Award Number: W81XWH-11-1-0367

TITLE: Analysis of Novel Prostate Cancer Biomarkers and Their Predictive Utility in an Active Surveillance Protocol

PRINCIPAL INVESTIGATOR: Adam S. Feldman, M.D., M.P.H.

CONTRACTING ORGANIZATION: Massachusetts General Hospital
Boston, MA 02114-2696

REPORT DATE: May 2016

TYPE OF REPORT: Annual Summary

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.
Analysis of Novel Prostate Cancer Biomarkers and Their Predictive Utility in an Active Surveillance Protocol

Adam S. Feldman, MD, MPH

E-Mail: afeldman@partners.org

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

The Research Project supported by this DOD PCRP Physician Research Training Award investigates novel biomarkers for prostate cancer and investigation of Active Surveillance of low-risk prostate cancer. In this fifth year of my DOD PCRP PRTA, I have continued my productivity from both a translational laboratory and clinical research standpoint. We have continued to investigate our list of biologically relevant candidate prostate cancer biomarkers and have demonstrated promising results. We have also continued to investigate the expression of these markers in prostate cancer tissue and normal prostate using immunohistochemistry. We have had promising results with Tissue Inhibitor of Matrix Metalloproteinase Type 1 (TIMP-1), Semenogelin-2, and Leukocyte Elastase Inhibitor (SERPINB1). With these three biomarkers, we have made significant strides in identifying differential expression patterns. In addition to success in our laboratory work, we have also continued to make significant accomplishments in analyzing our database of 469 men on active surveillance for prostate cancer over the past 15 years. Our analysis of this database has demonstrated freedom from intervention of 77% at 5 years and 62% at 10 years. Cancer-specific survival was 100% at 10 years, and overall survival was 95% at 5 years and 88% at 10 years. We are currently in the process of writing a manuscript to formally publish our results.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>4</td>
</tr>
<tr>
<td>Body</td>
<td>4</td>
</tr>
<tr>
<td>Key Research Accomplishments</td>
<td>13</td>
</tr>
<tr>
<td>Reportable Outcomes</td>
<td>13</td>
</tr>
<tr>
<td>Conclusion</td>
<td>14</td>
</tr>
<tr>
<td>Appendices</td>
<td>15</td>
</tr>
</tbody>
</table>
**Introduction:**

The Research Project supported by this DOD PCRP Physician Research Training Award investigates novel biomarkers for prostate cancer detection and prediction of disease outcome. The goals and objectives of this study are summarized by the Specific Aims: 1. Evaluate the relative levels of expression of our panel of candidate biomarkers in urine, tissue and serum from patients with prostate cancer compared with normal controls to identify prostate cancer specific biomarkers. 2. Evaluate the relative urine, tissue and serum levels of these prostate cancer specific biomarkers within our active surveillance (AS) cohort to identify accurate biomarkers predictive of indolent vs. progressive prostate cancer. The funding from this Physician Research Training Award provides salary support for me to secure protected time as a translational and clinical investigator in prostate cancer research. It also provides salary support for a Research Assistant for my research.

**Body:**

The first four years of my DOD PCRP PRTA involved the identification of differentially expressed proteins in the urine from men with prostate cancer compared to samples from men without disease through mass spectrometry analysis. In summation, I used mass spectrometry (MS) to quantitatively compare the entire urinary proteome and identify differentially expressed proteins in the urine from men with prostate cancer as compared with those found in controls. The MS analysis identified >1400 unique proteins, and comparative analysis revealed 55 potential prostate cancer specific proteins. Over the ensuing period of this grant, we extensively investigated these potential candidate biomarkers via Western Blot, ELISA (enzyme-linked immunosorbent assay) and Immunohistochemistry (IHC). Our investigation of potential biomarkers includes serpinB1, tissue inhibitor of matrix metalloprolinease type 1 (TIMP1), and semenogelin 2 (SEMG2); these three proteins represent only a select group amongst many other proteins of interest, which we have investigated and outlined in previous reports.

In this fifth year of funding, our focus on the validation of these three potential biomarkers, serpinB1, SEMG2, and TIMP1 as relevant prostate cancer biomarkers was continued. In addition to continuing our investigation of the expression of these proteins in patients with localized disease, we expanded our experiments to include patients with metastatic prostate cancer. Our patients are now divided into four groups; patients with no prostate cancer (control group), patients with Gleason score 6 prostate cancer, those with score 8 or above, and patients with metastatic prostate cancer. Using these four groups has allowed us to better observe differential expression patterns for our proteins of interest, specifically serpinB1, SEMG2, and TIMP1.
In addition to expanding our patient populations, this year has been marked by a heightened response to technical difficulties and shortcomings. Of utmost importance for the analysis and accurate representation of protein expression is the quantification of total protein in urine samples. While there are a number of methods to measure total protein concentration, some procedures are incompatible with protein sourced from urine samples and seemed to give inaccurate results. For this reason, it is imperative that an appropriate method of quantification is identified and consistently used. Some (but not all) previous data were generated using the widely applicable BCA assay for protein quantification, although recently we established this was a less acceptable way to measure the concentration in urine samples. While we do not have an exact mechanism for why the BCA assay fails to give accurate protein concentrations, our inconsistencies observed using this method were reflected in other partnering labs’ experiences with quantifying urine samples. A more robust method of protein quantification, based on the Bradford assay, is now being used and our results reflect more reliable data.

Our previous data suggested the protein TIMP1 was upregulated in prostate cancer. The ongoing exploration of this protein’s expression pattern uses Western blot and ELISA to observe levels in urine samples. We have repeatedly run into technical difficulties in Western blotting due to the nature of the samples, which have extremely variable concentrations and protein content. For this reason, we have coupled Westerns with ELISA, which serves as an accurate and fully quantitative method of protein identification. It is easily compatible with urine samples and has yielded especially interesting results for TIMP1 with the addition of the fourth patient population (men with metastatic disease).

