UMDNJ
UNIVERSITY OF MEDICINE & DENTISTRY OF NEW JERSEY

Institutional Review Board
Newark Campus

NOTICE OF APPROVAL OF MODIFICATION

IRB PROTOCOL NUMBER: 0120090320
(Refer to this number when making inquiries)

PRINCIPAL INVESTIGATOR/DEPT: **Zamir Brelvi, M.D., Ph.D.**
Medicine
New Jersey Medical School
MSB H-538
Newark, NJ 07101

CO-INVESTIGATOR(S): Dharmesh H. Kaswala, MD Nitin Patel, MD

TITLE: Role of ROX and BOX Biomarkers As Monitors Of Interferon Treatment Response In Hepatitis C Patients.

PERFORMANCE SITE(S): UMDNJ- Doctors Office Center/ New Jersey Medical School/ University Hospital

SPONSOR/PROTOCOL NUMBER: Air and Water Solutions, Inc.

TYPE OF REVIEW: Expedited

RISK DETERMINATION LEVEL: Minimal Risk

DEVICE DETERMINATION: Not Applicable

TYPE OF APPROVAL: MODIFICATION

MODIFICATION ITEMS: Correction of typo error within the Research Protocol

Document Versions Approved with this Modification:
☑ **Protocol Version:** Approved: 4/22/2010

Currently Approved Documents:
Protocol Version: - 10/8/2009
Consent Version: -10/8/2009

Vulnerable Population Code(s): No Children As Subjects; No Pregnant Women as Subjects; No Prisoners As Subjects

APPROVAL DATE: 4/22/2010 **EFFECTIVE DATE: 4/28/2010** **EXPIRATION DATE: 2/12/2011**

1. **Adverse Events:** Any on-site serious adverse events, or any unanticipated problems involving risk to subjects or others, or any serious or continuing non-compliance that occurs in relation to this study must be reported to the IRB Office (45 CFR 46, 21 CFR 50, 56) as outlined in the investigator instructions for adverse event reporting.
2. **Continuing Review**: Approval is valid until the protocol expiration date shown above. The IRB must review and approve all human subject research studies at intervals appropriate to the degree of risk, but not less than once per year, as required by 45 CFR 46 and 21 CFR 50, 56. In order to avoid lapses in approval of your research and the suspension of subject enrollment, please submit your continuation application at least eight weeks before the study expiration date.

3. **Consent**: The IRB has reviewed and approved the attached date-stamped consent/assent/parental consent form(s) for this study, as required by 45 CFR 46 and 21 CFR 50, 56, if applicable. Only the attached date-stamped consent/assent form(s) may be used to document informed consent of study subjects. All subjects must receive a copy of the approved date-stamped consent/assent form(s); a copy of the signed consent/assent form must also be filed in a secure place in the subject's medical/patient/research record.

   Number of consent forms approved: 1
   Subjects: Number of subjects approved at this site: 200

4. The investigator(s) did not participate in the review, discussion, or vote of this protocol.

5. **Amendments/Modifications/Revisions:** If you wish to change any aspect of this study, including but not limited to study procedures, consent form(s), principal investigator, co-investigator(s), advertisements, the protocol document or procedures, the investigator drug brochure, or accrual goals, you are required to obtain IRB review and approval under 45 CFR 46 and 21 CFR 50, 56. Implementation of these changes may not occur until you receive notice of IRB review and approval.

6. **Completion of Study**: Please notify the IRB when your study has been stopped for any reason. Include the following information in the written notification using a continuing review/final report form: number of subjects enrolled; number of subjects withdrawn from the study; and reason for study termination. Neither study closure by the sponsor or the investigator removes the obligation for timely continuing review or a final report.

7. **Modification: At the request of the PI, a correction of typo error within the Research Protocol needed to be corrected.**

Amy L M Lallier

Amy L.M. Lallier, B.A., C.I.M., C.I.P. - NWK IRB Assistant Director

Date: Apr. 28, 2010

Cc: Department Chair

DHHS Federal Wide Assurance Identifier: FWA00000036

Developing a Protocol

PROJECT OVERVIEW

***Title*: Role of ROX™ and BOX™ biomarkers as monitors of Interferon Treatment response in Hepatitis C patients.**

Investigators/collaborators/funding sources: There are no available funding sources.
PI:Zamir S Brelvi MD, PhD and Co investigators are as follows.
Dharmesh H Kaswala MD, Nitin Patel MD, Michael Demyen MD.

