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Investigating the Role of Radiation Therapy Breast Cancer Clinical and Translational Research

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The training grant goals are to provide a broad range of opportunities for undergraduate students to participate in general clinical and basic science breast cancer research under the mentorship of experienced physician scientists in an academic institution. In the second year of the training grant, from May to August of 2004, six undergraduate students participated in original clinical or basic science research projects in the Department of Radiation Oncology. The students met or exceeded their expected goals of learning the principles of research, completing data collection and proceeding to analysis of the results. All students presented their research results at a mini-symposium for the departmental staff and faculty. One student will be presenting his project as a poster presentation at the Era of Hope Conference in June 2005. One student who conducted basic science research is co-author on a published peer-reviewed paper, and the second student decided to continue in the same lab another year as a research fellow. Two students are co-author on a manuscript in progress for submission to peer-reviewed journals. One project is ongoing. The quality of applicants was high and their performance on their research projects was excellent.

Breast cancer, radiation therapy
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I. Introduction

This is the second annual summary report for the training grant, summarizing the first two years of the grant and primarily the accomplishments since the last annual report, for the summer 2004 time period. The training grant goals are to provide a broad range of opportunities for undergraduate students to participate in general clinical and basic science breast cancer research under the mentorship of experienced physician scientists in an academic institution. The students were exposed to the research process from design to analysis to authorship, with the goal of instilling both an understanding of the research process and of fostering a lasting commitment to the pursuit of breast cancer research. In the summer of 2004, from June through August, six undergraduates from the University of Pennsylvania and Haverford College participated in the research program. Each conducted an original research clinical or laboratory project under the supervision of a mentor. The majority of the students completed projects that have been or will be published. In their evaluations, the students expressed a high degree of satisfaction with their experience. As principal investigator, I was highly impressed with the quality of work and dedication these students exhibited. All six students exceeded expectations for the program’s goals.

II. Body

The goal of the training grant is to recruit six students per summer for the duration of the grant period, and to provide each the opportunity to participate in a research project from start to finish. The initial phase is recruitment, which began in January 2004. Through contacts at the University of Pennsylvania School of Arts and Sciences, I was able to recruit an excellent pool of applicants from which to choose the six trainees. Recruitment flyers (attached) were submitted to the chairman of the Biological Sciences department, to the undergraduate premedical advisors office and to the Center for Undergraduate Research and Fellowships, the office which coordinates all research opportunities for the school. I received 30 inquiries and 15 complete applications for the six positions. The number of applicants was slightly lower this year than last year, likely because the posting of the notice on the website for the Center for Undergraduate Research and Fellowships was inadvertently delayed by their office. However, the caliber of applicants remained highly academic and diverse. Among the applicants, ten were women and five were men. Most of the applicants would be entering their sophomore or senior year, although two upcoming juniors also applied. Prior laboratory experience and science course were required for any applicants wishing to conduct basic science research, but no specific background was required for students wishing to pursue clinical research.
selected six applicants from the pool and two alternates, and six students were accepted into the program. These included were four women and two men, four upcoming seniors, and two upcoming sophomores. Four students were assigned clinical research projects, and two were assigned laboratory projects.

Once the applicants were selected, they reviewed a project list giving the titles of possible research projects (attached). Prior to the beginning of the summer session, they were asked to review the list and select any projects titles they were interested in working on. I met with each candidate individually to discuss in greater detail the study questions and design involved in any of the projects they had found interesting. Students then submitted three choices for projects, and I assigned each one a specific topic. The students were then shown how to perform a Medline search and given copies of reference chapters from textbooks to read. Each was asked to independently research their specific topic by finding relevant background studies in the medical literature. I reviewed with them their background searches and provided them with any additional references they needed in order to learn about their topic. Once the students began their research in May 2004, they knew what their specific topic would be and had reviewed the relevant medical literature. During the summer session, all students were expected to spend at least one day shadowing a physician in the clinic to provide them a glimpse of the clinical nature of breast cancer patients’ experiences.

