 3) and, perhaps most importantly, our Department had growing concerns about Dr. Olufemi Ogunbiyi's ability to lead the Southwest

Nigeria consortia. By chance, the University of Ghana became a viable alternate recruitment site around this time. However, we made the decision to proceed with Dr. Ogunbiyi at the University of Ibadan (UI) as the on-site PI for Ibadan since 1) that was his home institution, 2) the ground work to rapidly activate the project at UI had already been laid, 3) we believed that the pathology component and prostate cancer disease focus would likely bring out Dr. Ogunbiyi's best since those were his reported areas of greatest interests and 4) we invited a more reliable collaborator, Dr. Clement Adebamowo, a cancer surgeons with research programs in breast and prostate cancer, to serve as Co-PI for Ibadan. These changes resulted the following organizational structure. (Figure 1.)

Figure 1. Organization structure



b. Recruitment strategies developed in each of the three sites. The protocol for recruitment at each study site is located at our website (www.luhs.org/depts/prevmed/pc_study). Since access beyond the project abstract and directory is restricted to co-investigators, we have included description of the recruitment process at site the appendix (pages 15-16). Data collection at all three sites commenced by May 22, 2000 (Mo. 2), with case eligibility retroactive to January 1st, 2000 in Kingston and Ibadan. Data collected to date are summarized below (Table 1).

Table 1. Subjects enrolled and data collected to date through March 31, 2001.

| | Study Sites | | | Total |
|-------------------------------|-----------------|-----------------|------------------|------------|
| | <i>Chicago</i> | <i>Kingston</i> | <i>Ibadan</i> | |
| Recruitment Dates | 4/10/00-3/31/01 | 5/22/00-9/1/00 | 4/17/00-9/1/00 | |
| Duration | 50 weeks | 35 weeks* | 30 weeks* | 115 weeks |
| Subjects | | | | |
| No. Eligible | 62 | 74 | 49 | 185 |
| No. Solicited (% of eligible) | 57 (91.9%) | 61 (82.4%) | 28 (57.1%) | 146 (77.8) |
| No. Enrolled (% of solicited) | 52 (93.0%) | 61 (100%) | 24 (85.7) | 137 |
| No. Enrolled per week | 1.2 | 1.7 | 0.8 | - |
| Data Elements | | | | |
| Consent | 52 | 61 | 0 | 113 |
| Case Registry | 52 | 61 | 0 | 113 |
| Histopathology slide | 52 | 59 | 0 | 111 |
| Leukocytes | 51 | 59 | 0 | 110 |
| Plasma/serum | 51 | 59 | 0 | 110 |
| All data elements above | 51 | 59 | 0 | 110 |
| Prostate tissue | 16 | 12 | Not applicable** | 28 |

*Subject eligibility retroactive to January 1st, 2000 at these sites

**Surgery for prostate cancer is generally not performed since most patient present with advanced disease.

PROBLEMS

a. Obtaining Single Project Assurances (SPA's) from our foreign sites. On September 1st, 2000, subject enrollment in Jamaica and Ibadan were suspended because Single Project Assurances had not been obtained. This oversight was due to multiple factors: 1) Ms. Sonya Lewis, our first Human Subjects Protections specialist, told us that recruitment at the foreign sites could begin as soon as the project was approved and that such assurances could be obtained at later date; 2) our Department has been engaged in multi-site research studies involving foreign site for nearly 15 years now - we should have been much more skeptical about such an allowance, especially given the PCRPR rigorous HSRR approval process up to that point. Although we did question her

recommendation on two occasions, we did not pursue the correct answer more aggressively; 3) I was not notified of the requirement until mid to late August. It was alleged by a member of the Human Subjects Research Review Board that notification was delayed because the new Human Subjects Protection specialist assigned to our project, Ms. Catherine Smith, somehow had the wrong email address for me. Furthermore, because of a physical limitation, she could not speak to me by (still, notification by fax would have been a good alternative). Therefore, all of us share responsible for this error. On or around September 15, 2000, Lt. Col. Peirson informed me over the telephone that due to the extenuating circumstances surrounding the error, patients enrolled and data collected in Kingston and Ibadan up until that time could be included in the study. However, no further enrollment at these sites would be permitted until SPA's had been approved by the Program. At present, only Jamaica is nearing completion of the necessary paper which we expect will be forwarded to OHRP for approval in the next few weeks.

