Award Number: W81XWH-11-2-0034

TITLE: Prediction of Acute Mountain Sickness using a Blood-Based Test

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REPORT DATE: January 2016

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

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1. REPORT DATE January 2016	2. REPORT TYPE Annual	3. DATES COVERED 20Dec2014 - 19Dec2015
4. TITLE AND SUBTITLE Prediction of Acute Mountain Sickness using a Blood-Based Test		**5a. CONTRACT NUMBER:**
		5b. GRANT NUMBER W81XWH-11-2-0034
		5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S) Roach, Robert, PhD email: robert.roach@ucdenver.edu		**5d. PROJECT NUMBER**
		5e. TASK NUMBER
		5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Colorado, Denver 13001 E. 17th Place, building 500, W1126 Aurora, CO 80045-25-5		**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. In the last year we have completed analysis of Phase II of the project. In Phase I we showed that an RNA-based gene signature from a sample taken at sea level could be used to successfully predict in 9 out of 10 individuals who went on to develop acute mountain sickness or who was AMS resistant. In Phase II, results suggest a completely independent sample was equally effective in predicting AMS susceptibility and resistance. These results were presented to the scientific leadership at Fort Detrick last spring. Under the advisement of that review process we have taken an expanded view of the data we are using for predicting AMS. Specifically, in addition to the data collected in this project for Phase I and Phase II, we will add several data sets to the overall analysis. New dataset #1 is analyses of an additional 70+ samples from Phase I. These samples represent subjects who were not very sick or not very well, the middle of the road group. The hypothesis is that a very effective predictive test would predict those who might get very sick or not at all sick, but also those who feel only a little bit impaired by high altitude. New dataset #2 is from a companion project called AltitudeOmics where we have samples from sea level subjects exposed to very high altitude. All of these data will be analyzed in one integrated test to assess effectiveness. Our team of bioinformaticians is working together to realize these analyses in the following year.

15. SUBJECT TERMS
Acute Mountain Sickness Prediction, Hypoxia, High Altitude

16. SECURITY CLASSIFICATION OF: U			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	6	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION:

The goal of this project is to design an easy-to-use cost-effective test that accurately predicts whether or not someone is likely to develop acute mountain sickness (AMS) when they travel to high altitudes.

OVERALL PROJECT SUMMARY:

Following program reviews over the last 18 months we have expanded the scope of the study and received a no cost extension to complete this expanded scope. We just in the last week have received our final no cost extension covering the scope of the project for the next two years. In addition, we are applying for additional funding through an independent DoD mechanism to cover some of the work we will do with US ARIEM on related projects.

QTR 17 Accomplishments (Jan-Mar 2015):
In quarter 17 we focused on two major tasks: getting the RNA purified and ready for chip analysis and working on the bioinformatics organization of all the data we will examine for this study. To remind the reviewer, we have a primary dataset of ~120 subjects who were studied in Dallas and Breckenridge, Colorado. The middle responders, about 80 individuals, did not have gene array chips run on them previously, and that is the focus of the above-mentioned gene chip tasks. But in addition we are adding a number of other studies to the analysis to get the most comprehensive and robust prediction signature that we can from these data. The additional datasets include the AltitudeOmics dataset. We have already run the prediction algorithm on that dataset as a test and found very good prediction capability. Then we have the original chamber dataset that we will add to this dataset, and one additional, independent dataset that can also be analyzed. Work is underway to standardize the organization of each dataset so we can compare all of them together. The actual comparison will begin as soon as the chips are back from the core for the new analyses. Also this quarter we had to work on IRB continuing review locally, and next quarter we have to work on the HRPO DOD process to keep the protocol open for research.

QTR 18 Accomplishments (Apr-Jun 2015):
In quarter 18 we focused on two major tasks. Number one was getting the RNA purified and ready for chip analysis. This is a time consuming, tedious task that we have almost completed. As soon as that is done the MicroArray Core will run the chips on all samples. We expect both of these tasks to be complete by the end of next quarter. All but 6 of the samples have been completed and are ready to be run through the core facility. The other six had degraded RNA and were re-purified. We are awaiting all those results. We expect those results within the next few days. As soon as we know what samples have high quality RNA we will order the correct number of chips and get the assays completed. We also spent about one month negotiating with Affymetrix to get chips that match the previous samples we have analyzed for this study. Finally we worked out the technical details and figured out a way to use current release chemicals and the old chips.

