

AD _____

Award Number: W81XWH-11-1-0487

TITLE: Development of Advanced Technologies for Complete Genomic and Proteomic Characterization of Quantized Human Tumor Cells

PRINCIPAL INVESTIGATOR: Dr. Robert Moritz

CONTRACTING ORGANIZATION: Institute for Systems Biology
Seattle, WA 98109-5234

REPORT DATE: July 2012

TYPE OF REPORT: Revised Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE July 2012		2. REPORT TYPE Revised Annual		3. DATES COVERED 15 June 2011 - 14 June 2012	
4. TITLE AND SUBTITLE Development of Advanced Technologies for Complete Genomic and Proteomic Characterization of Quantized Human Tumor Cells				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-11-1-0487	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dr. Robert Moritz E-Mail: Robert.Moritz@systemsbiology.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Institute for Systems Biology Seattle, WA 98109-5234				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT With the establishment of the program wide IRB agreement, we are able to begin our project by collecting tumor samples with consent in preparation to perform the molecular analysis of these. Our efforts in the development of whole genome sequencing from families have been published and our efforts in the development of quantitative assays for essentially every human protein have been successfully established. This program will be enabled through these significant developments and will provide the technical capability to provide significant results over the program in the next 2 years.					
15. SUBJECT TERMS Human cohorts, Glioblastoma, Genomic, Proteomic, Single-cell technologies, Hypothesis-driven, integrative systems approach, Early diagnosis, Patient stratification, Blood protein biomarkers, Quantized cell populations					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	7	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusion.....	6
References.....	7
Appendices.....	N/A

INTRODUCTION

We will develop quantitative tools for direct application to human cohorts with glioblastoma classified cancer. This program promises to deliver important insights to cancer mechanisms (disease-perturbed networks), as well as blood biomarkers to assess progression and stratification of human glioblastoma. This proposal will significantly advance genomic, proteomic and single-cell technologies, enabling the commencement of hypothesis-driven integrative systems approaches to disease (cancer). To this end, we are developing new strategies for advanced genome sequencing, new technologies for the analyses of transcriptomes, miRNAomes and single cells as well as multiplexed quantitative protein measurements including the measurement of isoforms, and post-translational modifications. The tools proposed here will be generally applicable to all cancer-based studies, as the nature of the tool development is designed to identify and quantify DNA, RNAs, proteins and cells, challenges ubiquitous to all human disease systems.

The expected outcomes and deliverables of this innovative program will be: 1) deeper understanding of human glioblastoma disease mechanisms; 2) blood protein biomarkers for use in early diagnosis, stratification of glioblastomas, assessment of the progression of a glioblastoma, assessment of effectiveness of drug treatment and detection of reoccurrence at an early stage; 3) new strategies for genomic sequencing of quantized cancer cells and their normal counterparts to identify cancer-driver mutations; 4) new technologies for transcriptome, miRNAome, proteome and single-cell analyses, and 5) the creation of quantized glioblastoma cell lines that can be used for general molecular characterization as well as to assess the biology of this cancer (drugs, RNAi's, natural ligands) and the effectiveness of existing drugs in reacting with these cell types.

BODY

On March 22, 2011, ISB was informed by Swedish hospital that Swedish's IRB-approved research protocol for collecting tissue and performing genetic analyses did not include any language for performing genetic testing on healthy family members. We received Swedish's local IRB approval in August 2011, and then pursued our own local IRB approval for ISB's engagement in the project. Western IRB deemed ISB's involvement "not human subjects" research under the Common Rule on August 18, 2011. Both local IRB reviews were sent to the DoD IRB for their review at that time. It is our understanding that the DoD IRB requested changes to the Swedish protocol and consent forms. Swedish re-submitted their protocol to their local IRB (twice) and received local IRB approval on 3/27/2012. The DoD IRB signed off on both protocols at Swedish & ISB. Specifically, on April 6, 2012, after approving the Swedish protocol, the USAMRMC ORP HRPO concurred with Western IRB's conclusion that this project does not involve human subjects at ISB.

Until this process was complete, we were not able to begin work or plan work procedures and personnel to start on the project. On April 6, 2012 we received notice that we could start and for logistical purposes, we have chosen July 1 as a start date for the project to get personnel for the project to coincide with our project plans.