b. Dr. Olufemi Ogunbiyi. Dr. Ogunbiyi has had great difficulty meeting the responsibilities of on-site PI for Ibadan. As Table 1 indicates, recruitment fell substantially short of targets put forth in the Scope of Work. While we anticipated that recruitment at each would experience a 'learning curve', the recruitment experience in Ibadan was more a symptom of Dr. Ogunbiyi leadership. His work has been uneven at best and remains unverifiable as indicated by the absence of original informed consent forms or tissue specimens. Furthermore, he failed to worked effectively with the other members of the consortium and has repeated engaged in other collaborations which conflict with the research interests of this project. Finally, he use of funds from our Department's research stimulation account has been highly circumspect. *We regret that Dr. Ogunbiyi has ultimately proved to be an unreliable and untrustworthy collaborator.*

Proposed Remedy

1.) We plan to transfer the West African component of the study from Ibadan, Nigeria to Accra, Ghana, the site originally considered as an alternative to Ibadan. During a visit to the University Hospital in Accra last summer, Dr. Richard Cooper, our Department Chairman, discussed with collaborators the possibility of extending recruitment for this project to there in the future. Although the response was positive, plans never went beyond the talking stage since we were busy assisting Dr. Ogunbiyi with the project in Ibadan. Since then, Dr. Charles Rotimi, Director of Genetic Epidemiology at Howard University and consultant to this project, has begun discussing the matter more seriously with possible collaborators in Accra, and it now appears that our effort has enormous potential there. During a visit to Ghana earlier this month, Dr. Rotimi met with urologists and pathologist on staff to discuss the project and recruitment. Interest in serving as the West African site for this study is great. Furthermore, they have just hired an academic urologist from the U.K. who will set-up his practice this summer. According to Dr. Rotimi, our timing could not have been more fortuitous. I have been asked to visit the hospital, formally present this research, and meet with potential collaborators. This will be essential to successfully establishing study there. **OUR GOAL IS TO BEGIN ENROLLING CASES IN ACCRA BY FEB. 2002.** In Ghana, conditions for successful conduct of epidemiologic research in cancer are quite good: 1) the government is relatively stable, 2) the country has good infrastructure for transportation and commerce, 3) the University is excellent and, 3) because of its historical significance in the slave trade, it remains an important and safe tourist destination.

Task 2. Characterize each case using a common protocol (Month 3-12)

- a. Convene pathologists for a review of the Gleason grading system and group reading of representative slide of cases diagnosed in each region.
- b. Determine histologic grades ('Gleason sums') of cases subsequently enrolled by consensus via the Internet, using whole slide images created by Bacus Laboratory Inc. (www.mcs.net/~basuclab) (Lombard, IL.) And posted on an access-restricted website (webslides).
- c. Identify and monitor adherence to a common set of tumor and lymph node staging procedures.
- d. Collect baseline demographic, clinical and pathologic data via medical records abstraction, supplemented by patient interviews as needed.

ACCOMPLISHMENTS

a. Pathology Consensus Meeting was held in February, 2000. Pathologist from each region (Drs. Kathleen Coard-Kingston, Jamaica, Ogunbiyi-Ibadan, Nigeria and Eva Wojcik-Chicago, IL) met at Loyola University for a review of the Gleason grading system and group slide reading. They also came to agreement on procedures for processing and grossing surgical specimens.

The following abstracts have been accepted:

1. Freeman, VL, Coard, K, Ogunbiyi, O, Wojcik, EM. "Gleason scoring system: high level of agreement between pathologist from three countries" 90th Annual Meeting of the United States and Canadian Academy of Pathology, Atlanta, GA March, 2001
2. Wojcik, EM, Coard, K, Freeman, VL "Prostate cancer in African Americans and Jamaicans" 90th Annual Meeting of the United States and Canadian Academy of Pathology, Atlanta, GA March, 2001

The following manuscript have been submitted or are in preparation

1. Wojcik, EM, Coard, K, Freeman, VL. Prostate cancer in African Americans and Jamaicans. (submitted to *Urology*)
2. Freeman, VL, Coard, K, Ogunbiyi, O, Wojcik, EM. Gleason scoring system: high level of agreement between pathologist from three countries. (submitted to *Prostate*)
3. Coard, K, Freeman, VL, Wojcik, EM. Prostate cancer histopathology in 90 consecutive cases from Jamaica. (In preparation)

b. The BLISS System is operational. Of the 111 slides collected to so far: 75 have been converted to weblides and posted on our access-restricted website; 45 (~40%) have been consensus graded by Dr. Coard and Wojcik.

c. Baseline demographic, clinical and pathologic data collected on nearly all cases enrolled. Completeness of data elements are shown below (Table 2).