The Western blot data for this protein, TIMP1, shows the protein may be generally expressed at higher levels in Gleason 6 samples than controls (fig. 1). Although we need to expand our enrollment numbers, preliminary ELISA results show these two groups may indeed differ in TIMP1 expression levels (fig. 2). Between localized disease groups, both Western blot and ELISA suggest there might actually be a significant decrease in TIMP1 expression in patients with Gleason score ≥8 as compared to those with Gleason 6 (fig. 1, 2). This downward trend in TIMP1 expression seems to continue to metastatic from Gleason ≥8 disease on ELISA, although Western blotting shows metastatic samples as having consistently moderate TIMP1 levels (fig. 1, 2). Because we only recently added this patient population, we need to replicate these experiments to confirm the correlation between TIMP1 and disease stage. In order to validate these observations and trends in Westerns, it will be necessary to compare quantifications of the band sizes between populations across all the membranes we generate. This is an ongoing next step of analysis. In ELISAs, we want to generate meaningful correlation coefficients for differences in median expression values, and for this more samples need to be analyzed. This reduced TIMP1 expression in high risk and metastatic disease may in effect allow a reduced
inhibition of matrix metalloproteinases (MMP), such as MMP-9, and has been demonstrated by others in tissue using IHC.\textsuperscript{1,2}

Figure 1 \textit{TIMP1 expression in four prostate cancer patient populations on two different membranes.} Four patients per population are represented in each blot. Populations are indicated by the following abbreviations; c- control (no prostate cancer), 6- Gleason score 6, $\geq$8- Gleason score of 8 or above, m- metastatic disease. The positive control, human recombinant TIMP1 protein, is indicated by “pc.” 40\textmu g of total protein was loaded into each lane. All Western blots in this study are performed using PVDF membranes. Please note the two different membranes with different exposure times. The expected band size for this protein is 29kD.


Figure 2 Percent TIMP1 of total protein measured by ELISA. Box plots give representation of median value, interquartile range, range, and outliers. Patient populations are indicated by the following abbreviations; c- control (no prostate cancer), 6- Gleason score 6, 8- Gleason score of 8 or above, m- metastatic disease. Each category contained either nine or ten patients. In this figure, the concentration of TIMP1 is expressed as a percent of total protein.

The next protein of interest, SerpinB1, also showed an interesting trend in expression pattern. On ELISA, the median value of SerpinB1 expression (represented as a percent of total protein) was highest in control samples, with a drop in value for all patients with any stage of prostate cancer (fig. 3). Because the ranges of SerpinB1 percent overlap for the groups, as well as the 95% confidence intervals for the median values, we will need to continue replications of this experiment to elucidate the consistency and significance of the trend (fig. 3). Of note, because the sample size is small (between six and eight patients per population), it is difficult to calculate a reliable confidence interval for the significance of the median values. For this reason, more repetitions of ELISA for SerpinB1 are warranted.

When visualized on Western blots, the pattern of SerpinB1 expression may not be as easily assessed. Similar to the TIMP1 immunoassays, these Westerns for SerpinB1 show nearly the opposite of what the ELISA analysis indicated. While the ELISA showed lower levels of SerpinB1 in prostate cancer as compared with disease-free urine samples, Western blotting had large bands indicative of high SerpinB1 expression in especially Gleason 6 samples while Gleason 8 or above and metastatic samples
may have lower than control levels (fig. 4). To make sure these qualitative observations are accurate, we will need to quantify Western bands using the computer program ImageJ and see if there is a consistent trend.

**PERCENT SERPINB1 OF TOTAL PROTEIN**

![Box plot showing expression levels of SerpinB1 in four prostate cancer patient populations. Each group consists of six to eight patients. The concentration of SerpinB1 is expressed in this figure as a percent of total protein.](image)

**Figure 3** Box plot showing expression levels of SerpinB1 in four prostate cancer patient populations. Each group consists of six to eight patients. The concentration of SerpinB1 is expressed in this figure as a percent of total protein.

Our inconsistencies with Western blots and ELISA data are not new challenges, but our progress with accurately measuring protein concentration and understanding discrepancies that may affect results has allowed for continued experimentation. One reason we may observe dissimilarities between these immunoassays is the different antibodies used; unfortunately, this is a factor over which we have very little control. Another difference in the two methods involves the preparation of samples. While both use concentrated proteins isolated from urine, under-concentrated samples are extremely difficult to use for Western blots, so it is important sufficient concentration is reached before using these samples. High loading volumes (corresponding to low concentrations) may affect how much protein actually runs down the lane, which may result in a smaller band for the target protein. This volume issue is not a concern for ELISA, which can detect target protein in the least concentrated of samples.
Figure 4 Three membranes showing SerpinB1 expression. Four patient populations are represented on each blot. In these three membranes, a total of 12 patients per population are assessed for SerpinB1 expression. The positive control, MCF7 cell lysate, is indicated by “pc.” 40µg of total protein was loaded into each lane. The expected size for this protein is 43kD.

The third protein of interest, SEMG2, has rarely been observed on Western blot by our group. This may be because there is very little SEMG2 in urine and Western blotting is not sensitive enough to detect it. We have tried a number of different antibodies and membrane blotting conditions, but consistently only the positive control of COLO205 cell lysate shows a band (fig. 5). However, the levels of SEMG2 are sufficient enough to be detected by ELISA. When this immunoassay was performed for SEMG2, a similar trend to that of TIMP1 was observed (fig. 6). According to our ELISA, SEMG2 is expressed in higher concentrations in patients with Gleason 6 disease and then drops off to the lowest median value in metastatic patients (fig. 6). Interestingly, the control samples seem to have SEMG2 levels that fall within the ranges of Gleason 6 to metastatic expression levels, but the metastatic patients have almost no overlap with Gleason 6 expression levels (fig. 6). In fact, basic statistical analysis on these two groups, Gleason 6 and metastatic, indicates that the median values differ with 95% confidence. Aside from one outlier, the metastatic samples are all lower in expression than Gleason 6 samples. This interesting
finding encourages repetition of this particular ELISA with new patients, as well as continued attempts at showing the trend in a Western blot.