The other major task has been to work on the bioinformatics organization of all the data we will examine for this study. To remind the reviewer, we have a primary dataset of ~120 subjects who were studied in Dallas and Breckenridge, Colorado. The middle responders, about 80 individuals, did not have gene array chips run on them previously, and that is the focus of the above-mentioned gene chip tasks. But in addition we are adding a number of other studies to the analysis to get the most comprehensive and robust prediction signature that we can from these data. The additional datasets include the AltitudeOmics dataset. We have run the prediction algorithm on that dataset as a test and found very good prediction capability. Then we have the original chamber dataset that we will add to this dataset, and one additional, independent dataset that can also be analyzed. Work was underway to standardize the organization of each dataset so we can compare all of them together. The actual comparison began when the chips are back from the core for the new analyses. Also we

worked on IRB continuing review locally, and in planned for more work in quarter 19 on the HRPO DOD process to keep the protocol open for research. This work is ongoing and will continue for remainder of the time we work on this grant.

QTR 19 Accomplishments (Jul-Sep 2015):
As of quarter 19, we were ahead of our projected timeline. All chips were run through the microarray core, with repeats of several chips due to quality control issues. We then did a study by study standardization procedure before combining all chips from all studies into one large database for AMS prediction. This will allow us over the next several quarters to address specific questions about generalizability of the prediction algorithms across AMS severity, altitude of origin, altitude of final study, and gender.

QTR 20 Accomplishments (Sep-Dec 2015):
As planned we are now addressing specific questions about generalizability of the prediction algorithms across AMS severity, altitude of origin, altitude of final study, and gender.

We continue work for IRB compliance, and we have to work on the HRPO DOD process to keep the AMS Prediction protocol open for research. This work is ongoing and will continue for remainder of the time we work on this grant.

We completed the request for a no cost extension. And we began the process of integrating some additional new data from US ARIEM into our workflow.

Throughout all four quarters of 2015 we have had to commit considerable time and resources to continuing IRB review, both locally here in Colorado and at HRPO, which we have done and will continue to do.

KEY RESEARCH ACCOMPLISHMENTS:

- confirmation of our ability based on a sea level test in Phase I to predict acute mountain sickness at high altitude

- design and execution of the Phase II field study

- successful RNA isolation and microarray analyses from the Phase II validation study data

- gaining additional funding to analyze 'middle of the road' samples from Phase I

- completion and approval of a no cost extension to allow the above described analyses to take place

- addition of data from our companion study, AltitudeOmics, to the database of gene studies to be analyzed for AMS prediction

- expansion of a bioinformatics team to include an additional consultant

- expansion of the bioinformatics dataset to include advanced clustering analysis which may lead to identification of biological factors linking the AMS prediction signature to biological mechanisms

REPORTABLE OUTCOMES:
Though the initial results are exciting, and verify our original hypothesis, it is premature to proclaim complete success. Today it is accepted practice to validate gene expression findings in an independent cohort before relying on the validity of a gene expression screening test. We are expanding this validation phase to include different samples from higher altitudes, and more samples from Phase I with less severe symptoms. In total now in our hands are data to analyze the prediction of AMS from samples collected from Denver residents studied in

a chamber at 4500m, sea level residents studied at 5200m, sea level residents in two different protocols studied at 2800m. And soon to be added are US ARIEM studies of sea level subjects studied in chamber and in the field at 4300m. This robust approach to validation will rigorously test whether AMS prediction from a sea level blood test is possible.

CONCLUSION:
The results are promising, the database of studies has been greatly expanded at no extra cost on this base contract, and in the next 12 months we should answer the question about the generalizability of the results of our previously successful tests--do they work at all altitudes in all combinations. This last step has potential to change the way we manage risk in people who have never before gone to high altitude.