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TITLE: Biomarker Development for Diagnosis, Surveillance, and Prognosis for Adrenocortical Carcinoma (ACC)

PRINCIPAL INVESTIGATOR: Tobias Else

CONTRACTING ORGANIZATION: University of Michigan, Ann Arbor

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14. ABSTRACT
We will assess the prospective utility of novel biomarkers in the clinical management of ACC. We hypothesize that serum steroids can specifically diagnose ACC, measure ACC burden, and detect recurrence; we also hypothesize that molecular biomarkers including tumor DNA methylation will predict ACC recurrence, progression, and selective response to adjuvant therapy. We will prospectively recruit ~200 participants with ACC or ACA from UM, A5, and ADIUVO-2. Diagnosis of ACC or ACA will be established by histology or imaging. Aim 1a: In patients with ACC or ACA, we will measure serum steroids by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify ACC-specific steroid markers. Aim 1b: In patients with ACC, we will measure serum steroids as in Aim 1a with parallel imaging surveillance to identify steroid markers that predict recurrence. Aim 2a: In patients with ACC, we will measure tumor DNA methylation and determine if it predicts recurrence, progression, response to systemic therapies, and death. Aim 2b: In patients from ADIUVO-2, we will measure tumor DNA methylation and determine if it predicts adjuvant therapy-specific recurrence/survival. We will perform exome sequencing of paired tumor/germline DNA to prospectively identify genetic factors that predict response to adjuvant therapies.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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TABLE OF CONTENTS

1. Introduction

The adrenal glands are paired endocrine organs that produce steroid hormones and catecholamines critical for life. Adrenocortical carcinoma (ACC) is a rare cancer of these glands affecting ~1 individual/million/year worldwide. While ACC is rare, ~10% of the population bear benign adrenal lesions (largely adrenocortical adenomas [ACA]). Differentiating localized ACC from ACA is challenging, requiring extensive imaging workup. However, imaging often cannot rule out ACC, and exposes patients to collateral radiation. Half of all patients with ACC are diagnosed with surgically resectable localized disease. However, up to ~75% of all patients with ACC develop metastatic disease for which therapies are limited and prognosis remains dismal; <10% these patients survive 5 years after diagnosis. Early diagnosis of a recurrence is essential for appropriate management. Currently, mitotic activity in the primary tumor is the best predictor of recurrence, but aggressive disease course is frequently observed among "low-risk" patients. Additionally, patients are usually surveilled with extensive imaging exams post-operatively, which is expensive and exposes patients to high doses of radiation. These statistics highlight significant gaps in the knowledge of optimal strategies for Diagnosis, Surveillance, and Prognosis of ACC. The goal of this proposal is to assess the prospective utility of novel biomarkers, including serum steroids and tumor DNA methylation, in diagnosis, risk stratification, and disease surveillance of patients with ACC This proposal will utilize samples prospectively obtained for the UM Endocrine Oncology Repository; the "American-Australian-Asian Adrenal Alliance" (A5), a large international collaborative network for adrenal research; and A5-initiated clinical trial ADIUVO-2 (NCT03583710), aiming to evaluate adjuvant therapies in patients with high grade ACC, randomized to receive mitotane alone or plus chemotherapy.

2. Keywords

adrenocortical carcinoma, ACC, DNA methylation, CIMP-high, steroidomics, LC-MS/MS, steroids, adjuvant therapy, adrenal cancer, adenoma, tumor, prospective, predictive, biomarker, adrenal, hormones

3. Accomplishments

The major goals of this project, as stated in two Specific aims in the SOW, are:

- Specific Aim 1. Evaluating the use of steroid profiles in Diagnosis (Aim 1a) and Surveillance (Aim 1b) of adrenocortical carcinoma (ACC) patients. In patients with ACC or adrenocortical adenomas (ACA), we will measure serum steroids by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify ACC-specific steroid markers (Aim 1a). In patients with ACC, we will measure serum steroids with parallel imaging surveillance to identify steroid markers that predict recurrence (Aim 1b).
- Specific Aim 2: Evaluating the use of tumor DNA methylation in stratifying ACC patients into risk groups (Prognosis). We will measure the methylation levels of a single locus, G0S2, and prospectively assess the utility of G0S2 hypermethylation in ACC risk stratification (Aim 2b), and in predicting response to different types of adjuvant therapy (Aim 2b).

For this reporting period, pertinent tasks as proposed in the SOW and **accomplishments** are described below:

- Specific Aim 1:
    - Major Task 1: Enrollment of 200 participants (ACA=100; ACC=100) and collection of relevant clinical data and biospecimens.
        - Subtasks 1 and 2 (also related to Specific Aim 2): Protocols review and approval by the University of Michigan IRBMED (approved on October 17, 2019) and by the USAMRMC Human Research Protection Office (approved on December 23, 2019).
        - Subtasks 3 and 4: Prospective enrollment of patients with ACC and ACA from the UM Endocrine Oncology Repository and American-Australian-Asian Adrenal Alliance (A5) that meet inclusion criteria for this study. To this date, we have enrolled 62 ACC patients and 49 ACA patients (total of 111 patients out of 192 aimed for this reporting period). Serum is available on a total of 57 of these patients. In addition, we received 72 additional samples from our collaborators at MD Anderson.
    - Major Task 2: Serum steroid profiling by LC-MS/MS.