Background and Purpose of Study:
Oxygen is a significant and potent tool in the body's defense against invading foreign chemicals and organisms. The storage and release of oxygen forms an integral part of the immune system. ROX™ is a marker for oxygen reserves in blood, and is hypothesized to be a measure of the innate ability of the patient to provide oxygen for attack. BOX™ is a marker for blood cellular oxidative capability and is hypothesized to be a measure of the adaptive capability of the immune system. ROX™ and BOX™ are numbers that are of potential clinical and diagnostic value for diseases in which immune system operation is of concern. Further work with human blood has led to development of ImmuniGraph™ test, which measures both the amount of Reservoir of Oxygen (ROX ™), and the rate at which it is built (BOX™).

The purpose of this study is to identify the patients who have low ROX™ and BOX™ levels. These patients may prove to be the non-responders for interferon treatment. Interferon is a standard treatment for hepatitis C, with the majority of patients being non-responders to interferon treament. The patients with low ROX™ and BOX™ levels are potential candidates for not responding to interferon treatment. Early identification of non-responders will lead to medical cost savings, will prevent patient suffering from severe side effects from interferon, and will save time and resources for physicians and patients

Summary of Proposed Project in Lay Terms and Scientific Terms:
The study will be done for early detection of non responders to interferon-the medication for treatment of hepatitis C. The study will be compared via measuring ROX™ and BOX™-the biological markers in blood. The Hepatitis C patients(Group B) and normal healthy individuals(Group A) will donate 3ml of blood after consent and we would measure ROX™ and BOX™ markers which will be indicators for interferon responders vs non responders. After collections of a baseline blood draw as a control, Group B will be started on interferon treatment. Then we will collect 3ml of extra blood, drawn monthly from group B during their standard monthly interferon treatment to monitor ROX™ and BOX™ level. We will take advantage of the existing venipuncture such that a new puncture will not be needed for the 3-ml sample. Group A will be donating their 3ml of blood every three months for 12 months, in order for us to obtain a longitudinal 'normal' baseline control.

INTRODUCTION

The existence of non-responders to interferon treatment of Hepatitis C patients is well known. An excellent review is provided by Dr. William M. Lee at the university of Texas Southwestern Medical School, Dr. Lee and colleagues at nine other institutions worked on the HALT-C study from 2002-2007. This study points out that there are 50-60% of non-responders to interferon plus ribavirin treatment. These non-responders are furthermore non-responsive to long-term interferon maintenance strategies in the sense that there is no significant difference in the rate of progression of liver disease between non-responders on interferon and non-responders not on interferon maintenance. The question that arises from this work is: Why is there a difference between responders and non-responders?

Hinshaw, Sofer, and coworkers[1] found when studying an extracorporeal blood recirculation system in canine endotoxic shock models, that the shear stress generated by the extracorporeal pump circulation system led to autoanticoagulation. That is, stressed blood would not clot, in the absence of external anticoagulants such as heparin, even in a vigorously agitated blood circulation system. They further isolated from stressed blood, HLF, a heparin-like factor, which was shown to prevent clotting. Hinshaw and coworkers[2] also noted that such stressed blood in canines led to a 'cure' in the dogs---the autoanticoagulated dogs were resistant to shock when injected with bacterial endotoxin. Sofer[3] pursued this problem as a New Jersey State Sponsored Research Professor of Biotechnology, and discovered MOPs, or molecular oxygen peaks (not radical oxygen species) that were generated from stressed blood[4]. Other published work based on thousands of runs by Sofer's NJIT Biotechnology group reinforce the presence of oxygen peaks generated by many other types of stress: chemical, thermal, pH, etc. The questions that arise here are: Where does the oxygen from the MOPs come from, in view of the fact that the MOPs are generated from blood at zero oxygen concentration, where the hemoglobin equilibrium oxygen content is zero? Why does stress release this oxygen? Why does stress strengthen the immune system against bacterial endotoxin attack?

AWSI

AWSI[5] is a private New Jersey corporation dedicated to biological applications involving enzymes and whole cells. Further development of this research after several years of work by Sofer at AWSI, with private funding for the IgX Division, has led to the discovery of markers that determine the amount (ROX™) of stress-released oxygen reserves available in the blood, and the rates at which this oxygen is released and built (BOX™). Currently further details of this technology are proprietary and confidential. The technology is supplied in kit form, along with technical consultations, at no charge.