Table 2. Completeness of Case Registry Form data elements

| | Study Sites | |
|-----------------------------------|--------------|---------------|
| | Chicago (52) | Kingston (61) |
| Demographics | | |
| Name & address | 100% | 100% |
| DOB | 100 | 100 |
| Marital Status | 100 | 100 |
| Health History | | |
| Smoking status | 100 | 70 |
| Family history of prostate cancer | 100 | 91 |
| Diagnosis | | |
| Date | 100 | 100 |
| Method | 96 | 84 |
| Gleason Grade | 100 | 100 |
| Differentiation | 100 | 100 |
| Metastatic evaluation | 97 | 82 |
| Stage | | |
| Summary | 100 | 100 |
| SEER | 100 | 100 |
| Whitmore-Jewitt | 100 | 100 |
| TMN | 100 | 100 |
| AJCC Group | 100 | 100 |
| Treatment | | |
| Date | 100 | 100 |
| Treatment | 100 | 100 |

PROBLEMS & PROPOSED REMEDIES

Most of the data are collected prospectively. Smoking history, family history of prostate cancer, method of diagnosis and metastatic evaluation are the least complete. In Jamaica, missing data elements tended to be from patients recruited from the retroactive period, reflecting some of the challenges of collecting these data retrospectively through medical record review. Continue prospective collection of data elements along with staff reminders may help minimize missing registry data.

Task 3. Create a centralized repository for serum, plasma, leukocytes and prostate tissue for biochemical and molecular studies (Month 3-6)

- a. Collect plasma, serum, and leukocytes on each case at the time of diagnosis, as well as fresh normal prostate tissue at the time of surgery from those undergoing radical prostatectomy.
- b. Bank all specimens in Chicago (Department of Preventive Medicine, Loyola University) using an existing barcode driven specimen identification and storage system.

ACCOMPLISHMENTS

a. Approximately on 97% subject had blood collected for biochemical and molecular studies.

The high rate is due to the enrollment process where blood is collected at the time of enrollment. Also, we have obtained local IRB approval to collect red blood cell clot for RBC membrane fatty acids as well.

b. Specimens collected to date have been archived in the Coordinating Center in Chicago.

For your convenience, our common protocols for blood, tissue collection, processing and shipment have been downloaded from our website and are included in the appendix (pages 16-26)

PROBLEMS & PROPOSED REMEDIES

Although we have collected fresh prostate tissue from most patient undergoing radical prostatectomy, some were missed because surgeries ran late, and gland had to be fixed in formalin overnight (~32% of radicals). Unfortunately, there really isn't a remedy for this as we have no control over this.

Task 4. Link case demographic, clinical and pathologic characteristics to corresponding tissue samples using a computerized database (Month 7-12)

- a. Establish a single computerized registry of demographic, clinic and pathologic data for cases recruited in each region.
- b. Combine tissue and registry data into a single electronic record, linking case registry information to corresponding tissue samples using their unique barcode identification number.

ACCOMPLISHMENTS

a. Approximately 80% of all Case Registry Form have been entered into an MS Access data using the double-keyed method. The rest of the forms are awaiting completion of missing data elements. These will be entered into the database as they are completed. We have differed combining tissue and registry data into a single record until SPA's are approve in Jamaica and molecular studies have been completed.

Task 5. Pilot Studies: Conduct comparative studies of genes, nutrition and histopathologic markers of prognosis. (Month 18-36)

- a. Compare androgen receptor gene CAG repeat sequence lengths and the distribution of CYP3A4 receptor gene variants among 50 cases vs. 50 age-matched controls in each region, and how they relate to stage at presentation within and between groups.
- b. In 20 of these men undergoing radical prostatectomy in each region, measure prostatic levels of carotenoids, tocopherols, retinol and fatty acids. Compare mean levels, and explore how they relate to markers and whether they modify a relation between androgen and CYP3A4 receptor gene variants and markers of progression, raising the possibility of gene-nutrient interactions.

ACCOMPLISHMENTS

No molecular or biochemical analyses were performed pending approval of single project assurance in Kingston and Ibadan. Once Jamaica receive approval will conduct these analyses on cases from Chicago and Kingston first. We hope to complete these during during the summer of Year 2 (2001).

