Award Number: W81XWH-04-1-0242

TITLE: Therapy Selection by Proteomic Profiling

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REPORT DATE: February 2008

TYPE OF REPORT: Final

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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Therapy Selection by Proteomic Profiling

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The long term goal of this work was 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 proteins 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. The collection of human prostate cancer tissue, its grafting to mice and treatment of these mice with Taxotere was completed. Tissues were harvested and MALDI-MS profiles generated from both sample epithelium and adjacent stroma. The main effort in the no cost extension year has been to try to establish methods to monitor responses to Taxotere and to critically examine the histopathology of the harvested samples. This has revealed problems relating to the nature of the tissues which have rendered a full bioinformatic analysis of the data moot, therefore funds reserved to pay for this service have been returned to DOD-PCRP. This report includes an analysis of these problems and a plan to process the existing data set.

proteomic profiling, taxotere, prediction of response, biostatics

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Introduction

In addition to describing activities in the last year of funding, this final report will summarize activities, problems and achievements over the duration of this grant. The long-term goal of this work was 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 proteins expressed in a prostate tumor can be used to predict response to specific therapeutic regimens. A potential subordinate aim was to catalogue proteins 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 purpose of this work was to generate predictive methods that will allow patients to be selected for specific treatment protocols. This project was 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 was linked to, and shared tissue resources with a related study DAMD 17-03-1-0047 which has similar aims in terms of gene expression. The idea behind funding these two projects in parallel using the same patient samples was to allow the possibility of mixed genomic/proteomic-based tool development. As a result of the parallel funding, the budget for this proteomics section was significantly reduced from the outset to take advantage of synergies between the projects. The parallel nature of the projects also subjected them to similar operational problems which became clear in the final year of funding of both projects (in the case of this project the first no cost extension year).

This proposal was recognized from its inception as high risk but potentially high gain. The model system which was proposed was to utilize a xenografting model developed by the P.I. as a platform for assessing the regulation of genes by Taxotere in susceptible versus non-susceptible tumors.

As noted in previous annual reports tasks one, two and task 3a were successfully completed. The funding of this proposal occurred at a time when two changes in clinical practice were ongoing. These impacted our collection of tissues, and were described in the final report for DAMD 17-03-1-0047 but
are repeated here. The first change was a general migration towards downstaging and grading of prostate tumors. This effect has been going on for some time, was recognized at the time of the proposal submission, and while adding some time to the sample collection, documented in previous annual reports provided no major problems. The issue is that, with the widespread use of PSA testing, the size of prostate tumors which are detected and the stage and grade of the disease generally seen clinically is slowly decreasing. Despite debatable issues of over treatment it would generally be argued that this is good news for patients. However, perversely, it is not positive news for researchers since the pool of tissue available for research is reduced. However as noted, while we did see a reduction in the number of samples from which the Tissue Acquisition Core could provide samples, this caused delays but was not otherwise a serious problem. This was documented in early annual reports.

The second clinical issue, the introduction of robotic laparoscopic surgery as the preferred means of prostatectomy at this institution (and many others), has turned out to be much more problematic. While the impact on clinical outcomes of this robotic surgery are unclear, heavy marketing to the patient population has resulted in high demand for the procedure. So, early samples used for this project, as with historical samples on which our preliminary data were based, were from prostates which had been removed by open prostatectomy. As time went on, however, the proportion of samples derived by the laparoscopic procedure rapidly increased. Our initial characterization of responses to Taxotere and the times needed to achieve these were performed using tissue from open prostatectomy samples.

We noted in previous annual reports that the quality of tissue derived from prostates which were resected by this technique was not as good as that seen in open prostatectomy samples. In response to this we modified our procedures to allow recovery of control tissue in vivo. What was not clear at that time, but has come into focus in the last year, is that the loss of blood supply seen by the prostatic tissue during laparoscopic surgery resulted in a preferential killing of tumor as compared to normal cells. Thus while the normal cells were able to recover reasonably well when grafted into SCID mouse hosts this was not true of the cancer tissues. As a result, there was a significant reduction in the proportion of cancer tissues seen in the samples derived from patients who underwent laparoscopic surgery and this has seriously undermined our ability to estimate the response of cancer tissues to treatment with Taxotere.

