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PRINCIPAL INVESTIGATOR: Bruce J. Trock, Ph.D.

CONTRACTING ORGANIZATION: Johns Hopkins School of Medicine
Baltimore, MD 21287

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Bruce J. Trock, Ph.D.

E-Mail: btrock@jhmi.edu

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14. ABSTRACT
This report covers the second 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 $\geq$ 7) tumor that was not sampled by the biopsy. This report deals largely with modifications to the study to accommodate the unfortunate death of the study Research Nurse, and the development of a metabolomics signature for aggressive disease by our industry partner, Metabolon, Inc. These events have caused a shift of focus for Aim 1 to validation of the Metabolon signature (rather than discovery of a new signature), and de-emphasized the need for frozen tissue in favor of matched urine and serum. With these changes we anticipate being able to complete the study and achieve our planned objective – determining whether a metabolomics signature can discriminate men who can vs. cannot safely be offered active surveillance - by the end of Year 3.

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Prostate cancer, active surveillance, Gleason grade, metabolomics

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FOREWORD

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NA In conducting research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

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.

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INTRODUCTION

This is the second Annual Report for this project. It provides a summary of progress and problems during the second year of funding, and plans for addressing these problems and ensuring progress during Year 3 to complete the study within the original time frame. In addition to problems encountered in Years 1 and 2 the state of the science has continued to progress. In response to both the problems and the changes in scientific knowledge the study aims are slightly modified. However, these changes do not affect the objective of the study, which continues to evaluate whether a metabolomic profile can distinguish prostate cancer patients with a phenotype indicative of low risk of disease progression (i.e. only Gleason 6 tumors in their prostate) who are candidates for active surveillance vs. patients with higher risk (Gleason 7 or higher) tumor in the prostate that was not captured by the biopsy specimen.

BODY

Study Progress

This section will describe the following:

(a) original study objectives and specific aims
(b) progress and problems during Year 2
(c) plans for completing the study during Year 3

Study Objectives and Aims

The objectives and aims were stated in the previous annual report but are repeated here for context and to emphasize minor changes to the aims.

Objective. 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.

Aim 2. Determine whether the metabolomic profile developed in Aim 1, when measured in baseline urine or serum samples from active surveillance 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 active surveillace men who do and do not progress.

Rationale. The presence of any Gleason grade 4 (Gleason score ≥7) is indicative of a phenotype that is potentially lethal and requires treatment. Decisions for active surveillance are based on largely on the Gleason score in biopsy tissue. However, approximately 25% of men whose biopsy shows only Gleason 6 tumor harbor undetected Gleason 7 tumor elsewhere in the prostate that was not sampled by the biopsy; such men should probably receive treatment instead of active surveillance. Thus, our goal was to determine whether a metabolomics profile measured in urine or serum could supplement the information from the biopsy to identify men with Gleason 7 tumor missed by biopsy.

Our original plan was to develop metabolomic profiles from Gleason 6 and Gleason 7 prostatectomy tissue. Matched urine and serum samples from these same patients would then be evaluated to see if the profile developed in tissue could also distinguish Gleason 6 vs. 7 tumors when measured in urine or serum. However, our industry collaborator who will perform the actual metabolomics analyses on this project (Metabolon, Inc.) **has recently identified a metabolomic profile associated with aggressive prostate cancer**, derived from prostate tissues from 2 distinct patient cohorts with a larger sample size than we had originally proposed in our study (McDunn 2013). Thus, rather than Aim 1 being a discovery aim to identify metabolites in tissue that distinguished low vs. higher risk, we now propose to validate – in tissue, and in urine or serum - the metabolomic signature identified by McDunn et al.

Thus, our *revised* Aim 1 will compare the metabolomics profile developed by McDunn et al. in urine and serum from prostatectomy patients with Gleason 6 vs. 7 tumors. We currently have identified frozen tumor samples matched to urine and serum from approximately 70 patients, roughly split between Gleason 6 and 7, so we will be able to do additional tissue-based discovery if necessary, i.e. if the McDunn signature does not distinguish Gleason 6 vs. 7 cases when measured in urine or serum.

Aim 2 will not change, and will be based either on the original McDunn signature, or if a modified signature is developed in Aim 1.

Progress

In the Year 1 progress report we detailed a number of unforeseen problems that had seriously reduced out expected rate of enrollment of patients with sufficient frozen tissue meeting our

stringent requirements. Because of the delay in the study caused by these problems we had decided to relax 2 of our eligibility criteria:
(1) no previous cancer (non-prostate), and (2) the biopsy indicates only Gleason score 6 or only Gleason score 7 for an individual patient (i.e. no patients with biopsies containing both Gleason 6 and 7). We also indicated that if the accrual rate was still too low after these changes we would 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. For a large number of patients who have frozen tissue available in the PCBN there are matching serum and urine samples that had been collected by our colleague Dr. Alan Partin as part of his NCI Early Detection Research Network project.

However, shortly after we submitted the progress report the Research Nurse for the study, Patricia Kolmer, who was unfortunately suffering from a serious chronic disease during the previous year experienced a downward course and passed away in May 2013. With her loss we were unable to rely on prospective enrollment being sufficient to complete the study and we began identifying patients with frozen prostatectomy tissue and matched urine and serum already available in the PCBN. We discussed this change with our collaborators at Metabolon and it did not present any technical problems for the metabolomics assays.

We also decided to begin assembling the urine and serum samples from the active surveillance patients for Aim 2 because these samples have already been collected from the large cohort (more than 1200) of active surveillance patients managed by urologists at Johns Hopkins. We have identified 150 patients who were upgraded (Gleason 7 or higher) at an annual surveillance biopsy, and 100 patients who have been followed for at least 5 years without an unfavorable biopsy (i.e. all annual surveillance biopsies have been Gleason 6, with no more than 2 cores positive for cancer and no more than 50% of any biopsy core involved with tumor).

We anticipate being able to send all of the samples for Aims 1 and 2 to Metabolon by the end of April or early May. They anticipate 1-2 months to analyze all of the samples, and we anticipate an additional month for us to analyze the data. Thus, we should be able to complete the study by the end of August 2014.

KEY RESEARCH ACCOMPLISHMENTS

None since the previous report.

REPORTABLE OUTCOMES

None

CONCLUSIONS

The difficulties in reaching accrual goals from Year 1 were compounded by the serious illness and subsequent death of Pat Kolmer, the Research Nurse for the study. During the period of the study our industry collaborator, Metabolon, Inc. derived a prognostic metabolomic signature using 2 large independent cohorts. As a result we have shifted emphasis in Aim 1 to *validating* the signature derived by Metabolon using matched urine and serum samples, with additional discovery in frozen tissue if necessary. At the same time we are assembling the matched urine and serum samples from active surveillance patients for Aim 2 and will send out samples for both aims by the end of April or early May. We believe these changes will allow us to finish the study in a timely fashion and will not alter the objective of the study, which is to determine whether metabolomics profiling can define a signature to distinguish men who can safely enroll in active surveillance from those who should be referred for treatment.

REFERENCES

McDunn JE, Li Z, Adam KP, et al. Metabolomic signatures of aggressive prostate cancer. Prostate 2013; 73:1547-60.

APPENDICES

1. List of abbreviations and acronyms (p. 11).
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LIST OF ABBREVIATIONS AND ACRONYMS

PCBN	Prostate Cancer Biorepository Network

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

Personnel receiving pay from this negotiated effort:

Bruce Trock, PhD
Ballentine Carter, MD
Zhen Zhang, PhD
Zhaoyong Feng, MS
Patricia Kolmer, RN, BSN