AD_____

Award Number: DAMD17-03-1-0047

TITLE: Therapy Selection by Gene Profiling

PRINCIPAL INVESTIGATOR: Simon W. Hayward, Ph.D.

CONTRACTING ORGANIZATION: Vanderbilt University
Nashville, TN 37232-2103

REPORT DATE: April 2004

TYPE OF REPORT: 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. 074-0188*



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | April 2004 | Annual (1 Apr 2003 - 31 Mar 2004) |

**4. TITLE AND SUBTITLE**

Therapy Selection by Gene Profiling

**5. FUNDING NUMBERS**

DAMD17-03-1-0047

**6. AUTHOR(S)**

Simon W. Hayward, Ph.D.

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Vanderbilt University
Nashville, TN 37232-2103

E-Mail: simon.hayward@vanderbilt.edu

**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. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

Original contains color plates: ALL DTIC reproductions will be in black and white

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**12b. DISTRIBUTION CODE**

**13. ABSTRACT (Maximum 200 Words)**

The **long term goal** of this work is to develop a new prognostic tool with which to determine the response of a patient to a given therapy, with the view of providing the most appropriate treatments tailored to individual patients. The **central hypothesis** of this proposal is that a subset of the genes expressed in a prostate tumor can be used to predict response to specific therapeutic regimens. The **purpose** of this work is to generate predictive methods which will allow patients to be selected for specific treatment protocols. In this year, per or proposed schedule, we have focused on acquisition of tissue samples and their grafting and treatment in SCID mouse hosts. A modification of the control arm of the study has been made to reflect changes in clinical practice occurring around the inception of the study which are likely to persist for the foreseeable future. Other minor technical improvements have also been made relating to the microarray plans and the method of detecting apoptotic cells. The project is proceeding on its predicted timeline in the accepted statement of work, the first microarray analysis should be initiated within the next three months.

**14. SUBJECT TERMS**

Stromal-epithelial interactions, Carcinogenesis, Insulin-like growth factors, Telomerase

**15. NUMBER OF PAGES**

9

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

## Table of Contents

**Annual Report**

PCRP Idea Development Award

DAMD 17-03-1-0047

Therapy selection by gene profiling

**P.I. Simon W. Hayward, PhD**

## Introduction

The **long-term goal** of this work is to develop a new prognostic tool with which to determine the response of a patient to a given therapy, with the view of providing the most appropriate treatments tailored to individual patients. The **central hypothesis** of this proposal is that a subset of the genes expressed in a prostate tumor can be used to predict response to specific therapeutic regimens. The **purpose** of this work is to generate predictive methods that will allow patients to be selected for specific treatment protocols. A subordinate aim is to catalogue genes that are regulated in response to treatment with Taxotere, in both responding and non-responding human prostate cancer tissue samples, since these genes might suggest additional targets for therapeutic intervention. The **rationale** is to utilize a novel method of tissue grafting in combination with state of the art microarray, biostatistical and bioanalytical analysis to generate new prognostic tools. This project is an essential "proof of principle" step in the sense that if this methodology is successful with Taxotere it should be applicable to any new therapeutic approach that exists or which will be developed in the future. This project is divided into four specific aims. The first three aims will primarily generate data on the predictive value of gene expression profiles in samples derived from patients in determining the response of those patients to treatment with Taxotere. This work will allow us to design new predictive microarray or multiplex real time RT-PCR assays to determine whether specific patients will respond to Taxotere. The fourth specific aim will use existing samples from patients engaged in an ongoing clinical trial to test whether these predictions are valid in a clinical setting.

**Original Statement of Work**

**Therapy selection by gene profiling.**

Task 1

Generate DNA Microarray patterns for prostate cancer samples from 150 patient tumor samples

a) As cases present, collect 150 histopathologically-confirmed prostate cancer tissue cores. Snap freeze core fragments (months 1-24)

b) Prepare RNA from snap frozen core fragments (months 1-25)

c) Run 150 comparative DNA microarrays using 12k human chip against a mixed sample human prostate standard (months 1-25)

Task 2.