The summer session was opened with an orientation session (agenda attached). This included an orientation to the hospital and Radiation Oncology Department, regarding payroll procedures, hospital and departmental policies, HIPAA training, departmental and hospital database access and utilization procedures, and the students were taken for hospital identification badges. The orientation included a series of lectures given by members of the Cancer Center faculty on breast cancer, radiation therapy and systemic therapy for breast cancer, clinical and laboratory research practices and documentation, clinical trials, and biostatistics. The trainees also heard presentations from residents and medical students who had done research work in the department as an example of the types of projects they would themselves be working on. The students then began their individual projects under the direction of their individual mentors. Three mentors participated in the program: Eleanor Harris MD, Lawrence Solin MD, and Gary Kao MD PhD; each mentor was assigned two students.

For clinical research projects, the first stage of work was to develop a study hypothesis and study design under the guidance of the faculty mentor. This step was completed in the first week of the project. For each clinical project, the students were provided with the pertinent patient list and an Excel spreadsheet containing numerous data points derived from the department’s extensive breast cancer database. These data files were saved to a password protected departmental server and each student was assigned a unique username and password through which to access their specific data files and on which to save their work. Students were assigned desk with a computer workstation at which to work. Students then developed a list of data points they needed for their study and edited their spreadsheets. Students were also given the opportunity, with guidance,
to write and submit standard requests for Institutional Review Board approvals, which in all cases involved a simple expedited review process.

The second stage of work was data collection. Each student searched through the relevant medical records for the needed data and entered that data on their spreadsheets on an ongoing basis. During this phase, the students needed to think about their hypothesis and ensure that the data being obtained was adequate to analyze the study question. Each student kept a diary of their daily activities and met once a week with their mentor to discuss their progress and ask any questions that had arisen. I met with my own mentees as well as all four students weekly as well in order to assess the progress of each project. Either the faculty mentor or I met once weekly with the two students in the lab, to review their weekly logs and assess their progress. These students were under daily supervision of their faculty mentor, as they worked side by side with him in the lab.

The third stage of work was data analysis. For this, most of the students worked with a statistician. The students discussed with the statistician how to organize their data files for statistical analysis, then submitted their data files to her for computation. Simple analyses like descriptive statistics were sometimes performed by the student. Each student began work on an abstract in standard format (Introduction; Methods; Results; Conclusions).

The final stage of work was abstract production and presentation of their work. A research symposium was organized and held within the department at which each student gave a 20 minute Powerpoint presentation of their research project (attached). Each gave an introduction, followed by methods, results and conclusions. Although not a requirement, one student was second author on a manuscript by the completion of the summer project, and is now in press in Cancer Biology & Therapy.

At the end of the summer session, each student turned in their diaries and were asked to complete an evaluation form (attached) of the program. Their comments were generally positive, rating the quality of the introduction and orientation, mentoring and oversight, scope and interest level of the projects, experiences shadowing a clinician, interaction with other students and residents and facilities all in the excellent or good category. Some students expressed some frustration at obtaining their study charts for data collection in a timely manner. We have addressed this issue by relocating all of the study charts to a central location at the hospital and thus will not rely on delivery from a storage facility any longer. Many students felt that the research experience had solidified their interest in a medical or research career. Comments included: “I was previously turned away from laboratory research but I had a wonderful time and am really interested in the research we performed”; “The program increased my interest in Radiation Oncology as a field of medicine to pursue, specifically in regards to breast cancer research and treatments;” and “After this program I am considering getting my MD PhD”.

All but one of the student’s projects either has been presented or published, or is anticipated to be published in 2005.
III. Key Research Accomplishments:

Undergraduate researchers, 2004:

1. Jessica Liao: "Late Cardiac Effects of Breast Irradiation: An Analysis of Electrocardiograms"
Mentors: Eleanor Harris, MD and Candace Correa (medical student)
Status: Jessica completed data collection and preliminary analysis. She is co-author on a manuscript currently under revision, entitled "Late cardiac effects in left versus right sided early stage breast cancer patients treated with contemporary breast conservation therapy", to be submitted for peer-review publication by summer 2005. I anticipate several additional manuscripts from this data set, including one specifically analyzing the EKG findings that Jessica collated.

