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14. ABSTRACT This report covers the first year of funding of this project. The goal of the project is to identify a metabolomics signature, measured on biopsy tissue, serum, or urine, that can distinguish men with only Gleason score 6 tumor in their prostate vs. men whose biopsy indicates Gleason score 6 but who harbor higher grade (Gleason score > 7) tumor that was not sampled by the biopsy. This report deals largely with unanticipated problems resulting in patient and tissue accrual that are behind schedule, and our plans to rectify these problems and get the study on track during Year 2.					
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FOREWORD

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NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

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NA In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI – Signature

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INTRODUCTION

This is the first Annual Report for this project. It provides a summary of progress and problems during the first year of funding, and plans for addressing these problems and ensuring progress during Year 2 to meet the study objectives. The objective of this case-control study is to determine whether a metabolomic profile can identify prostate cancer patients with a phenotype indicative of low risk of disease progression who can safely be followed with active surveillance (AS). In the Johns Hopkins Active Surveillance program (JHAS) men are eligible only if they have biopsy Gleason score of 6; any presence of Gleason pattern 4 or Gleason score 7 or higher in the biopsy makes them ineligible for AS. However in approximately 20-25% of men whose biopsy indicates only Gleason score 6 tumor there is higher grade tumor in the prostate that was not sampled by the biopsy. Thus, the goal of this project is to determine whether metabolomic profiling can identify a signature in biopsy tissue, serum, or urine that can distinguish men with prostate cancer with only low grade (Gleason 6) tumors in their prostate from cases with Gleason 7 or higher tumors that were not captured by the biopsy. Development of the signature will also consider potential confounding variables such as age, body mass index, medication usage, and dietary patterns.

BODY

Study Progress

This section will describe the following:

- (a) original study objectives and specific aims
- (b) progress during Year 1
- (c) delays in obtaining tissue from eligible participants
- (d) plans for improving the participant/tissue accrual rate during Year 2

Study Objectives and Aims

Develop a metabolomic profile that can be measured in urine or serum and which identifies men with a low risk phenotype who can safely be followed with active surveillance.

Specific Aims:

Aim 1. Develop distinct metabolomic profiles to discriminate pure Gleason 6 tumors (without grade 4) from pure Gleason 7 (3+4 or 4+3) tumors in tissue from men undergoing prostatectomy, and determine whether the profile can be detected in matched urine or serum.

Rationale. The presence of any Gleason grade 4 (Gleason score ≥ 7) is indicative of a phenotype that is potentially lethal and requires treatment. Metabolomic profiles will be developed from tissue derived from prostatectomy specimens that exhibit only a single Gleason score category (Gleason score 6 vs. 7). Matched urine and serum will be collected at the same time, and profiles will be developed from these samples as well. To ensure that the profile captures the phenotype of Gleason grade 4 we will use clinically significant tumors from men who underwent immediate surgery (i.e. not AS patients). The goal is to develop profiles that can be used subsequently with AS patients as indicators at entry to AS of Gleason grade 4 that may have been missed on biopsy, or evaluated during follow-up to detect Gleason grade progression.

Aim 2. Determine whether the metabolomic profile developed in Aim 1, when measured in baseline urine or serum samples from AS men, can distinguish those who do vs. do not progress. Also, correlate *changes* in urine or serum metabolomic profiles from baseline to follow-up samples in AS men who do and do not progress.