**Activities in Final Year**

As described in the previous annual report we saw very low numbers of samples with evidence of apoptosis following Taxotere treatment when the tissue samples were collected and stained (examples
shown in figure 1). This was in contrast to both our preliminary data and the optimization experiments, which were performed early in the study (importantly these studies used samples collected from open prostatectomy). It was important, for comparison purposes to perform all of the apoptosis staining (our chosen measure of response to Taxotere) at the same time, therefore the first comparisons could not be made until after the final tissues were harvested.

![Figure 1. Examples of TUNEL (green) and DAPI (blue) staining of grafted human prostate tissue samples in mice treated as noted on the figures. The robust Taxotere response in well defined cancer tissue seen in preliminary studies was absent in the vast bulk of the tissue sections examined. Of note there was a marked absence of tumor tissue in most of the samples (quantitated in table 2).](image)

The goals for the final year of funding were, as stated in the previous report, therefore to ascertain the best method of assessing response to Taxotere, given the poor response seen when apoptosis was assessed. Once this was achieved we planned to hire a data manager as a part of DAMD 17-03-1-0047 to select from banked tissue samples for the work proposed in the final specific aim. We therefore optimized immunohistochemical staining protocols for the expression of a series of markers with the potential to identify cells responding to Taxotere. At the time of submission of the last annual we had
identified some of these markers and others were still being selected. The final chosen markers were:

Thymidine phosphorylase (figure 2), which is induced in a number of tumor types following Taxotere treatment, typically 5-10 fold. Increased levels are long-lasting (peak levels >10 days). This protein is controversial as a predictor for Taxotere response in a number of cancers, but was expected to be useful to confirm exposure of xenografts to therapeutic levels of Taxotere by comparison of treated and non-treated patient matched samples.

Figure 2. Relative thymidine phosphorylase staining intensity in stromal and epithelial compartments of grafted tissue samples with and without both testosterone and Taxotere, averages of samples. No trend was seen between enzyme staining and treatment protocol (shown here) and furthermore no consistent observations were made between patient matched treated and untreated pairs of samples (not shown) therefore no conclusions could be drawn in regard to Taxotere response.

Stabilized detyrosinated (Glu) microtubules, which are increased with Taxotere treatment. It was anticipated that this marker could be used in conjunction with thymidine phosphorylase levels to confirm exposure of tumor xenografts to therapeutic levels of Taxotere, and might also be useful for detection of resistant tumor cells, as resistant cells often have beta-tubulin mutations that prevent microtubule stabilization.

Tubulin stabilization, which is a definitive positive response to Taxotere was also examined to determine which tumors contained cells in which microtubule structure has been locked, as a
The consequence of successful Taxotere treatment.

Thioredoxin, glutathione-S-transferase pi 1, and peroxidoxin 1 levels were also determined from microarray data, as these have been found in breast tumor biopsy samples to correlate with resistance to Taxotere response in patients (table 1 and figure 3).