Figure 5 Western blots probing for SEMG2. Even after 10 minutes of exposure, the target protein is only observable in the positive control, COLO205 cell lysate, and MCF7 cell lysate lanes. This is suggestive of insufficient protein levels in urine samples. 40ug of total urinary protein was loaded into each lane. The expected band size for this protein is 65kD.
Figure 6 **Box plots of ELISA data for SEMG2 in four patient categories. Basic statistical analysis indicates the median values for 6 and metastatic categories are different with 95% confidence. The concentration of SEMG2 is expressed in this figure as a ratio against the concentration of total protein.**

Aside from focusing on these three protein biomarkers for prostate cancer, our group also has continued to expand our work in metabolomic evaluation of urine and tissue in prostate cancer patients. This work is in collaboration with Leo L. Cheng, Ph.D., here at the Massachusetts General Hospital. Our group has a long history of have investigating metabolomic signatures in prostatectomy specimens using *ex vivo* MR spectroscopy.\(^3\)\(^4\)\(^5\) Results from these studies of radical prostatectomy samples suggested the existence of metabolic or metabolomic fields, i.e. PCa metabolic information are observed to delocalize from PCa glands and into the surrounding structures that are benign tissue according to histology. These field effects likely create PCa “metabolomic lesions” that are larger than the histologic lesions. We have also continued to utilize multiparametric prostate MRI and MRI-Ultrasound fusion biopsy of the prostate to investigate biopsy cores taken from MRI targetable lesions and uninvolved prostate regions that appear normal by imaging criteria. Our current cohort of subjects in this study includes 25 patients, with suspicious lesions (PIRADS ≥ 3) undergoing MRI-Ultrasound fusion biopsy. From each patient, one targeted core biopsy and one standard template biopsy from a region appearing normal on MRI, immediately undergoes *ex vivo* MR spectroscopy to assess metabolite levels. Each specimen then is submitted for standard histopathologic analysis. Patient voided urine specimens, obtained just prior to the prostate biopsy, also undergo MR spectroscopy to assess metabolite levels. We are currently evaluating our data to assess for metabolic biomarkers, to distinguish malignant from benign, and clinically significant disease (Gleason ≥ 7) from clinically insignificant disease (Gleason 6). We will investigate the presence of potential metabolomic biomarkers in tissue, but also in urine from the same patients. We also will compare *ex vivo* biopsy metabolomic data with the *in vivo* pre-biopsy multiparametric prostate MRI data to assess for metabolomic characteristics of various PIRADS lesions. We look forward to analyzing and reporting these data in the near future.

---


My clinical research in active surveillance for low risk prostate cancer has also continued and we have made further progress in our investigation of our database of nearly 1000 men on active surveillance. In our work on assessing outcomes of men who initiate active surveillance when they are under 60 years of age, we have begun a collaborative analysis combining our cohort with that of Dr. Lawrence Klotz at Sunnybrook Health Sciences Centre in Toronto. We are currently performing the data analysis and expect manuscript submission within the next few months.

We also have investigated our cohort of men on active surveillance for those whom we would consider to be “borderline” candidates. Our guidelines for AS eligibility, formalized in 2008, include Gleason ≤6, stage ≤cT2a, PSA <10 ng/mL, ≤3 of 12 cores positive at diagnosis, and ≤20% of any core involved at diagnosis. For this analysis, we defined borderline cases for AS as those patients with one or more of either Gleason score 7, PSA >10, stage cT2a, >33% of cores positive at diagnosis, or >20% of any core involved at diagnosis.

In the entire cohort (n=990), mean age at diagnosis was 66.9 years (±7.9) and median PSA 5.1 (IQR 4-6.87). While the majority met all AS criteria, 312 patients (31.5%) met at least one of the borderline AS criteria; 2.4% of patients had Gleason 7, 7.6% had PSA>10, 8.0% were cT2a, 4.1% had >33% of cores positive at diagnosis, and 18.4% had >20% of any core involved. With mean follow-up 5.2 years, univariate survival analysis demonstrated no difference in freedom from treatment (FFT) between patients with Gleason 7 vs. ≤6, >33% vs. ≤33% cores involved, or PSA >10 vs. ≤10. Lower FFT was noted among patients with cT2a vs. ≤cT1c disease (59.4% vs. 70.6%, P=0.04) and >20% vs. ≤20% of any core involved (56.5% vs. 69.5%, P=0.01). In multivariate analysis, >20% core involvement remained a significant predictor for treatment, adjusting for PSA>10, Gleason>6, >33% cores involved, and stage. Among the 312 borderline AS cases, there were only 5 (1.6%) cases of metastasis and 1 (0.3%) prostate cancer-specific death. These adverse outcomes were equivalent to the remainder of the cohort meeting strict AS criteria, which included 10 (1.5%) cases of metastasis and 2 (0.3%) prostate cancer-specific deaths.

We concluded that active surveillance remains a viable option for select patients who are borderline cases per current AS criteria. However, patients with higher volume disease may be more likely to progress to treatment. It is clear that long-term clinical outcomes in these patients should continue to be investigated, and we will continue to observe their outcomes over time. These analyses and results are being prepared as a manuscript for publication, which we anticipate in the next several months.
In addition to significant research accomplishments, I continue to meet my goals within the training program of this grant. I meet regularly with my two mentors, Drs. Matthew Smith and Bruce Zetter. In our regular meetings, we not only discuss research progress, but also focus on career planning and guidance. I attend regular urologic oncology clinical and research conferences at our institution and both attend and present at regional and national scientific meetings. I attend regular laboratory research meetings both for our own research progress, as well as reviewing other associated research in the current literature.