*In vivo* studies

a) Perform preliminary study to determine optimal post-treatment timepoint for determining histopathological response to Taxotere (months 1-3)

b) Graft tissues from the cores used in task 1 to pairs of SCID mice (months 1-24)

c) Treat one of each pair of mice with Taxotere for 30 days (months 1-25)

d) Sacrifice mice and harvest tissues. Snap freeze tissues, make RNA for microarray analysis, take representative tissue samples for histology (months 2-26)

e) Run 300 comparative microarrays of untreated vs standard and Taxotere-treated vs standard samples (months 3-28)

This task requires the use of 195 male SCID mice

Task 3

Biostatistical analysis

a) Identification of gene expression patterns which predict histopathologic response to Taxotere. Biostatistical analysis to determine a pattern of gene expression in tissue cores which predict histopathologic response to Taxotere in a xenograft model (months 26-32).

b) Identification of genes regulated by Taxotere in responsive and non-responsive tissues. Biostatistical and bioinformatic analysis will be used to identify genes regulated by Taxotere in responsive and non-responsive tissue samples (months 28-34).

c) Design of assays microarrays to predict response to Taxotere. Custom microarrays or assays (depending upon the number of prognostic genes identified in 3a) will be designed in which expression patterns of a limited number of genes should predict the response of human prostate cancer to Taxotere (months 32-35).

Task 4

Prediction of response of patients in a clinical trial setting (months 35-36)

Based upon microarray analysis of archived snap frozen tissue the ability of the arrays designed in task 3 to predict response in a clinical trial will be tested. Prepare RNA from archived tissue cores. Run microarrays, predict response based upon data acquired in earlier tasks. Test results by breaking patient code and correlating actual and predicted responses.

**Work Ongoing and Completed**

The main tasks for the initial year of this proposal have been, 1) to collect and graft tissues into SCID mice, and 2) to treat the mice with Taxotere and subsequently (six days post-treatment) to harvest the tissues for analysis. This procedure has been followed for a total of 41 patient samples. Control samples have been snap frozen and preserved in RNA later for processing which will commence in the near future. Dr. Shawn Levy director of the Vanderbilt Microarray Shared Resource has requested that as far as is practical we perform microarray analyses in batches of 48 or 96 as this allows each batch to be performed with a single lot of reagents maximizing internal consistency. Therefore an initial batch of microarray analyses will be performed as soon as we reach 48 samples. Formalin fixed sections of treated tissues have been prepared for analysis of apoptotic indices.

**Technical modifications:**

Clinical Practice Changes.

One major technical issue has caused us to modify the originally proposed procedure. Early in this study there was a technical modification to the process of prostatectomy at VUMC with the introduction of robotic techniques. This reflects changes in surgical practice occurring at many major hospitals across the USA. A consequence of this change is that the tissues spend significantly more time inside the body of the patient between their surgical removal from the blood supply and final delivery to Pathology. This can compromise the quality of the tissues by the time they become available to investigators, and certainly will change the profile of genes expressed by the cells. Therefore we have modified the protocol to use tissues grafted to untreated mice as the source for test genetic profiles for this study. The act of grafting into a SCID mouse host provides an opportunity for the tissues to recover and regain their original profile of markers. This modification to task one does not change the overall goals of the project.