► KEY RESEARCH ACCOMPLISHMENTS IN YEAR 1

- Established reliable recruitment and data collection strategies in Chicago and Kingston.
- Established a centralized data repository in Chicago consisting of demographic and clinico-pathologic history and tissue (serum/plasma, leukocytes, erythrocytes and prostate tissue) for biochemical and molecular studies.
- Have met solicitation and recruitment target in Chicago and Kingston.
- Successfully applied secure web-based technology to solve the problem of pathologists determining histologic grade of cases enrolled by consensus.
- Positioned to conduct preliminary biologic and molecular comparison of cases in from Chicago and Kingston, pending approval of the latter's assurances with OHRP.
- 2 manuscripts have been submitted, and 1 is in preparation from our pathology working group (Dr. Coard, myself and Dr. Wojcik).

► REPORTABLE OUTCOMES

1) Centralized data registry consisting of baseline demographic, clinical and pathologic data collected on each case enrolled. The database is located in Chicago (see Table 2)

2) Centralized tissue repository in Chicago consisting of serum/plasma, leukocytes, erythrocytes and prostate tissue (see Table 1)

3) Abstracts:

A. Freeman, VL, Coard, K, Ogunbiyi, O, Wojcik, EM. "Gleason scoring system: high level of agreement between pathologist from three countries" 90th Annual Meeting of the United States and Canadian Academy of Pathology, Atlanta, GA March, 2001

B. Wojcik, EM, Coard, K, Freeman, VL "Prostate cancer in African Americans and Jamaicans" 90th Annual Meeting of the United States and Canadian Academy of Pathology, Atlanta, GA March, 2001

4) Manuscripts:

A. Wojcik, EM, Coard, K, Freeman, VL. Prostate cancer in African Americans and Jamaicans. (submitted to *Urology*)

B. Freeman, VL, Coard, K, Ogunbiyi, O, Wojcik, EM. Gleason scoring system: high level of agreement between pathologist from three countries. (submitted to *Prostate*)

C. Coard, K, Freeman, VL, Wojcik, EM. Prostate cancer histopathology in 90

consecutive cases from Jamaica. (In preparation)

► CONCLUSIONS

Implementing the project has been very successful in Chicago and Kingston. Processes essential to reliable recruitment, data collection and storage are now in place in these sites, moving us well along toward our goal of building the infrastructure to conduct comparative studies of prostate cancer disease determinants in each region. Our next challenge is to extend this apparatus to a suitable site in West Africa. Accra in Ghana is presently the best candidate, and we have already begun taking the necessary steps. It is hoped that recruitment can begin there by February 2002.

Finally, we would like to change the name of the study to: "Prostate Cancer in *West Africans*, Jamaicans and U.S. Blacks.

► REFERENCES

1. Greenlee, R.T., Murray, T., Bolden, S., Wingo, P.A. Cancer statistics 2000. (2000) 50:7-33. *Cancer J Clin*

APPENDIX

Case identification and enrollment, Pgs. 15-16

Blood and prostate tissue collection, processing storage and shipment, Pgs.17-26

Case Identification and Enrollment

Case identification and enrollment begins with the assembly of individual folders consisting of an informed consent document, questionnaires and all relevant intra- and inter-department forms. Each folder is pre-assigned a unique bar code ID generated by computer at the Coordinating Center in Chicago and its contents labeled accordingly. Folders will be mailed out to each site in batches. Additional forms may be added in each site depending on the needs of that site. Age range of subjects: age \geq 18 years old. Exclusion criteria: histologic diagnosis cannot be confirmed.

Enrollment includes subject identification and contact, informed consent, specimen collection and registry data collection. Specimen and registry data collection will be discussed in a separate section.

CHICAGO

Incident cases of prostate cancer are identified by the Project Coordinator as they are histologically confirmed. With the assistance of the clinical research nurse in department urologists, the Project Coordinator determines which newly diagnosed cases are African-American. The rest of the procedure is as follows:

1. When a potential subject is identified, he is assigned a folder and its unique bar code ID and entered into the subject log.
2. *Mail contact.* The project coordinator mails a cover letter from the subject's attending physician which describes the purpose of the study, the informed consent process and the procedure for data collection. The informed consent document and blood draw request form are sent for his review. The subject will have the option of waiting until the coordinator calls him within 5 to 7 days of his receiving the letter or contacting the coordinator sooner at the program office at (708) 327-9001.
3. *Telephone contact.* The subject is contacted by the project coordinator within 5 to 7 days of receiving the mailing. When making this follow-up telephone call, the project coordinator uses the same simple script to solicit participation from and assess the eligibility of each.
4. *Informed consent.* When a patient is deemed eligible, the project coordinator reviews the informed consent with the patient over the telephone. Afterwards, the subject is asked to sign the informed consent in the presence of a witness, complete return it to us in the mail using an envelop provided. He is instructed to keep the blood draw collection for future use per protocol.
5. *Completing Parts 1 & 2 of the Case Registry Data Collection Form.* Information regarding date of birth, marital status, smoking status and family history of prostate cancer can be collected over the telephone at this time.
6. If a patient declines, the recruitment process ends and he will not be contacted about this study in the future.