**Key research accomplishments:**

- Expanded analysis of three potential biomarkers to high score prostate cancer (Gleason score 8 or above) and metastatic disease
- Produced continued data indicative of differential expression between control and cancer groups for SerpinB1, and between disease progression for TIMP1 and SEMG2
- Optimized protocols to overcome previous setbacks and challenges in order to accumulate reliable and reproducible data
- Began metabolomic evaluation of tissue and urine in pilot cohort of men undergoing MRI-Ultrasound fusion targeted prostate biopsy
- Investigated outcomes of active surveillance in young men and men with borderline clinical features

**Reportable Outcomes:**

  *Co-first Authorship
Conclusion

In summary, this fourth year of my DOD PCRP PRTA has been very productive. After identifying a number of potential biomarkers pertaining to prostate cancer, I have worked to explore the expression patterns’ correlations with disease progression. In particular this year, I expanded my patient populations to include metastatic disease and conducted ELISAs on a number of proteins to get quantitative results. The quantifications allowed unambiguous comparisons across patient populations and revealed previously unobserved expression trends.

In addition to our work in proteomic biomarker evaluation, we have begun to explore metabolomic biomarkers and their relationship with MRI-based imaging. This collaborative project is very exciting and will complement our current work in proteomic biomarkers and active surveillance very well.

All of our projects are extraordinarily clinically relevant and have no trouble applying to “so what” criteria. While the prostate specific antigen (PSA) serves as the predictive biomarker for prostate cancer, new diagnostics with more consistent predictability and enhanced performance characteristics are sorely needed. The work funded by this grant directly addresses the challenge of discovering more predictive, dependable, and informative biomarkers; we are in the process of producing results to meet this goal.

Appendices:
Curriculum Vitae for Dr. Adam S. Feldman is included in this annual reporting.
Curriculum Vitae

Date Prepared: October 26, 2016

Name: Adam S. Feldman, M.D., M.P.H.

Office Address:
Department of Urology
Massachusetts General Hospital
55 Fruit Street, GRB 1102
Boston, MA 02114 United States

Home Address:
29 Lovett Rd.
Newton, MA 02459

Work Phone: 617-643-1955

Work E-Mail: afeldman@partners.org

Work FAX: 617-726-9150

Place of Birth: New York, NY

Education
1994  B.A. - Biological Basis of Behavior  University of Pennsylvania
1996  M.A. (Alpha Epsilon Lambda) - Medical Sciences  Boston University School of Medicine
2000  M.D. (Alpha Omega Alpha)  University of Massachusetts Medical School
2009  M.P.H. – Clinical Effectiveness  Harvard School of Public Health

Postdoctoral Training
07/00-06/01  Intern in Surgery, Massachusetts General Hospital
07/01-06/02  Resident in Surgery, Massachusetts General Hospital
07/02-06/05  Resident in Urology, Massachusetts General Hospital
07/05-06/06  Chief Resident in Urology, Massachusetts General Hospital
07/06-06/08  Fellow in Urologic Oncology, Massachusetts General Hospital

Faculty Academic Appointments
2000-2006  Clinical Fellow in Surgery, Harvard Medical School, Boston, MA
2006-2010  Instructor in Surgery, Harvard Medical School, Boston, MA
2010-present  Assistant Professor of Surgery, Harvard Medical School, Boston, MA

Appointments at Hospitals/Affiliated Institutions
2006-present  Assistant in Urology, Massachusetts General Hospital, Boston, MA

Major Administrative Leadership Positions
2011  Scientific Program Chair, American Urological Association, New England and Mid-Atlantic Sections, Annual Meeting
2015-present  Director, Combined Harvard Urologic Oncology Fellowship
**Other Professional Positions**
2012 Member: Scientific Program Committee, American Urological Association, New England Section Annual Meeting
2012-present Board Member: Sean Kimerling Testicular Cancer Foundation
2013-present Co-Leader of the Career Development Program: DFCI/HCC Prostate Cancer SPORE
2016 Scientific Co-Chair: DF/HCC Kidney Cancer Program Retreat

**Committee Service - Local**
2012-present Member: Surgical Coordination Committee, Department of Urology, MGH
2013-2015 Member: MGH eCare Big Data and Data Repository Workgroup
2013-present Urology Representative: Clinical Research Workgroup of the Continuous of the Continuous Research Operations Improvement (CROI) Task Force
2016-present Urology Representative: MGH Research Council

**Committee Service - Regional**
2012-present Member: Massachusetts Medical Society Committee on Men’s Health

**Committee Service - National**
2013-present Member: Eastern Cooperative Oncology Group (ECOG) Genitourinary Committee

**Professional Societies**
1998-present Massachusetts Medical Society, Member
2002-present American Urological Association, Member
2004-present American Association of Clinical Urologists, Member
2009-present Society of Urologic Oncology, Member

**Grant Review Activities**
2012-13 Prostate Cancer Foundation Young Investigator Awards Review Committee
2013-15 Bladder Cancer Advocacy Network Young Investigator Awards Review Committee
2013-14 Prostate Cancer Foundation Challenge Awards Review Committee
2013-16 DFCI/HCC Prostate Cancer SPORE Review Committee

**Editorial Activities**
2006 Ad-Hoc Reviewer, International Braz J Urol
2010-present Ad-Hoc Reviewer, Urology
2010-present Ad-Hoc Reviewer, Prostate Cancer and Prostatic Diseases
2010-present Ad-Hoc Reviewer, Urologic Oncology
2011-present Ad-Hoc Reviewer, BJU International
2012-present Ad-Hoc Reviewer, Molecular Cancer Research
2013-present Ad-Hoc Reviewer, European Urology
2015 Ad-Hoc Reviewer, JAMA

**Editorial Board**
2015-present Editorial Board Member, BMC Urology

**Honors and Prizes**
1996  Alpha Epsilon Lambda - Graduate Honors Society, Boston U. School Of Medicine
2000  Senior Scholar - Department of Surgery, U. Of Massachusetts Medical School
2000  Alpha Omega Alpha Honor Medical Society, U. Of Massachusetts Medical School
2003  Resident Abstract Travel Award, American Urological Association - New England Section
2005  Merit Award for Outstanding Abstract, The ASCO Foundation Grants Program – Multidisciplinary Prostate Cancer Symposium
2006  Gerald P. Murphy Scholar, American Urological Association
2008  Merit Award for Outstanding Abstract, The ASCO Foundation Grants Program – Multidisciplinary Genitourinary Cancers Symposium
2009  AUA Foundation Research Forum – AUA New England Section Nominee
2008  Prostate Cancer Foundation Young Investigator Award
2011  CINE Golden Eagle Award – CBS Public Service Announcement on Prostate Cancer
2012  AUA Foundation Research Forum – AUA New England Section Nominee