Objectives:

To identify the patients with low ROX™ and BOX™ levels. These patients may prove to be the non-responders for interferon treatment in Hepatitis C patients. The patients with low ROX™ and BOX™ levels are potential candidates for not responding to interferon treatment. Early identification of non-responders will lead to medical cost savings, will prevent patient suffering from severe side effects from interferon, and will save time and resources for physicians and patients

Hypotheses:

ROX™ and BOX™ biomarkers as monitors of Interferon Treatment response in Hepatitis C patients: is there a role?

ROX™ is a marker for oxygen reserves in blood, and is hypothesized to be a measure of the innate ability of the patient to provide oxygen for attack. BOX™ is a marker for blood cellular oxidative capability and is hypothesized to be a measure of the adaptive capability of the immune system. ROX™ and BOX™ are numbers that are of potential clinical and diagnostic value for diseases in which immune system operation is of concern. Immunotherapy such as with the addition of immunomodulators such as interferons or activated T-cells seeks to trigger the immune system to fight a given disease.Our hypothesis is that the lack of adequate oxygen availability for use, by for example CD8+ cytotoxic T-cells, could be the reason that for many populations, interferons and activated T-cells do not work. For true immunomodulation, interferons and activated T-cells are a necessary, but not sufficient condition to activate the immune system. Oxygen availability at the site is necessary.

PROCEDURES/METHODS

RESEARCH DESIGN

Oxygen is a significant and potent tool in the body's defense against invading foreign chemicals and organisms. The storage and release of oxygen forms an integral part of the immune system. ROX™ is a marker for oxygen reserves in blood, and is hypothesized to be a measure of the innate ability of the patient to provide oxygen for attack. BOX™ is a marker for blood cellular oxidative capability and is hypothesized to be a measure of the adaptive capability of the immune system. ROX™ and BOX™ are numbers that are of potential clinical and diagnostic value for diseases in which immune system operation is of concern. Further work with human blood has led to development of ImmuniGraph™ test, which measures both the amount of Reservoir of Oxygen (ROX ™), and the rate at which it is built (BOX™).

The test typically works by adding a 0.05-0.5 ml sample of patient blood to a well. BOX and ROX numbers are output by a reader. The reader is calibrated at zero and 100%, and standardized for a baseline against a standard supplied by AWSI.

ROX™ and BOX™ exist at high baseline levels in homeostasis. Unlike PSA and c-reactive protein, the baseline is reads '100' with respect to the standard. Therefore both up- and down-fluctuations are readable and of potential value.

Immunotherapy such as with the addition of immunomodulators such as interferons or activated T-cells seeks to trigger the immune system to fight a given disease.Our hypothesis is that the lack of adequate oxygen availability for use, by for example CD8+ cytotoxic T-cells, could be the reason that for many populations, interferons and activated T-cells do not work. For true immunomodulation, interferons and activated T-cells are a necessary, but not sufficient condition to activate the immune system. Oxygen availability at the site of action is hypothesized to be a MUST!

The purpose of this study is to identify the patients who have low ROX™ and BOX™ levels. These patients may prove to be the non-responders for interferon treatment. Interferon is a standard treatment for hepatitis C, with the majority of patients being non-responders to interferon treament. The patients with low ROX™ and BOX™ levels are potential candidates for not responding to interferon treatment. Early identification of non-responders will lead to medical cost savings, will prevent patient suffering from severe side effects from interferon, and will save time and resources for physicians and patients.

In our study we will have 40 total patients of which 20 patients will be normal healthy volunteer patients which we will call group A, and 20 patients will be newly diagnosed hepatitis C, which will be designated as Group B. Group A will be selected by the Principal investigator and they will be counted as healthy only those who are not taking any prescribed medications, and who describe themselves as not being sick. Group B inclusion criteria will be patients with Hepatitis C, newly diagnosed and never been treated. We will exclude those patients with Diabetes Mellitus, Hypothyroidism or Depression and subjects who are Immunocompromised or those who are taking Immunosuppressive drugs.

Individuals in both groups will donate 3ml of blood, which is the size of the smallest readily available vacuum container, for each test. After collections of a baseline blood draw as a control, Group B will be started on interferon treatment. Then we will collect 3ml of extra blood, drawn monthly from group B during their standard monthly interferon treatment to monitor ROX™ and BOX™ level. We will take advantage of the existing venipuncture such that a new puncture will not be needed for the 3-ml sample. Group A will be donating their 3ml of blood every three months for 12 months, in order for us to obtain a longitudinal 'normal' baseline control.