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Table 1. Microarray results (from project DAMD 17-03-0047, which shared samples with this project) comparing Taxotere treated samples with elevated apoptotic activity and those whose activity was not elevated. The purpose of this comparison was to determine whether this “response” seen in a small number of samples, was supported by changes in other potential markers of Taxotere action. The conclusion from the data presented here is that there was no correlation, suggesting, as we suspected, that the apoptotic response in these samples was not a reliable marker of Taxotere action. These data are shown graphically in pooled form in figure 3. GSTP1=glutathione-S-transferase pi 1, INDO=indoleamine-pyrrole 2,3 dioxygenase, PRDX1=peroxidoxin 1, TXN=thioredoxin, and TDO2=tryptophan 2,3-dioxygenase.
Once optimized, slides were appropriately stained and examined critically in collaboration with a pathologist. This revealed that the lack of response seen in apoptosis was mirrored in these additional markers, however more importantly it also showed that there was little or no tumor tissue in many of the recovered samples, providing a reason for the lack of response. Given that our methods of assessing tumor phenotype prior to grafting into the mice was constant throughout the study (assessment of frozen sections immediately adjacent to the grafted samples) we did not think that the histopathology of the grafted tissue was likely to have changed. This loss of tumor tissue in the rescued fragments correlated with the switch to laparoscopic surgery, suggesting that the poor condition of the tissue presented after this procedure (as noted in earlier reports) was a much more significant problem than we had previously supposed. Our observation that tissues recovered to a condition that allowed the generation of good RNA samples and the generation of MALDI-MS traces did not take into account the nature of the cells from which such samples were derived.
<table>
<thead>
<tr>
<th># of xenograft samples examined</th>
<th>132</th>
</tr>
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<tbody>
<tr>
<td># negative for malignancy</td>
<td>111</td>
</tr>
<tr>
<td># with confirmed carcinoma</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 2. Summary of PCa xenograft pathology. Examination of samples from 132 evaluable xenografts showed that tumor was confirmed in only 8.3% of samples. As noted above this is in contrast to our preliminary studies, and also our published work in this area, where tumor in grafted samples was represented at almost 100% reliability. This change reflects alterations in clinical practice resulting in less well preserved tissue samples, as described in the text.

Given that we were unable to identify sufficient tumor tissue to complete the proposed studies a decision was made to terminate this aspect of the study and to return to DOD-PCRP the funds which had been earmarked for the full bioinformatic analysis of the MALDI-MS data set which we are therefore unable to complete. As noted in the previous report, this aspect of the work is expensive and we believed that it would not be responsible or prudent to proceed with this analysis without a firm grounding relating to the validity and response of the samples.

At this point in time we have a series of MALDI-MS profiles taken from stromal and epithelial tissues which reflect the treatment of human prostate tissue with Taxotere in an in vivo environment. We also have, as a result of the work in DAMD 17-03-1-0047 full gene profiles of the overall tissues from the same samples. Clearly these are potentially useful data sets and should be placed in the public sphere. We therefore plan to write a paper summarizing the two experiments which will allow us to place the whole data set into one of the public databases for future reference for other workers in the area. This will also allow us to air the technical difficulties associated with the surgical changes which are currently sweeping the field. We are working to educate our local surgeons as to the issues and to develop approaches which might sidestep this issue. These approaches include removal of the resected tissue as soon as possible, which represents a very minor change to surgical procedure which could profoundly alter outcomes in a favorable direction.

**Personnel Changes**

None since last report

**Research Goals/Accomplishments**

- Task 1 completed.
- Task 2 completed.
- Determined that we are unable to proceed with bioinformatic analysis as proposed and have
terminated the protocol.

- A set of MALDI-MS profiles has been generated representing protein expression in stromal and epithelial tissues of human prostate in vivo. These illustrate both baseline expression and response to Taxotere.

**Reportable Outcomes.**
None

**Conclusions.**
On a positive note, this project has generated a large data set showing a profile of proteins which are altered in human prostate tissues in vivo following challenge with Taxotere. In particular, we were able to examine both stromal and epithelial tissues to generate a data set which will be useful for future data mining. We believe that these data should be in the public sphere and for this reason we are preparing a paper describing the experiments performed and the results obtained. This will make the full data set available publicly for metaanalysis and for data mining by other interested groups.

The failure to achieve the main aim of the project is a great disappointment, especially since this was apparently due to changes in clinical practices which were well beyond our control, but which have implications for many other studies using human prostate tissues.

This work was always perceived as high risk but potentially very high gain. Clearly the failure to achieve the high gain is not to our satisfaction. It is particularly frustrating that this apparently results from a change in clinical practice which was beyond our control, which does not have established benefits to patients and whose adoption was largely driven by direct marketing to patients.