Report of Funded and Unfunded Projects

Funding Information

Past:

1997  Student  Institutional Grant, Joseph P. Healy Grant, Pre-clinical Intercultural Program, University of Massachusetts Medical School
  • Summer intercultural immersion program in clinical medicine in Latino community in Miami, FL

1997-1998  Project Director  Institutional Grant, Community Service Grant funding Creating Our Future Program, University of Massachusetts Medical School
  • Program in which medical students tutored and mentored children of homeless families in Worcester, MA

2007-2008  P.I.  Claire and John Bertucci Prostate Cancer Research Fund, A Proteomic Approach to Prostate Cancer Biomarker Discovery
  • Use proteomic techniques for urine biomarker discovery in men with prostate cancer
  • $25,000 award

2007-2009  P.I.  Company – Predictive Biosciences; Evaluation of Urine Based Protein Biomarkers in Bladder Cancer
  • Analyze urinary proteins as novel diagnostic and surveillance markers in bladder cancer
  • Sponsored Research Agreement

2009-2010  P.I.  Claire and John Bertucci Prostate Cancer Research Fund - Active Surveillance for Prostate Cancer: Management Patterns, Outcomes, and Quality of Life
- Funding supports research personnel for data mining and management
- $25,000 award

2008-2012  P.I.  Prostate Cancer Foundation – Young Investigator Award; Proteomic Discovery and Analysis of Novel Biomarkers in Prostate Cancer

- Use proteomic mass spectrometry techniques for identification of novel prostate cancer biomarkers in urine and serum

$75,000 per year for 3 years.

2009-2010  Investigator  Harvard Catalyst Pilot Grant Program

NIH UL1 RR 025758-02 Clinical and Translational Science Center Grant

Sonoelastography for Tumor-Targeted Prostate Biopsy

- This study is a pilot study of the utility of sonoelastography for targeting biopsy to foci of cancer in the prostate.

2015  P.I.  Project Title: A Collaborative Study Using Primary Prostate Cells and their Reprogramming for the Study of Progression to Castrate Resistant Prostate Cancer
Role on the Project: Site PI
Supporting Agency: Georgetown University/GHUCCTS/Clinical and Translational Science Awards
Level of Funding: $10,000

Myriad Genetic Labs, Inc.
The objective of this registry is an estimation study intended to evaluate the impact of genomic test results towards selecting a first-line therapy option for newly diagnosed localized prostate cancer patients

Current:
2009-present  Investigator  RTOG 0712: A Phase II Randomized Study for Patients With Muscle-Invasive Bladder Cancer Evaluating Transurethral Surgery and Concomitant Chemoradiation by Either BID Irradiation Plus 5-Fluorouracil and Cisplatin or QD Irradiation Plus Gemcitabine Followed by Selective Bladder Preservation and Gemcitabine/Cisplatin Adjuvant Chemotherapy

2011-present  P.I.  Department of Defense Prostate Cancer Research Program - Physician Research Training Award; Analysis of Novel Prostate Cancer Biomarkers and Their Utility in an Active Surveillance Protocol
The research project will investigate novel biomarkers in prostate cancer detection and prediction of disease outcome.
$130,000 per year for 5 years
2013-present  P.I.  Project Title: Validating Conditionally Reprogrammed Cells to Advance Personalized Medicine for Prostate Cancer  
Role on the Project: Site PI  
Supporting Agency: Georgetown University/DoD (W81XWH-12-PCRP)  
Level of Funding: $50,000

2013-present  Investigator  RTOG0938: A Randomized Phase II Trial of Hypofractionated Radiotherapy for Favorable Risk Prostate Cancer

2013-present  Investigator  Phase III randomized clinical trial of proton therapy vs IMRT for low or low-intermediate risk prostate cancer

2013-present  Investigator  Characterizing Prostate Cancer by ex vivo MRS Signature (Cheng)  
NIH/NCI, R01CA115746  
The proposed project is aimed at permitting translation of our pre-clinical human study results into new diagnostic and evaluation paradigms for the PCa clinic

2014-present  P.I.  Prognostic Utility of CCP Score in Patients with Renal Cell Carcinoma  
Myriad Genetics, Inc.  
The specific aims are: 1) to evaluate the prognostic utility of the CCP score generated from nephrectomy to predict recurrence and cancer-specific mortality in patients who have undergone radical nephrectomy; 2) to evaluate the correlation between CCP scores generated from biopsies and nephrectomy tissue in patients with paired samples; and 3) to evaluate the association between CCP score form biopsy and observed tumor growth rate in patients with RCC managed by active surveillance.

2015-present  Site-P.I.  Tissue-based Genomics for Risk Stratification in Localized Renal Cell Carcinoma  
University of Michigan/NCCN  
The goal of this subcontract work is to collaborate with University of Michigan to provide clinical specimens and clinical data to Myriad Genetics on the clinical management of patients with RCC.

Unfunded Projects
Past:
1991  Research Assistant  Isolation and sequencing of a conserved domain of the DnaJ family of chaperonins. Department of Surgical Research, Children’s Hospital, Boston, MA.

1994-1995  Research Assistant  Evaluation of Critical Pathways for CHF, DVT, and Normal Vaginal Delivery with 24 hour LOS. Brigham and Women’s Hospital, Boston, MA.


1999-2000  Research Fellow  Characterization of Angiogenic Markers in the Rat Genitourinary System. Laboratory for Cellular Therapeutics and Tissue Engineering, Department of Urology, Children’s Hospital, Boston, MA.

2002-2004  Investigator  Development of bladder cancer in a murine model for Cables knock-out mice exposed to N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN). Laboratory of Urology/Pathology, Massachusetts General Hospital, Boston, MA.