The Principal Investigator will arrange for monthly meetings to evaluate the progress of this project, and to review the data. Standard evaluations such as for Rapid-, Early-, and Sustained-Viral Responders (RVR, EVR, SVR) will be compared to their respective ROX™ and BOX™ levels. The chi-squared test will be used to determine any correlations.

Study time line: The study duration is expected to be for about 12 months

STUDY POPULATION

The patients will be recruited from Physician's outpatient's office (DOC-UMDNJ) at The University Hospital, UMDNJ, Newark

Case definitions:

Criteria for healthy volunteer subjects: Only those subjects will be included who will not be taking any prescribed medications

Criteria for Hepatitis C subjects:

Inclusion Criteria: Newly diagnosed hepatitis C only subjects

Exclusion criteria: Immunocompromised, Hypothyroidism, Depression and subjects on Immunosuppressive drugs

Recruitment: The patients will be recruited from Physician's outpatient's office(DOC-UMDNJ) and The University Hospital, UMDNJ, Newark . The patient will be explained in detail about

the study by Dr. Nitin Patel/Dr. Dharmesh H Kaswala/Dr. Zamir Brelvi prior to recruitment for study.

Consent Process: The patient will be informed about the risks and benefits of study. The written consent form will be explained prior to getting signed consent form from patients. Patients will also be informed in detail about research design in lay terms and the authority of patients regarding research study for the documents.

Study instruments, including questionnaires, laboratory instruments, and analytic tests: The consent forms and project hypotheses will be described in detail for the patients. The test kit will also be described as stated above. Any proposed changes to the test protocol or study will be documented and submitted to the IRB prior to taking any action.

DATA HANDLING AND ANALYSIS

Data analysis plan, including statistical methodology and planned tables and figures: The Principal Investigator will arrange for monthly meetings to evaluate the progress of this project, and to review the data. Standard evaluations such as for Rapid-, Early-, and Sustained-Viral Responders (RVR, EVR, SVR) will be compared to their respective ROX™ and BOX™ levels. The chi-squared test will be used to determine any correlations.

HANDLING OF UNEXPECTED OR ADVERSE EVENTS

Response to new or unexpected findings and to changes in the study environment: If any report of adverse outcomes from study if we find the study will be terminated.

Identifying, managing, and reporting adverse events: Patients will be exposed to certain rare risks of physical injury associated with blood draws---bruising or infection at the site of the venipuncture. Patients are not giving up any of their legal rights by signing the informed consent form or by taking part in this research study.

Emergency care: Medical treatment will be arranged by UMDNJ for participants who sustain physical injuries or illnesses as a direct consequence of participation in this research. The patients' insurance carriers or other third party payers will be billed for the cost of this treatment. No additional financial payment to patient is available.

DISSEMINATION, NOTIFICATION, AND REPORTING OF RESULTS

Notifying participants of their individual results: Study participants will not be notified of their individual results, as these will not be individually calculated.

Notifying participants of study findings: Participants will not be notified of the study findings. The target audiences of this study are physicians treating hepatitis C patients.

Disseminating results to public: The results of the study will be reported in refereed academic journals, if accepted for publication.

PROTECTION OF HUMAN RESEARCH PARTICIPANTS

***Description of risks (physical, social, psychological) to the individual or group. Include methods to minimize risks*:**

The patients will be thus informed: When your blood is drawn, there may be a bruise, or bleeding, or infection, at the place of venipuncture. However, infection is rare. The benefits of taking part in this study: for healthy volunteer subjects will be potential benefits for humanity by this study; for potential newly diagnosed hepatitis C subjects will be helping identify the non responder for hepatitis C treatment, leading to potential cost savings, time savings for the physician and patient alike, and preventing side effects from hepatitis C treatment. However, you might receive no direct benefit from taking part in this study.

References

1.Am J Physiol Heart Circ Phys:H742-750 (1980)

2.Circ. Shock 1979; 6(3)261-9

3.Brief bio of Sam Sofer: BS Honors Chem E, U of Utah, PhD U of Texas at Austin, Post doc Clayton Foundation Biochemical Institute. Funded Research: Steroid biosynthesis, hepatic assist device using liver enzymes,drug biosynthesis using enzymes, development of a test for mutagenicity, applied biochemistry. Industrial experience, Professor and Department Chair, Research Chair Professor of Biotechnology, President, AWSI, Inc.

4.Comparative Haematology International (1999) 9:68-71

5.Incorporated in 1979 as SRE, moved to New Jersey and adopted new name: Air & Water Solutions, Inc.,US National Science Foundation Grantee, US DOE grantee, Specialist in applied biochemistry, formed IgX Division as a research arm.