7. The dates and results of all contacts are recorded in the subject log.

Nigeria

New cases are identified in the urology clinic when patients are referred because they are symptomatic of prostate disease (local or complicated). Subsequent to consultations, the research assistant approaches the patient to discuss the study in progress. Patients are given the questionnaire to study, it is appropriately interpreted, and consent for participation is then sought. They are allowed to take the questionnaire away and return it at the next follow-up appointment. Those who agree to participate have their forms (case registry form etc.) filled and surgical specimens reported. Prostate tissue is obtained via surgery (transrectal Tru-Cut Needle biopsy blind) and sent to the pathology department where it is fixed in formalin.

Jamaica

The plan is for the urologists to see the patients in the clinic and they will biopsy cases they suspect of having prostate cancer and that biopsy will be sent to pathology. After confirming a case as positive for cancer, the urologist (or the project coordinator) will approach the patient about enrollment in the project and will start with the data collection.

As soon as the pathologist receives confirmation that a case is to be included in the project, they will collect those particular biopsies, review and follow criteria for selecting slides to send to the coordinating site; to be posted on the web site for consensus grading by the others.

Blood and Prostate Tissue Management: Collection, Processing, Storage and Shipment

The specimens to be collected and their use is presented in the table below:

| Specimen | | Mode of collection | Collection vehicle | Studies |
|----------|------------------------------|---|---|---|
| 1 | Serum | Phlebotomy | red-top, EDTA (lavender-top) | Serum makers (eg. PSA) Lipids Hormones |
| 2 | Plasma | Phlebotomy | Li-Heparin green-top (foil wrapped) EDTA (lavender-top) | Antioxidants |
| 3 | WBC ("Buffy Coat") DNA | Phlebotomy | EDTA (lavender-top) | Genotyping |
| 4 | RBC | Phlebotomy | Li-Heparin green-top (foil wrapped) EDTA (lavender-top) | Fatty acids |
| 5 | Prostate <i>Normal</i> | Sextant biopsy Radical prostatectomy | | Fatty acids Antioxidants Other micronutrients Hormones |
| 6 | Prostate <i>Malignant</i> | Sextant biopsy Radical prostatectomy | | DNA mRNA (protein expression studies) |

Blood can be collected (in 10 cc collection tubes) at anytime and does not have to be a fasting specimen. If Collecting malignant tissue (tumor blocks) has not been discussed previous. A protocol shall be developed over the next by the end of the first year.

BLOOD AND PROSTATE TISSUE MANAGEMENT:

BLOOD PROCESSING:

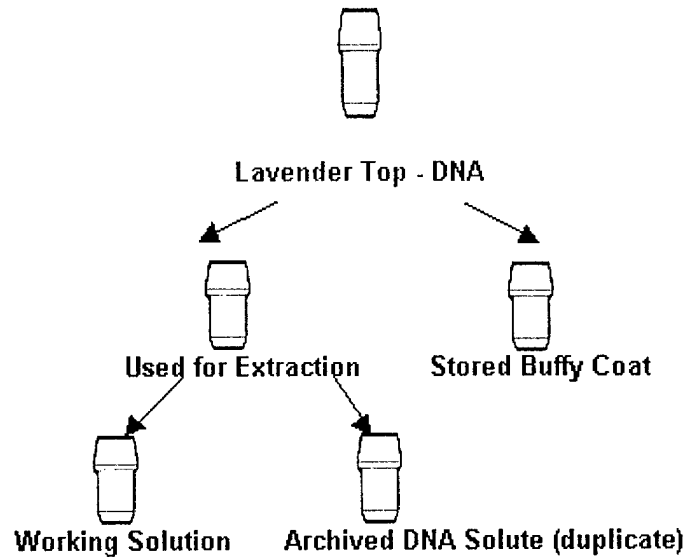
1 red top (10cc), 1 green top (10cc) and 1 lavender top (10cc) tubes.