        - Subtask 1: We are proceeding with steroid measurements from serum samples, as stated in the proposed SOW. We have processed 174 samples from patients with ACC and benign adrenal nodules. Serum was extracted using liquid-liquid extraction protocols – chloroform:2-butanol extraction for sulfated steroids, and methyl-tert-butyl-ether (MTBE) for Δ4 steroids. Steroids were analyzed and quantified using 2D liquid chromatography-tandem mass spectrometry scanning for 4 sulfated steroids and 25 Δ4 steroids in MRM mode. A third method to analyze Δ5 steroids is now established and samples are being extracted.
- Specific Aim 2:
    - Major Task 1: Enroll 100 participants with ACC; collect relevant clinical data and biospecimens.
        - Subtask 1 and 2: We have procured 40 fresh-frozen ACC samples, and 152 formalin-fixed paraffin-embedded (FFPE) samples (we have successfully developed an optimized protocol that enables the use of FFPE samples to study DNA methylation) from our retrospective cohort. We are ready to start processing tissue samples for DNA extraction and determination of G0S2 methylation, which we expect to send as a single batch to the external facility that will perform targeted bisulfite sequencing.

Training opportunities and professional development:
Graduate student research assistant Dipika Mohan has trained with members of Adrenal Research Group at the University of Michigan to develop an improved technique for extracting DNA from FFPE tissue for DNA methylation analysis. Desmare van Rooyen is being trained as a post-doctoral fellow.

Dissemination of results to communities of interest:
Nothing to report at this time.

4. **Impact:** Nothing major to report at this time.

5. **Changes/Problems:** We experienced a significant delay in patient enrollment, sample collecting and processing due to lockdowns and access restrictions to facilities related to COVID-19. As a result, we fell short of our target of 192 samples for this reporting period. We are working with our collaborators to fill this gap and will in addition use retrospectively stored samples for the analysis (partly included – current number 174 samples). For aim 2, we were able to substantially increase the number of cases by procuring frozen and FFPE samples from our retrospective cohort. With the inclusion of retrospective cases, we were able to compensate for the slow accrual rate of prospective cases that we unexpectedly experienced during this time.

6. **Products:** Nothing to report.

7. **Participants & Other Collaborating Organizations**

Individuals who have worked in this project during this reporting period are listed below. There are no changes from the previous submission of this information and no change in active or other support of the PD/PI or senior/key personnel since the last reporting period.

Name: Gary D. Hammer
Role: Initiating Principal Investigator
Research Identifier: http://orcid.org/0000-0001-6843-3628
Nearest person month worked: 1
Contribution to Project: Dr. Hammer has supervised the entire study and lead meetings with all members of the research team.

Name: Richard J. Auchus
Role: Partnering Principal Investigator
Research Identifier: https://orcid.org/0000-0001-6815-6181
Nearest person month worked: 1
Contribution to Project: Dr. Auchus has supervised his arm of the study and shared his expertise regarding LC-MS/MS analysis as we plan for sample acquisition during team meetings.

Name: Tobias Else
Role: Partnering Principal Investigator
Research Identifier: https://orcid.org/0000-0002-2262-0011
Nearest person month worked: 1
Contribution to Project: Dr. Else has supervised his arm of the study, facilitated acquisition of IRB approval, shared his expertise regarding serum sample analysis.

Name: Antonio M. Lerario
Role: Co-Investigator
Research Identifier: https://orcid.org/0000-0002-8336-6432
Nearest person month worked: 1
Contribution to Project: Dr. Lerario has worked on optimizing sample acquisition for G0S2 methylation analysis and has shared bioinformatics expertise to evaluate study design and number and type of samples required for biological/clinical significance during team meetings.

Name: Dipika R. Mohan
Role: Graduate Student
Research Identifier: https://orcid.org/0000-0002-6334-9416
Nearest person month worked: 1
Contribution to Project: Ms. Mohan has worked with Dr. Lerario to optimize sample acquisition for G0S2 methylation analysis and evaluate study design, and has worked with Ms. Brand and Dr. Else to obtain IRB approval.

Name: Sarah Brand
Role: Coordinator of A5
Research Identifier: N/A
Nearest person month worked: 1
Contribution to Project: Ms. Brand has led submitting and obtaining IRB approval, and will also assist in enrolling and consenting patients at the University of Michigan and managing clinical data.

Name: Patrick O'Day
Role: Technician
Research Identifier: N/A
Nearest person month worked: 1
Contribution to Project: Mr. O'Day has prepared and optimized mass spectrometer for measurement of steroid profiles from plasma samples.