2002-2004  Investigator  The Role of Cables, a novel cell-cycle regulatory protein in human transitional cell carcinoma and prostate cancer. Laboratory of Urology/Pathology, Massachusetts General Hospital, Boston, MA.

2004-2005  Investigator  Proteomic analysis of voided urine specimens for biomarker discovery and validation in prostate and bladder cancer. Laboratory of Urology/Pathology,
Massachusetts General Hospital. Department of Vascular Biology, Children’s Hospital, Boston, MA.

2007-2008 Investigator Laparoscopic and Open Radical prostatectomy after laparoscopic inguinal hernia repair. Massachusetts General Hospital, Boston, MA.

2010 Investigator Outcomes of Organ Sparing Surgery in Penile Cancer. Massachusetts General Hospital, Boston, MA.

2010-2012 Investigator Multi-Institutional Bladder Cancer Quality Care Initiative for non-metastatic muscle invasive transitional cell carcinoma of the bladder.

Current:
2008-present P.I. A comparison of nephron sparing techniques: percutaneous radiofrequency ablation (RFA) vs. open and laparoscopic partial nephrectomy. Massachusetts General Hospital, Boston, MA.

2009-present P.I. Active Surveillance in Prostate Cancer: Retrospective analysis of quality of life and outcomes and development of a prospective cohort. Massachusetts General Hospital, Boston, MA.

2010-present P.I. Renal Biopsy for Small Renal Masses. Massachusetts General Hospital, Boston, MA.

2013-present Investigator PARTIQoL (Prostate Advanced Radiation Technologies Investigating Quality of Life) Registry

Report of Local Teaching and Training

Teaching of Students in Courses
2006-present Urologic Surgery

<table>
<thead>
<tr>
<th>Year</th>
<th>Group</th>
<th>Contact Time</th>
<th>Prep Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008-2010</td>
<td>Attending 30 Medical Students 8 Residents</td>
<td>10 hours/week for 50 week(s)</td>
<td>none reported</td>
</tr>
<tr>
<td>2010, 2015</td>
<td>Patient Doctor II 5 Medical Students</td>
<td>8 hours/year for 1 year(s)</td>
<td>none reported</td>
</tr>
</tbody>
</table>

2013-present HMS Surgical Clerkship Lecture on Urologic Surgery

<table>
<thead>
<tr>
<th>Year</th>
<th>Group</th>
<th>Contact Time</th>
<th>Prep Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013-present</td>
<td>Attending 10 Medical Students</td>
<td>4 hours/year for 1 year(s)</td>
<td>3 hours</td>
</tr>
</tbody>
</table>
Formal Teaching of Residents, Clinical Fellows and Research Fellows (post-docs)

2007
**Surgical Chief's Rounds** - Department of Surgery - Injuries to the Urogenital Tract

<table>
<thead>
<tr>
<th>Lecturer</th>
<th>25 Residents</th>
<th>contact time</th>
<th>prep time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hour</td>
<td>5 hours</td>
</tr>
</tbody>
</table>

2008-present
**Ambulatory Teaching Rounds** - Department of Medicine – Uro-oncology for the primary care physician; Management of Small Renal Masses

<table>
<thead>
<tr>
<th>Lecturer</th>
<th>30 Residents</th>
<th>contact time</th>
<th>prep time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 hours/year</td>
<td>10 hours/year</td>
</tr>
</tbody>
</table>

2010
**General Surgery Teaching Rounds** – Department of Surgery – Bladder Cancer Review

<table>
<thead>
<tr>
<th>Lecturer</th>
<th>25 Residents</th>
<th>contact time</th>
<th>prep time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 hour</td>
<td>3 hours</td>
</tr>
</tbody>
</table>

Clinical Supervisory and Training Responsibilities

2006-present
Urological Surgery – Training of Residents/Fellows 15 hours/week

2008-2012
Sub-specialty Faculty Advisor for the Acute Care Surgery fellow 10 hours/year

2015-present
Director, Combined Harvard Urologic Oncology Fellowship

Laboratory and Other Research Supervisory and Training Responsibilities

2007-present
Supervision and mentoring of Research Fellow 5 hours/week

Formal Teaching of Peers (e.g., CME and other continuing education courses)

1996-1997
Worcester, MA
Teaching Assistant/Tutor in Biochemistry, University of Massachusetts Medical School
Responsibility: Tutor fellow medical students in Biochemistry.

2009
Las Vegas, NV
Faculty (CME Course): Maximizing Bone Health for Patients With Prostate Cancer: Establishing the "Who, What, Why & How?"

2009
Scottsdale, AZ
Faculty (CME Course): Maximizing Bone Health for Patients With Prostate Cancer: Establishing the "Who, What, Why & How?"

2010
San Francisco, CA
Faculty (CME Course): Master Class on Integrating Novel Antiresorptive Agents into the treatment of Prostate Cancer

2010
Boston, MA
Faculty (CME Course): Trauma and Critical Care Symposium – Penile and Genitalia Trauma

2011
Boston, MA
Faculty (CME Course): Society of Translational Oncology Prostate Cancer Symposium – Prostate Cancer: Progress and Promise

2011
Cambridge, MA
Faculty (CME Course): Primary Care Internal Medicine: Principles & Practice – Case Studies in Urology [Invited Lecture]

2013
Ft. Lauderdale, FL
Faculty (CME Course): Winter Oncology Symposium – Holy Cross Hospital

2013
Waltham, MA
Faculty (CME Course): Men's Health Symposium – Prostate Cancer: Screening, Management and Controversy

2013
Chicago, IL
Faculty (CME Course): Radiologic Society of North America –
Refresher course: Small renal mass (T1a) – the case for resection

2014 Cambridge, MA Faculty (CME Course): Primary Care Internal Medicine: Principles & Practice – Male Urology [Invited Lecture]

2014 Boston, MA Faculty (CME Course): 17th Biennial Urologic Cancer Course – Bladder Cancer Biomarkers

2014 Chicago, IL Faculty (CME Course): Radiologic Society of North America – Refresher course: Small renal mass (T1a) – the case for resection