- 1) Green- and Lavender-topped tubes are refrigerated for ½ - 3 hours.
- 2) Red-top tube sits at room temperature for 1 hour.
- 3) Tubes are then inverted 5x, then centrifuged for 12 minutes at 2500 rpm.
- 4) Using sterile plastic pipettes, the tissue is distributed as follows:

| Tube | Expected Minimum # of vials | Aliquot volume |
|------------------|------------------------------------|--|
| a. Green top: | 4 1-2 | 1-ml aliquots (foil-wrapped) in tubes marked "hep plasma" (in green top cryovials) 1-ml aliquot in tubes marked "hep buffy coat" (in green top cryovials) |
| b. Red top: | 3 | 1-ml aliquots - "serum" (in red top cryovials) |
| c. Lavender top: | 3 2 | 1-ml DNA ** (in yellow top cryovials) Labels: Store DNA (buffy coat), Duplicate (archived solute), & DNA WKG SOL (Working Solution DNA), 1-ml plasma (in Blue top cryovials) |

** Buffy coat for DNA will be split in half. One half will undergo DNA extraction the other half will be stored frozen for future genetic studies. The DNA that is extracted will be further divided into two samples. One sample will serve as the 'working' solution, the other will serve as a duplicate.

BLOOD AND PROSTATE TISSUE MANAGEMENT:



- 5. Storage:** Cryovials are labeled with the corresponding barcodes, covered with freezer tape, and placed into a plastic ziplock bag and refrigerated in a -70°C freezer until it is time for shipment to the coordinating site.

At the coordinating site, the blood samples are stored in a -70°C freezer in cryoboxes that have a corresponding grid which specifically maps out the location of unique specimen in the cells of the cryobox using the barcode numbers.

| | | | | | | | | | Barcode | Cell Location |
|----|----|----|----|----|----|----|----|----|---------|---------------|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | | |
| 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | | |
| 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | | |
| 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | | |
| 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | | |
| 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | | |
| 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | | |
| 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | | |
| 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | | |

DNA EXTRACTION

1. Gather the following supplies:

BLOOD AND PROSTATE TISSUE MANAGEMENT:

- a. One 15 mL Falcon tube per sample
 - b. A handful of disposable pipettes
 - c. Vials or containers for discarded RBC
 - d. One pipeter and two plastic pipettes (label one 'RBC' and another 'Lysis')
 - e. Styrofoam rack for Falcon tubes
 - f. Absorbent sheet for counter area
1. Add 10 mL of "RBC Lysis Solution" to each 15 mL Falcon tube (label each tube accordingly)
 2. Transfer *at least* 1 mL of blood sample to each Falcon tube; cap tubes
 3. Set timer for 10 minutes. Invert 2x, let sit. Invert 2x again at 5 minute mark
 4. Spin at 3200 rpm for 10 minutes
 5. Pipette off lysed RBC, **LEAVE WHITE BLOOD CELLS IN BOTTOM**
 - If unclear, add between 4-6 mL of "RBC Lysis Solution"
 - Repeat steps 3-5
 6. Vortex each Falcon tube for 5 seconds
 7. Add 5 mL "Cell Lysis Solution"
 8. Vortex to homogenize solution (or pipette solution up and down until homogeneous)
 - Does not need to be perfectly homogeneous, clumps will dissolve in bath
 9. Place in water bath overnight

NEXT DAY

1. Add 2 mL "Protein Precipitation Solution"
2. Cap, vortex each sample for 20 seconds
3. Spin at 3200 rpm for 30 minutes
4. In a **NEW** Falcon tubes, add 6 mL of isopropanol
5. Pour off the liquid of sample into isopropanol tubes (leave solid – discard)
6. Invert repeatedly until solution is clear and DNA is clumped
7. Spin at 3200 rpm for 3 minutes
8. Pour of solution in sink (be careful – leave pellet!!)
9. Place upside-down in rack and let air dry over paper towel for a few minutes
10. Add 5 mL 70% EtOH to pellet
11. Invert to loosen pellet from the bottom
12. Spin for 1 minute at 3200 rpm
13. Pour off solution in sink, repeat step 9, dry for 15 minutes
14. Add 500 μ L "DNA Hydration Solution"

PROSTATE TISSUE COLLECTION (Overview):

Upon surgical removal, the prostate gland will be placed into a container of sterile saline and

BLOOD AND PROSTATE TISSUE MANAGEMENT:

delivered to the pathology department at each site. The specimen is then assessed with sterile instruments and gloves for the presence of palpable tumor. Two to three grams of fresh normal prostate tissue will be harvested from the peripheral zone prior to fixation in formalin according to the method described by Bova et. al.