Our collaborating partner, PI Foltz, has also chosen July 1 to begin this project to coincide with ourselves in a coordinated fashion.

We have not encountered problems and at this stage do not anticipate problems with the proposed work schedule for the next 12 months. Of course, in consideration of availability of human tumor samples, there may be small delays due to the availability of patients being seen by our collaborative group (CA100459P1, Award Number W81XWH-11-1-0488, Swedish Health Services, Dr. Gregory Foltz), but there is a number of patients that are being seen regularly as we expected and we assume this will continue as the current rate of patients presenting to our collaborator, PI Foltz.

Our efforts in whole genome sequencing have advanced since the proposal and bode well for the upcoming work to be completed. In addition, as part of a large NIH ARRA funded program, we have now completed creating multiple assays for essentially every human protein. With this tremendous resource, we will be easily able to select assays to detect the identified proteins in patient samples and we develop signatures from the genomic analysis of quantized cell populations from human glioblastoma patients. These signatures have the potential to be early indicators of disease and will be tremendously valuable to detect the disease early in future patient cohorts.

With the establishment of the program wide IRB agreement, we are able to begin our project by collecting tumor samples with consent in preparation to perform the molecular analysis of these. Our efforts in the development of whole genome sequencing from families have been published [1] and our efforts in the development of quantitative assays for essentially every human protein have been successfully established. This program will be enabled through these significant developments and will provide the technical capability to provide significant results over the program in the next 2 years.

In summary, over the last year two key technical pieces of work required for this program have been completed and will be applied to the research program of this grant. These are:

- development of whole genome sequencing from families
- development of quantitative assays for essentially every human protein

KEY RESEARCH ACCOMPLISHMENTS

None

REPORTABLE OUTCOMES

No reportable outcomes have been established for 2011/2012 period

CONCLUSION

Description of work to be performed during the next reporting period.

Over the next 12 months starting in July 2012 to June 2013 we will concentrate on the following aims:

In the first aim, we will isolate up to 1000 cells from each of five human glioblastomas and quantify initially 500 different transcripts from each cell (transcription factors, CD molecules, relevant signal transduction pathways, etc). GBM patient sample collection. Through the collaboration with Dr. Greg Foltz, a neurosurgeon and Director of the Ivy Center for Advanced Brain Tumor Treatment at Swedish Neuroscience Institute in Seattle, we will access freshly excised GBM tumor samples for single cell analysis. The same samples will also have 1000 GBM-related genes mapped by in situ hybridization at the Allen Institute for Brain Research—an Ivy Foundation funded project. Our initial analysis of 5 GBM patients will be selected out of these 64 patients, maximally leveraging data generated from the TCGA and Allen atlas projects. Promising candidate target proteins will be validated on all 64 patient tumor and blood samples during the latter half of this proposal by using targeted proteomic approaches. We will add 36 patients to this list to bring our sample population to 100.

As a second aim of the work to be conducted over the next 12 months, we will sort the disassociated tumor cells from several glioblastomas into their quantized cell populations using cell sorting/CD antibodies to each quantized cell type for functional analyses and establish primary cell lines. These will be used for molecular analyses—at the genome, transcriptome, miRNAome and selected proteome levels.

As we establish the major quantized glioblastoma cells, we will start into aim 4 as we originally proposed as we select current signatures for subsequent proteomics efforts by selecting 10 to 20 cells from each major quantized glioblastoma cell type from two patients to be used to determine the complete genome sequences. We will also determine the normal genome sequences of each patient and their family members to enable the Mendelian-based error correction process recently described in our recently published Science paper [1]. The mutations will be analyzed against quantitative changes in the transcriptomes, miRNAomes and proteomes and against the relevant biological networks. This aim will continue into year 2 but

expect the capability of the work to begin at the end of year 1 or earlier dependent on the success of the establishment of quantized glioblastoma cells.

REFERENCES

1. Roach JC, Glusman G, Smit AF, Huff CD, Hubley R, Shannon PT, Rowen L, Pant KP, Goodman N, Bamshad M, Shendure J, Drmanac R, Jorde LB, Hood L, Galas DJ. Analysis of genetic inheritance in a family quartet by whole-genome sequencing. *Science*. 2010; 328 pg 636-639.

APPENDICES

N/A