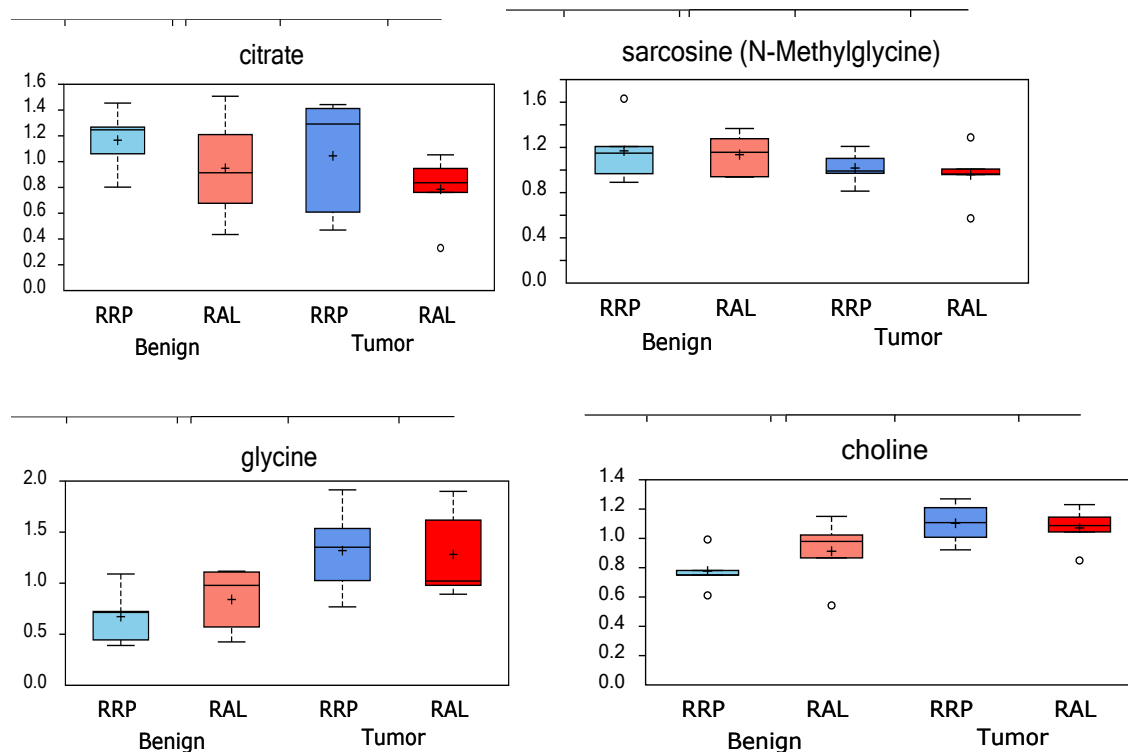
Rationale. The primary risk in classifying eligibility for AS based on needle biopsy is that the biopsy may miss a dominant focus of Gleason grade 4 elsewhere in the tumor, or that a Gleason 3+3 tumor with a *true progression phenotype* cannot be distinguished from one that will not progress. This aim will determine whether the metabolomic profile for Gleason grade 4 developed in Aim 1 from clinically significant tumor samples can detect the high grade or progression phenotype in urine or serum samples (whichever performed best in Aim 1) from an independent group of AS patients with apparently low grade small volume tumors.

Progress

Initial activities. During the first 6 months of the study we developed a database for tracking enrollment (see Informed Consent Process, below), established the Material Transfer Agreement (MTA) with our industry collaborator, Metabolon, established the process for enrolling participants, and coordinated with the Pathology Department the protocol for harvesting frozen tissue samples from enrolled participants with the Pathology Department.

Pilot study. We also implemented a pilot study during the initial study period. In February 2012 we sent matched tumor and benign tissue samples from 5 open prostatectomy (RRP) cases and 5 robotic assisted laparoscopic prostatectomy (RAL) cases (including 3 Gleason 6 and 7 Gleason 7 cases) to our collaborators at Metabolomics to ensure that their laboratory procedures worked well with our samples and to optimize the protocol. In particular, we wanted to determine whether samples obtained from RRP cases differed from RAL cases; the latter now comprise the majority of prostatectomies performed here.

Data were received in March 2012. A total of 405 biochemicals were identified in this data set (304 named biochemicals + 101 structurally unnamed biochemicals). There were no apparent differences between specimens obtained by RRP vs. RAL; this was true for tumor as well as normal. Given the large number of metabolites measured and the small sample size it was expected that some differences would be detected purely by chance. The number of specimens is too small for inference, and the pilot sample set was assembled only to ensure that adequate signal was obtained and to look for consistent differences between RRP and RAL, which were not observed. Below are box plots showing comparison of RRP vs. RAL, in tumor and normal, for a few exemplar metabolites. These results demonstrated that the metabolomics analysis performed by our collaborator Metabolon was feasible with our samples, and suggested that differences in surgical technique were unlikely to induce artifact.