- Frozen section histologic controls will then be obtained from the same areas to ensure that apparently "normal" tissue does not contain cancer.
- The tissue is then placed into a one inch plastic bag and stored frozen in a larger ziplock bag. Each large ziplock bag will contain 20 prostate tissue samples. On the outside of the bag, the barcode ranges will be written in permanent marker (eg. 40001-40020).
- Every two months, samples from Jamaica and Nigeria will be shipped on dry ice to the laboratory of the Chronic Disease Network located in the Department of Preventive Medicine, Loyola University for frozen storage.
- Specimen collection will commence in Year 1.

Prostate, Radical Prostatectomy

The specimen is received (*fresh, fixed*) in a single container labeled with (patient name and identification number) and consists of a prostate gland measuring (*Xcm x Xcm x Xcm*) and weighing (*X g*). The attached (seminal vesicle, vas deference, urethra) measure (*Xcm, Xcm, (Xcm)*), respectively. The gland is (*symmetrical, asymmetrical, nodular*). The external surface is (*pink – tan, smooth, with areas of yellow discoloration measuring up to X cm and located at...*). The left lobe is inked yellow and right lobe is inked blue. Apex and bladder neck are amputated and serially sectioned at vertical parasagittal plane at 3 mm intervals. The remaining prostate is coronally sectioned at 3-5 mm intervals. Cross sections revealed (*areas of yellow discoloration and softening, well circumscribed, firm, bulging nodules, areas of red-brown discoloration, necrosis measuring up to ...cm and located in the*).

RADICAL PROSTATECTOMY – GROSSING PROTOCOL

- Orient the specimen (remember – apex is at the bottom, posterior {rectal} surface is flat and smooth)
- Measure the specimen (3 dimensions: Xcm by Xcm by Xcm)
- Measure seminal vesicles, urethra, vas deferense
- Weigh the specimen
- Ink the specimen; two different colors for left and right lobe
- Amputate apex and bladder neck and serially section in the vertical parasagittal plane (same as cone)
- Amputate tips of seminal vesicles - margins of resection (to fit a cassette) cut longitudinally
- Serially section the remaining gland perpendicularly to the long axis of the gland at 3mm intervals, from apex to upper base.
- The last slice (upper base) with attached seminal vesicles section sagittally

Sections should include:

- Entire apex (coned)
- Entire bladder neck (coned)
- Entire upper base with inserted seminal vesicles (sagittally sectioned)
- Submit 4 cassettes per 1 cross section and indicate R & L anterior and R & L posterior

PROSTATE, RADICAL PROSTECTOMY

- If large prostate – submit every other slice (not to exceed 3 slices)
- If small prostate (approximately 30 grams) – submit the entire specimen

PROSTATE CANCER STAGING SUMMARY

| | Possible Responses |
|---|---|
| Histologic type | Acinar or other |
| Tumor grade | Well, Moderate, Poor, or Undifferentiated |
| Gleason score | Total (X + Y) eg. 7(3+4) |
| Tumor amount (percent) | X% |
| Tumor location (multifocal, solitary) | Multifocal or Solitary |
| Extracapsular extension (≤3mm, >3mm) | Present/Not Present |
| Margins | |
| Apex | Yes, No, Unknown |
| Bladder neck | Yes, No, Unknown |
| Posterior (right, left) | Yes, No, Unknown |
| Anterior (right, left) | Yes, No, Unknown |
| Seminal vesicles (base, margin) | Yes, No, Unknown |
| Other organs | Yes, No, Unknown |
| Perineural invasion | |
| Within prostate gland | Yes, No, Unknown |
| Outside prostate capsule | Yes, No, Unknown |
| Lymphovascular invasion | Yes, No, Unknown |
| Prostatic intraepithelial neoplasia (amount) | Yes (%), No |
| Regional lymph nodes (metastasis/total) | Yes, No, Unknown |
| Right pelvic | X/Y eg. 0/6 |
| Left pelvic | X/Y eg. 0/6 |
| Maximum dimension | X/Y eg. 0/6 |
| Extranodal extension | X/Y eg. 0/6 |
| Ancillary studies | Yes, No, Unknown |
| pTNM | eg. pT3b, N0, Mx |

GRADING

The histologic grade of each new case will be determined by consensus over the Internet using the Gleason system.

Protocol for consensus grading.