2015 Video Series Faculty (CME Course): Comprehensive Review of Urology – Penile and Urethral Cancer

2015 Boston, MA Faculty (CME Course): UroTrack – Renal Mass Biopsy

MGH Experience in MRI Fusion Prostate Biopsy

2014 Cambridge, MA Faculty (CME Course): Primary Care Internal Medicine: Prostate in the Aging Male [Invited Lecture]

2015 Chicago, IL Faculty (CME Course): Radiologic Society of North America – Refresher course: Small renal mass (T1a) – the case for resection

2015 Boston, MA Faculty (CME Course): UroTrack – Renal Mass Biopsy Debate - Pro

Report of Regional, National and International Invited Teaching and Presentations

Local Invited Presentations and Courses

2008 Boston, MA Comparative Analysis of Nephron Sparing Techniques. Update on Urologic Oncology – Massachusetts General Hospital, Harvard Medical School [Invited Lecture]

2008 Boston, MA Prostate Cancer: Diagnosis and Management. Prostate Cancer Support Group, Massachusetts General Hospital [Invited Lecture]

2011 Boston, MA Controversies Around the Management of Small Renal Masses – DF/HCC Kidney Cancer Program [Invited Lecture]

2011 Boston, MA Proteomic Discovery of Novel Biomarkers in Prostate Cancer – Massachusetts General Hospital Department of Urology Centennial Academic Program [Invited Lecture]

2011 Cambridge, MA Management of Small Renal Masses – Harvard University Health Services Grand Rounds [Invited Lecture]

2011 Boston, MA Incidental Radiologic Findings: "Incidental Renal Masses" – Massachusetts General Hospital Medical Grand Rounds [Invited Lecture]

2012 Concord, MA Controversies in the Management of the Small Renal Mass – Emerson Hospital Medical Grand Rounds [Invited Lecture]

2014 Boston, MA Management of Renal Lesions in Tuberous Sclerosis Complex – Massachusetts General Hospital Department of Pathology Grand Rounds [Invited Lecture]

2015 Boston, MA Management of the Small Renal Mass – Massachusetts General Hospital Department of Urology Grand Rounds [Invited Lecture]
<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Title</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>Boston, MA</td>
<td>Prostate Cancer: Facts and Misconceptions – Massachusetts State House, Prostate Cancer Awareness Day</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2016</td>
<td>Cambridge</td>
<td>Evaluation and Management of the Small Renal Mass – Cambridge Health Alliance, Department of Surgery Grand Rounds</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2016</td>
<td>Boston, MA</td>
<td>Evaluation and Management of the Small Renal Mass – Massachusetts General Hospital Department of Urology Grand Rounds</td>
<td>[Invited Lecture]</td>
</tr>
</tbody>
</table>

**Regional Invited Presentations and Courses**

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Title</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>Dedham, MA</td>
<td>Urologic Oncology: An Overview. Massachusetts Health Information Management Association</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2010</td>
<td>Mt. Kisco, NY</td>
<td>Controversies in the Management of Small Renal Masses</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2011</td>
<td>Dedham, MA</td>
<td>Penile Cancer. Urology Nursing Society</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2012</td>
<td>Boston, MA</td>
<td>AUA Update in Bladder and Prostate Cancer. AUA New England Section, Annual Meeting</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2013</td>
<td>Ft. Lauderdale, FL</td>
<td>Faculty (CME Course): Winter Oncology Symposium – Holy Cross Hospital</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2013</td>
<td>Waltham, MA</td>
<td>Faculty (CME Course): Men’s Health Symposium – Prostate Cancer: Screening, Management and Controversy</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2015</td>
<td>Bahamas</td>
<td>Renal Mass Biopsy Should Be Used Selectively Prior To a Treatment Decision</td>
<td>[Invited Lecture]</td>
</tr>
</tbody>
</table>

**National Invited Presentations and Courses**

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Title</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>Boston, MA</td>
<td>Renal Cell Carcinoma: Surgical Management at Massachusetts General Hospital. Exchange Experience Program on Renal Cancer</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2009</td>
<td>Las Vegas, NV</td>
<td>Faculty (CME Course): Maximizing Bone Health for Patients With Prostate Cancer: Establishing the &quot;Who, What, Why &amp; How?&quot;</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2009</td>
<td>Scottsdale, AZ</td>
<td>Faculty (CME Course): Maximizing Bone Health for Patients With Prostate Cancer: Establishing the &quot;Who, What, Why &amp; How?&quot;</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2010</td>
<td>San Francisco, CA</td>
<td>Faculty (CME Course): Master Class on Integrating Novel Antiresorptive Agents into the treatment of Prostate Cancer.</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2010</td>
<td>Boston, MA</td>
<td>Faculty (CME Course): Trauma and Critical Care Symposium – Penile and Genitalia Trauma.</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2011</td>
<td>Boston, MA</td>
<td>Faculty (CME Course): Society of Translational Oncology Prostate Cancer Symposium – Prostate Cancer: Progress and Promise</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2011</td>
<td>Cambridge, MA</td>
<td>Faculty (CME Course): Primary Care Internal Medicine: Principles &amp; Practice – Case Studies in Urology</td>
<td>[Invited Lecture]</td>
</tr>
<tr>
<td>2013</td>
<td>New Orleans,</td>
<td>Faculty – 3D Laparoscopic Urology: Surgical Techniques and Hands-On</td>
<td>[Invited Lecture]</td>
</tr>
</tbody>
</table>
2013 Chicago, IL
Faculty (CME Course): Radiologic Society of North America – Refresher course: Small renal mass (T1a) – the case for resection

2014 Cambridge, MA
Faculty (CME Course): Primary Care Internal Medicine: Principles & Practice – Male Urology [Invited Lecture]

2014 Boston, MA
Faculty (CME Course): 17th Biennial Urologic Cancer Course – Bladder Cancer Biomarkers

2014 Chicago, IL
Faculty (CME Course): Radiologic Society of North America – Refresher course: Small renal mass (T1a) – the case for resection