Participant enrollment and tissue collection. Upon completion of the above activities, patient enrollment began in April 2012. To date, the study has enrolled 31 cases, where enrollment encompasses participant consent, satisfaction of eligibility criteria, collection of sufficient frozen tissue from the prostatectomy, matched to serum and urine collected prior to surgery, and collection of clinical, pathology, epidemiology and dietary data.

This number is clearly much lower than we anticipated, and lower than needed to complete

accrual of our target sample size of 50 men with pure Gleason 6 tumors and pure Gleason 7 tumors within the 3 year time frame. The reasons for the low enrollment primarily reflect reduced number of prostatectomy cases meeting the stringent requirements for frozen tissue harvesting (as described below), and some change in personnel.

Fewer cases than anticipated were harvested for frozen tissue: As a general rule, prostatectomy cases are only selected for harvesting of frozen tissue if the prostatectomy specimen weighs more than 25 grams (with smaller specimens there is concern that taking the research samples may compromise appropriate pathologic evaluation for patient care), and also meets one of the following criteria:

- a. Diagnostic biopsy specimens have at least 3 cores containing cancer
- b. At least one diagnostic biopsy core has $\geq 50\%$ of the core occupied by tumor.
- c. Gleason pattern 4 or 5 in the biopsy

Of these cases, 4 punch biopsies (7 mm diameter) are taken from a palpable nodule if present in the prostatectomy specimen, or if there is no palpable nodule, punch biopsies are taken from the area in the prostate where the biopsy report indicated the presence of cancer.

Because men undergoing prostatectomy are increasingly being diagnosed with smaller tumors with no clinically evident palpable nodule based on digital rectal exam, and only 1 or 2 positive biopsy cores, there are fewer tumors that meet the criteria for harvesting. Typically, about 15-20% of prostatectomies at Johns Hopkins meet these criteria and are selected for harvesting frozen tissue. When we factor in our study-specific criteria that the biopsy indicate ONLY Gleason score 6 or ONLY Gleason score 7 for an individual patient the number of eligible patients for harvest gets even smaller, since many men with Gleason 7 on biopsy also have at least 1 core with Gleason 6 tumor. Once the punch biopsies are taken an H&E stained slide is prepared to determine whether the core contains cancer. Of patients enrolled in this study whose tumors have been harvested, 65% yielded at least one core with cancer.

Personnel changes: The Pathology Fellow (Dr. Bora Gurel), who was to oversee the Pathology Tech performing harvesting for this study, left Johns Hopkins to return to his home country. The process of recruiting a new Pathology Fellow took some time, and this process is driven by the Pathology Department, not this project. Furthermore, the general frozen tissue harvesting activity performed by the Pathology Department was also slower than normal because 2 of the pathology technicians who assist in the general harvesting effort (although these positions are not funded by this grant) went on maternity leave during Year 1. In addition, the study Research Nurse is dealing with a serious chronic disease and had to take more sick leave than usual, which also reduced the number of eligible patients who could be consented (see next section).

Additional considerations:

Informed consent process. The informed consent process is also very time sensitive. Because HIPAA requirements prevent us from enrolling men in the clinic when they come to discuss and schedule their surgery we use the following process:

- The Research Nurse reviews the surgery schedule weekly to identify men who have scheduled a prostatectomy (we have a HIPAA Waiver of Authorization for this activity)
- A packet is mailed to eligible men describing the study and including a consent form for them to sign and mail back in a postage-paid envelope
- When the consent form is received in the mail, the Research Nurse checks the biopsy log maintained by the Pathology department to determine if the biopsies meet study eligibility criteria
- If the consented patient meets eligibility criteria, the Pathology Tech is notified to harvest the specimen on the date of surgery (if it meets harvesting requirements, above)
- If the consent form is not received by 1 week before surgery, the Research Nurse calls the participant and asks them to bring the consent form with them when they come for pre-operative blood testing (day before surgery), when she will arrange to collect the form.

If the consent form is not received in time, the patient cannot be included in the study.

Other exclusion criteria. Patients with a history of any previous non-skin cancer or a history of transurethral resection of the prostate (TURP) for benign prostatic hypertrophy are excluded. Somewhat surprisingly, approximately 25% of men who meet other eligibility criteria for the study have a history of previous cancer or TURP.