1. Each month, pathologists in each region will select the slide that they believe best characterizes the Gleason sum of each new case and send them to the project coordinator in Chicago.
2. The project coordinator will hand deliver the slide to Simon Saraugh in Cytometry (708 327-2627) who will create and mount web-images of each slide using the BLISS system. The website will be accessible only to pathologist and selected study personnel. Each slide will be labeled in a way that does not identify site of origin.
3. Simon will notify the project coordinator when slide are ready for viewing over the Internet. The project coordinator will notify each pathologist by email that the slides are available for grading. (Need Web address.)
4. (Contact Dr. Wojcik for protocol for reporting results and reaching consensus per February meeting.)
5. Slides will be returned to pathologists approximately every 6 months.

STAGING OF TUMOR AND LYMPH NODES

1. *Tumor*-(clinical/pathologic): digital rectal examination/ sextant biopsy of gland, including seminal vesicles.
2. *Lymph nodes*-computerized tomography if PSA > 20/bilateral dissection of pelvic lymph nodes (N=2) at the time of radical prostatectomy. This reflects the current practice in each Chicago and Kingston.

Adherence will be monitored by the project coordinator at the Coordinating Center. Since bone radionucleotide scanning will not be available at the Nigerian sites at this time, a standard for evaluating distant metastases has not been set as yet. Stage will be assigned using the 1987 version of the TNM classification system of the International Union Against Cancer and American Joint Committee on Cancer.

Protocol for Shipping Biological Samples - International

1. Insure there is enough dry ice to maintain proper temperature for several days – to accommodate potential shipping delays (20 kg if ice is chipped, 10-15 kg if ice is in solid block). In no instance should any other freezer materials (ie, chemical ice packs) be used in lieu of dry ice. The box used should be a styrofoam-lined shipping box designed just for this purpose. The size chosen should be appropriate for the amount of ice and samples, but not so large that there is a lot of dead air space.
2. **Blood:** For shipping purposes the cryovials will be stored in the Nunc* Cryostore* Polyethylene-Coated Chipboard Storage Boxes. Each Box has a 81 vial capacity; at 12 vials per patient, approximately 6 subject's entire blood sample can be stored consecutively per box. Each box will be assigned a number.

There is a grid that identifies the location of each subject's specimen by barcode will provide inventory information concerning the specimen (i.e. box number, the number of cryovials, bar code range, site, and shipping date). The grid is saved on the zip disk in the data management folder. A hard copy of the grid is also enclosed in the appendix.

Put all the grids in a ziplock bag and ship them with the tissue in the Multipurpose Bio-mailers. Approximately 5 storage boxes can fit in the bio-mailers with ample room for dry ice.

Prostate: Group the prostate tissue in sturdy plastic bags that will not break apart when put on dry ice - this will minimize the need to dig through the ice to find all samples upon arrival and help insure all samples are found. Along with the prostate tissue samples send an inventory list of barcodes (in a ziplock bag) that correspond to the tissues being shipped.

3. After samples and dry ice are in the box, seal around the styrofoam box lid with packing tape. This will minimize evaporation. On the top of the styrofoam box lid, on the inside and the outside of the cardboard box, tape complete shipping information directly to the styrofoam (ie, shipper name and address and ship to name and address). This is in case the air-bill falls off outside of cardboard box.
4. Complete air-bill as required by shipping company.
5. Create an invoice for US customs*. On this sheet include shipper name and address, ship to name and address, number of samples and type of samples (ie, plasma, DNA, fixed tissue, etc). Also include on this invoice and on the air-bill – these samples are not infectious and do not carry HIV or HBV, etc. It's also good to include a line about collaborative research between so-and-so and so-and-so. They also require a dollar value for the shipment (usually say the shipment has no commercial value).

*The Customs Declaration is enclosed in the appendix along with the Shipment schedule.

SHIPPING ADDRESS:

The different sites will ship tissue in coolers with dry ice to the coordinating site.

Dr. Vincent Freeman

Department of Preventive Medicine & Epidemiology
Room 2880-1
2160 South First Avenue BLG 105
Loyola University Medical Center
Maywood, IL 60153-9931

Please contact the project coordinator Janet Baatile via e-mail to inform her that a shipment will be sent and attach the inventory list of all specimen to be shipped.

Data Management:

Each subject's identification number, tissue and blood inventory, demographic information, Case Registry and Abstraction Form, and shipment dates to Loyola will be documented in the Prostate Cancer Patient Log at the coordinating site.