Name: Desmare Van Rooyen
Role: Post-doctoral Fellow
Research Identifier: N/A
Nearest person month worked: 3
Contribution to Project: Dr. Van Rooyen optimizes the mass spectrometry analysis and extraction. She will prepare samples for further analysis and interpret results.

Partner organizations which are collaborating with this project:

- Dr. Mouhammed Habra, MD (**MD Anderson Cancer Center**, Houston, TX).
- Dr. Anand Vaidya, MD **(Brigham and Women's Hospital** Boston, MA).
- Dr. Diane Reidy, MD (Memorial Sloan Kettering Cancer Center, New York, NY).
- Dr. Nitya Raj, MD (Memorial Sloan Kettering Cancer Center, New York, NY).

8. Special Reporting Requirements: Not needed.

9. Appendices:

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eResearch.umich.edu

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To: Dr. Gary Hammer

FROM:

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Diane	Reidy
Dipika	Mohan
Tobias	Else
Richard	Auchus
Nitya	Raj
Gary	Hammer
Sarah	Brand
Antonio	Marcondes Lerario

Subject: Scheduled Continuing Review [CR00093292] Approved for [HUM00166409]

SUBMISSION INFORMATION:
Study Title: Biomarker Development for Diagnosis, Surveillance, and Prognosis for Adrenocortical Carcinoma (ACC)
Full Study Title (if applicable): CA180751 - Biomarker Development for Diagnosis, Surveillance, and Prognosis for Adrenocortical Carcinoma (ACC)
Study eResearch ID: HUM00166409
SCR eResearch ID: CR00093292
SCR Title: HUM00166409_Continuing Review - Fri Mar 25 12:36:09 EDT 2022
Date of this Notification from IRB: 5/25/2022
Date Approval for this SCR: 5/25/2022
Review: Expedited
Expiration Date: Approval for this application expires on **11:59 p.m. on** 5/24/2023
UM Federal Assurance: FWA00004969 (For the current FWA expiration date, please visit the UM HRPP Webpage)
OHRP IRB Registration Number(s): IRB00001995

Continuing Review Required: Yes

NOTICE OF IRB APPROVAL AND CONDITIONS:
The IRBMED has reviewed and approved the scheduled continuing review (SCR) to the study referenced above. The IRB determined that the proposed research continues to conform with applicable guidelines, State and federal regulations, and the University of Michigan's Federalwide Assurance (FWA) with the Department of Health and Human Services (HHS). You must conduct this study in accordance with the description and information provided in the approved application and associated documents.

APPROVAL PERIOD AND EXPIRATION:
The approval period for this study is listed above. Please note the expiration date. If the approval lapses, you may not

conduct work on this study until appropriate approval has been re-established, except as necessary to eliminate apparent immediate hazards to research subjects. Should the latter occur, you must notify the IRB Office as soon as possible.

RENEWAL/TERMINATION:
The IRB has determined that annual review and approval is required for this research. At least two months prior to the expiration date, you should submit a continuing review application either to renew or terminate the study. Failure to allow sufficient time for IRB review may result in a lapse of approval that may also affect any funding associated with the study.

IMPORTANT REMINDERS AND ADDITIONAL INFORMATION FOR INVESTIGATORS

APPROVED STUDY DOCUMENTS:
You must use any date-stamped versions of recruitment materials and informed consent documents available in the eResearch workspace (referenced above). Date-stamped materials are available in the "Currently Approved Documents" section on the "Documents" tab.

AMENDMENTS:
All proposed changes to the study (e.g., personnel, procedures, or documents), must be approved in advance by the IRB through the amendment process, except as necessary to eliminate apparent immediate hazards to research subjects or others. Should the latter occur, you must notify the IRB Office as soon as possible.

AEs/ORIOs:
You must inform the IRB of adverse events (AEs) and other reportable information and occurrences (ORIOs) according to your IRB's required reporting timetable (IRBMED and IRB-HSBS/Flint/Dearborn).

UNANTICIPATED PROBLEMS INVOLVING RISKS TO SUBJECTS OR OTHERS (UPIRSOs or UaPs)
Investigators must continue to inform the IRB via eResearch submission of any potential Unanticipated Problems (UaPs or UPIRSOs) that come to the attention of the study team. Unanticipated Problems meet all of the following criteria:

1. **Unexpected** (in terms of nature, severity, or frequency);
2. **Related or possibly related to participation in the research**; and
3. Suggests that the research places subjects or others at **a greater risk of harm** than was previously known or recognized.

See U-M HRPP Operations Manual Part 12.III.B.1.a. Routine AEs and ORIOs after Termination need not be reported.

SUBMITTING VIA eRESEARCH:
You can access the online forms for continuing review, amendments, and AE/ORIO reporting in the eResearch workspace for this approved study, referenced above.

MORE INFORMATION:
You can find additional information about UM's Human Research Protection Program (HRPP) in the Operations Manual and other documents available at: http://research-compliance.umich.edu/human-subjects.

Michael Geisser **Co-chair, IRBMED**	**Alan Sugar** **Co-chair, IRBMED**	**Robertson Davenport** **Co-chair, IRBMED**