Since April 2012 there have been 481 prostatectomy cases reviewed. Among these 112 patients met eligibility criteria, 55 were consented, and 31 had tissue harvested for this project.

Strategy to improve accrual rate in Year 2

Clearly, this rate will not allow the study to achieve the enrollment goals. Some of these factors are beyond our control, i.e. the pathology criteria for prostate size and extent of tumor, and the percentage of cores obtained that don't have tumor. However, 44% of cases that could not be harvested were due to our study-specific criteria requiring biopsies to contain only Gleason 6 or only Gleason 7, and excluding men with previous cancer or TURP. An additional 5% of patients were lost because the consent form arrived too late. Therefore, we have decided to drop our requirement for pure Gleason category in the biopsy, and also to allow enrollment of men with prior cancer (as long as they have not had any systemic treatment in the last 6 months and do not have progressive disease) or TURP. Although we hypothesized that the biology of a tumor with

only Gleason 6 or only Gleason 7 might be different from that of a tumor with both Gleason score categories, the Gleason score really reflects the Gleason grade (pattern) of individual glands, and we dissect both Gleason pattern 3 and pattern 4 separately from the frozen cores. So the metabolomics analysis will still be able to compare metabolites in pattern 3 glands vs. pattern 4 glands. We can also compare metabolomics patterns of pattern 3 or 4 cores from cases with both Gleason 6 & 7 to those from cases that only have Gleason 7 to see if there are consistent differences. Regarding previous cancer, it is unlikely that a previous cancer will greatly affect the metabolomic pattern, and we can similarly compare patterns from men with and without previous cancer to see if there are consistent differences. While both of these changes to the criteria are a departure from our ideal study design, they will allow the study to be completed and are not likely to have a substantive effect on our results.

Finally, to try to decrease the number of patients whose consent arrives too late, we will call patients 1 week after sending the packet (rather than 2) to check if they received it and have questions. This will provide a stimulus while receipt of the package is fresh in their minds and likely will speed up the process of submitting the consent form. It also may decrease the number of patients who decline to participate.

We will evaluate these changes after the first 3 months to determine if we are significantly improving our accrual rate. If the accrual rate is still too low we will consider using previously collected frozen tissue obtained as part of the Prostate Cancer Biorepository Network (PCBN), a prostate tissue bank, funded by a Dept. of Defense grant to Dr. Trock, that provides tissues to prostate cancer researchers. Although these tissues may not have matched serum or urine, they will still allow the analysis of tissue, which is the focus for developing the metabolomics signature. Another potential source of frozen tissue would be the Johns Hopkins Prostate SPORC Tissue Bank, which is funded by the National Cancer Institute Prostate Cancer Specialized Program of Research Excellence (SPORC) award to Johns Hopkins.

KEY RESEARCH ACCOMPLISHMENTS

Demonstrated feasibility of measuring metabolite profiles in our frozen tumor samples, and lack of artifact induced by differences in surgical technique.

REPORTABLE OUTCOMES

None

CONCLUSIONS

During the first year of this project we encountered significant difficulties in reaching accrual goals. Some of these are system-related and cannot be improved upon. However, we may be able to improve upon approximately 50% of the patients lost to accrual by changing our eligibility criteria and our procedure to follow-up on invitations to men to participate in the study. We will review the impact of these changes after they have been implemented for 3 months, and if the accrual rate is still too low we will consider supplementing with tissues from the PCBN and the prostate SPORC.

REFERENCES

None

APPENDICES

1. List of abbreviations and acronyms (p. 16).
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LIST OF ABBREVIATIONS AND ACRONYMS

AS	Active surveillance
MTA	Material Transfer Agreement
PCBN	Prostate Cancer Biorepository Network
RAL	robot assisted laparoscopic prostatectomy
RRP	radical retropubic open prostatectomy
SPORE	Specialized Program of Research Excellence
TURP	transurethral resection of the prostate

Meeting abstracts during reporting period: None in connection with this project

Publications during reporting period: None in connection with this project

Manuscripts in preparation: None in connection with this project

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