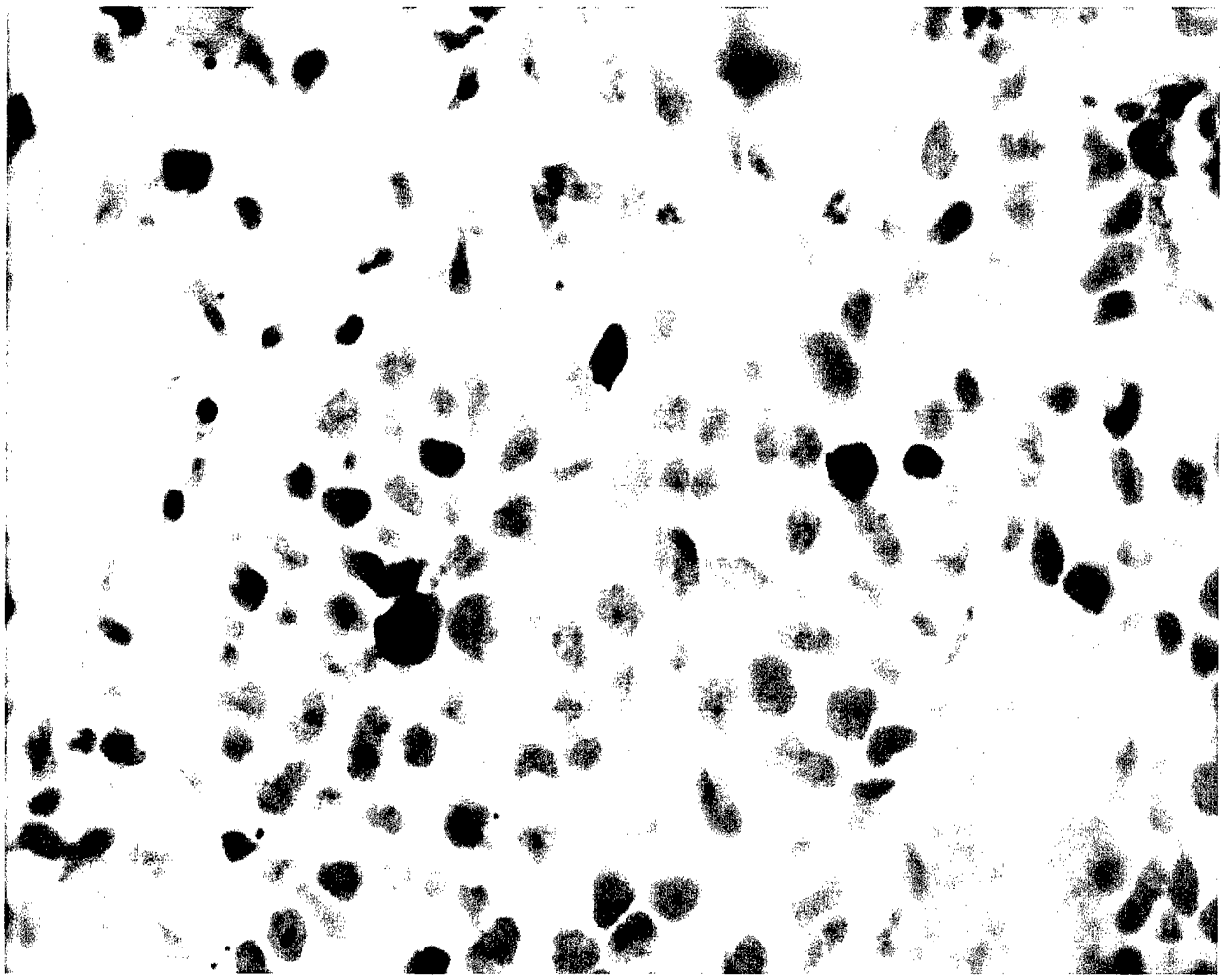
Microarrays.

The Vanderbilt Microarray Shared Resource has recently switched from the initially proposed 11k cDNA arrays to 29k oligoarrays. These are made of short oligonucleotides instead of big cDNAs, insuring a more specific hybridization. This is a much better annotated and much more

cost effective array providing data on approximately 4.5x more unique genes for only marginally greater cost than the 11k chips. For this reason all analysis will be performed with these chips.

Analysis of apoptotic index.

In the grant proposal we suggested that TUNEL assays would be used to determine apoptotic index following Taxotere treatment. TUNEL has well-recognized technical difficulties and while we have a significant level of experience with the technique we have decided to use a newly available immunohistochemical approach based upon antibodies against activated caspase 3, which is localized to the nucleus specifically in cells undergoing apoptosis (see figure below). This method is both technically simpler and more reliable than TUNEL.



Activated caspase 3 staining in an LNCaP tumor. Note blood lakes characteristic of an LNCaP tumor. Intense brown stained nuclei indicate active apoptosis.

**Personnel Changes**

Dr. Scott Shappell recently left Vanderbilt to enter private clinical practice. Human prostate cancer tissues are now obtained on a recharge basis through the VICC Tissue Acquisition Core under the directorship of Dr. Kay Washington. Dr. Washington ensures that tissue cores are collected by the same protocol used by Dr. Shappell and coordinates delivery of samples from the OR through Pathology to the laboratory. Dr. Richard Roberts (Department of Pathology) provides a timely, core-by-core, histopathologic analysis based upon frozen sections of fractions of each core as previously performed by Dr. Shappell.

**Key Research Accomplishments**

- Collection of tissues from the first 41 patients in the series.
- Grafting of tissue samples to SCID mice in control and treatment study arms.
- Treating mice with Taxotere.
- Harvesting and subsequent processing of tissues.

**Reportable Outcomes.**

None

**Conclusions.**

This work is proceeding on the predicted timeline. A number of changes to the specific details of the original statement of work are noted. These reflect changes in clinical practice that is beyond the control of the investigators and technical methodological improvements that enhance the overall quality of the proposal. None of these changes alters the overall aims and long-term goal of the proposed work.