Jessica Liao initially completed this clinical research project in the summer training program; during the course of the program she was exposed to laboratory research, which kindled such interest that she performed Independent Study of mechanisms in cancer cells with Dr. Kao as part of her academic course load. This too has been successful and will likely result in a first-author manuscript to be submitted soon for consideration of publication.

2. Chandresh Ladva: "The Survival of Patients with Past Histories of Malignancies Prior to Early Stage Breast Cancer".
Mentor: Eleanor Harris, MD
Status: Chandresh completed all data collection and statistical analysis. His project has been accepted for poster presentation at the Era of Hope Conference in Philadelphia in June 2005. Chandresh wrote a draft manuscript, and the data is currently being updated with a revised manuscript in progress.

3. Rahn Voong: "Death and Degradation: Discovering the Molecular Determinants of Taxol Sensitivity"
Mentor: Gary Kao, MD, PhD

Rahn's research contributions were considerable and enabled not only her co-authorship on this project but also several others that have been submitted or are nearing completion including one in which she will be first-author. Rahn's experiences kindled such a strong desire to pursue cancer research, that she will devote a year following graduation to continue her work investigating mechanisms relating to breast and other forms of cancer.

Mentor: Lawrence Solin, MD
Status: Kristin completed the majority of data collection and preliminary analysis. Final data collection is underway and manuscript preparation is to begin this year.

5. Andrea Denunzio: “Investigation of Cosmesis and Complications in Patients with DCIS”
   Mentors: Lawrence Solin, MD and Neha Vapiwala, MD (resident)
   Status: Andrea completed data collection and analysis. She will be co-author on a manuscript being written by a resident, Neha Vapiwala, with anticipated submission this year for peer-review publication.

   Mentor: Gary Kao, MD, PhD
   Anil Maggee, the first student from outside Penn, solidified his desire to pursue a career as a physician. However, Anil was not able to complete a sufficient body of work for publication.

Update on Undergraduate Projects from 2003:

1. Jill Starzyk: “Analysis of biopsies performed after definitive irradiation for early stage breast cancer”.
   Mentor: Lawrence Solin, MD
   Status: Accepted for poster presentation at the American Society for Clinical Oncology (ASCO) meeting in June 2004. Manuscript in preparation by a current resident.
   Jill is currently a second year medical student at Northwestern University.

2. Eric A. Lee: “Outcomes after breast conservation therapy relative to Her2 expression” and “Factors that determine breast cancer cell resistance to the microtubule-disrupting drugs”.
   Mentors: Eleanor Harris, MD and Gary Kao, MD, PhD
   Status: Clinical project accepted for oral presentation at the American Society for Therapeutic Radiology and Oncology (ASTRO) meeting in October 2004; “The Impact of Her2/neu Status on Local Recurrence in Women With Stage I-II Breast Cancer Treated With Breast Conservation Therapy”, presented by Eleanor Harris, MD
   Co-author on manuscript submitted to the International Journal of Radiation Oncology, Biology and Physics in May 2005 entitled: “The Impact of Her2/neu Status on Local Recurrence in Women With Stage I-II Breast Cancer Treated With Breast Conservation Therapy”, Authors: Eleanor E. R. Harris, MD, Wei-Ting Hwang, PhD, Eric A. Lee, James L. Rembert, MD, Michael D. Feldman, MD, PhD, Angela DeMichele, MD, Gary Kao, MD PhD, and Lawrence J. Solin, MD
   Eric is currently a first year medical student at Duke University.
Mentor: Gary Kao, MD, PhD 
Michael is completing his senior year at the University of Pennsylvania.

Mentors: Eleanor Harris, MD and Andrea Cheville, MD 
Status: After Jordan completed his phase of the project, we decided to add some additional data points to the analysis. Data collection was continued in 2004 by a medical student, Neha Amin, who was supported by a grant from the NIH-funded Short Term Training Grants. She presented the updated work at the University of Pennsylvania School of Medicine Short Term Summer Research Student Symposium in August 2004. Statistical analysis is under way.