2015 New Orleans, LA
Society of Urologic Oncology, May 2015 – Primary Penile Sparing: [Invited Lecture]

2015 Chicago, IL
Faculty (CME Course): Radiologic Society of North America – Refresher course: Small renal mass (T1a) – the case for resection

2016 Boston, MA
Faculty: World Conference on Interventional Oncology – Partial Nephrectomy Remains the Gold Standard

2016 Baltimore, MD
Faculty (CME Course): UroTrack – Renal Mass Biopsy Debate – Pro

2016 Boston, MA
Faculty: First Global Summit on Precision Diagnosis for Prostate Cancer – Clinical Discussant

**International Invited Presentations and Courses**

2011 Mallorca, Spain
5th International Urology Forum – The Potential of Nanoparticle Enhanced Imaging in the Accurate Detection of Lymph Node Metastases [Invited Lecture]

2012 Mallorca, Spain
6th International Urology Forum – Renal Mass Biopsy [Invited Lecture]

2016 Tel Aviv, Israel
Faculty: Friends of Israel Urology Symposium – Nephron sparing surgery for multiple renal tumors [Invited Lecture]

Partial Nephrectomy: How I do it with less than 20 minutes warm Ischemia time [Invited Lecture]

Session Chair: Oligometastases in Prostate Cancer

**Report of Clinical Activities and Innovations**

**Current Licensure and Certification**

2002 Diplomate, National Board of Medical Examiners
2004 Massachusetts Registered Physician

**Practice Activities**

Urology/Urologic Oncology, Laparoscopy and Endourology Massachusetts General Hospital
Attending Urologic Surgeon, Polycystic Kidney Disease Clinic Massachusetts General Hospital

**Report of Technological and Other Scientific Innovations**

**Patents**

Potential use of biomarkers as diagnostic or prognostic markers in bladder cancer. These are currently under investigation and are not yet being used in clinical care

My contribution was and is the discovery and analysis of the patented biomarkers

**Report of Education of Patients and Service to the Community**

**Activities**

**Educational Material for Patients and the Lay Community:**

**Report of Scholarship**

**Peer Reviewed Publications in print or other media:**

**Research Investigations:**

7. Coen JJ, **Feldman AS,** Smith MR, Zietman AL. Watchful waiting for localized prostate cancer in
the PSA era: what have been the triggers for intervention? BJU Int. 2010 Sep 22. Epub ahead of print.


    *Co-first Authorship


*C/o-first Authorship


Other peer-reviewed publications:


Non-peer reviewed scientific or medical publications/materials in print or other media:


**Thesis**


**Abstracts, Poster Presentations and Exhibits Presented at Professional Meetings:**

27. Gershman B, **Feldman A**, Zietman A, McDougal WS. Transperineal template-guided prostate biopsy for persistently elevated PSA following multiple negative biopsies. Presented at the
American Urological Association, 2011.


Narrative Report

My activities at the Massachusetts General Hospital and at Harvard Medical School are in the field of Urologic Oncology. My clinical service activity in both operative and office urology requires approximately 70% of my time. My research activities in clinical and translational research comprises about 30% of my time. Teaching residents and medical students clinical and operative urology is integrated into my clinical time and a significant proportion of my research time is devoted to mentoring and working with my research fellows and residents on our clinical and translational research projects.

My exposure to basic science and clinical research have fostered my interests in combining these skills in translational and clinical research endeavors. As an undergraduate, I worked in the Department of Surgical Research at Children's Hospital learning molecular biology and basic science methodology. After my undergraduate years I conducted clinical outcomes research at Brigham and Women's Hospital and learned fundamentals of answering a research question in my Master's thesis at Boston University School of Medicine. As a Senior Scholar medical student, I worked as a Research Fellow in the Laboratory for Cellular Therapeutics and Tissue Engineering at Children’s Hospital, Boston. This research experience gave me a solid foundation in molecular biology, immunohistochemistry and tissue culture methods.

As a Urology resident at MGH, I continued to develop basic science techniques with the development of a murine model of bladder cancer in Cables (novel cell regulatory protein) knock-out mice and investigated the expression of Cables in bladder and prostate cancers using immunohistochemistry. Later in residency I moved into translational biomarker research, mentored by Dr. Bruce Zetter at Children’s Hospital and Dr. Matthew Smith at MGH. This research focused on the development of novel biomarkers for bladder and prostate cancers. I continued to develop these investigative projects throughout my Fellowship in Urologic Oncology and as a member of the MGH faculty. I also have formed a close collaborative relationship with Dr. Steven Gygi at the Taplin Mass Spectrometry Facility at HMS, working on biomarker discovery and investigating kinase activity in prostate and kidney cancers. My research projects have led to the presentation of several research abstracts at national meetings and publications, a patent on a novel biomarker, a peer-reviewed institutional grant, and corporate sponsored research funding. In 2008, I was awarded a three year Young Investigator Award from the Prostate Cancer Foundation for the discovery and development of novel biomarkers in prostate cancer and in 2011 I was awarded a five year Department of Defense Physician Research Training Award to continue my biomarker research in prostate cancer.

In an effort to further my education in sound clinical and translational research, I pursued a Masters degree in Public Health in Clinical Effectiveness at the Harvard School of Public Health. Awarded in November 2009, this degree is helping me achieve my academic goals by refining my ability to design and implement translational and clinical research and produce high quality independent investigations.

My clinical focus has been in Urologic Oncologic surgery. During my residency and fellowship I learned and developed my skills in both open and laparoscopic techniques and in the clinical management of patients with genitourinary cancer. As a clinician interested in research, I have also directed some of my research efforts toward clinical projects, including investigations of active surveillance for prostate cancer, nephron sparing techniques in renal cell carcinoma, the utility of renal mass biopsy and outcomes in penile sparing surgery for penile cancer. Clinical teaching has always been an integral part of my roles as resident, chief resident, fellow and now attending urologic surgeon. In clinical research, I have mentored residents, fellows, medical students and our research associates, overseeing and teaching investigational method and presentation of data. On a national level, I have served on the faculty for several CME courses and served as the Scientific Program Chair for the 2011 American Urological Association New England and Mid-Atlantic Sectional Meeting.