IV. Reportable Outcomes:

A. Publications or Meeting Presentations:


B. Manuscripts in Progress:

1. Harris EE, Correa C, Hwang W-T, **Liao J**, Litt HI, Ferrari V, Solin LJ. “Late cardiac effects in left versus right sided early stage breast cancer patients treated with contemporary breast conservation therapy”. Manuscript in progress.

V. Conclusions:

In the second year of the training grant, encompassing the summer of 2004, I was able to recruit an academically stellar group of student researchers of diverse interests and backgrounds, all of whom worked diligently and who in many cases exceeded the expectations for the training program goals. One is a co-author on an in-press peer-reviewed manuscript, one will be presenting his work at the 2005 Era of Hope Conference, and several manuscripts based on these students work are being analyzed or written for peer-review submission by me or residents in the department.

The students seemed highly satisfied with their experience and all stated on their evaluations that the program increased their interest in pursuing a career in medicine and biomedical research, and specifically their interest in working in the area of breast cancer research.

I have addressed the main area of concern for the students, which was easy access to their study charts, by bringing all the archived study charts into a single repository in our hospital offices. This year we have nearly doubled our requests for applications, and have another high caliber group of students poised to begin their summer projects.

VI. References:


VII. Appendix

1. Recruitment Flyer

2. Orientation Agenda

3. Evaluation Form

4. Symposium Schedule and Research Student Powerpoint Presentations
Undergraduate Breast Cancer Research Opportunity
Release: December 2004

The Department of Radiation Oncology at the University of Pennsylvania School of Medicine is pleased to announce a new research opportunity for undergraduates at the University of Pennsylvania to participate in investigative projects in the area of breast cancer research. The training program will provide a broad range of opportunities for students to participate in general clinical or basic science breast cancer research. The student will be exposed to the research process from design to analysis to authorship, with the goal of instilling both an understanding of the research process and of fostering a lasting commitment to the pursuit of breast cancer research.

Due to a generous grant from the Department of Defense Breast Cancer Research Program, we are able to offer a $4000 stipend to up to six undergraduates students able to spend 12 weeks participating in a full-time mentored research project. (For interested students, additional independent study during the academic year may be available.) The program director is Eleanor Harris, MD, assistant professor of Radiation Oncology. In order to apply for the training program, students must be in good academic standing at the university. Students are required to fill out an application form and to submit an unofficial transcript. Students must also submit a one-page essay describing their reasons for applying to the training program, in particular their motivation for conducting breast cancer research. Applications will be due no later than April 9, 2004.

Students requesting laboratory projects involving bench research will be required to have some prior experience in basic laboratory techniques involved in their experiments, as 12 weeks is too brief to allow training in techniques and the completion of a set of experiments. For students interested in clinical projects, no prior research experience will be required. In fact, it is anticipated that the training program will provide many of these students with their first exposure to high quality scientific research with expert faculty mentoring.

Dr. Harris will review the applications and choose participants based on their interests, commitment, academic record, motivation and future goals. Applicants will be informed of their acceptance by mid-April 2004. Shortly afterwards, each participant will meet with Dr. Harris to discuss their background, areas of interest and potential research ideas. If the student has a specific research project or faculty member in mind, he or she will be put in contact with the appropriate mentor. While the primary mentor must be a training program faculty, students may be assigned a co-mentor from another department if beneficial. If the student does not have a specific research goal at the initial meeting, he or she will be given a list of projects with brief descriptions to review and will be asked to choose three projects from that list. Dr. Harris will review all the requests and assign a project to each participant. The student will then meet with that faculty mentor to discuss the specific project. The student will be assigned background reading, and with the mentor’s guidance will be asked to develop a timeline for research plan. It is anticipated that participants will complete these steps during the spring semester and prior to beginning their project in the summer session.

To request an application, please contact Ms. Betsy Patton in the Department of Radiation Oncology by phoning 215-662-3094, or by writing to email address: patton@xrt.upenn.edu.
Orientation
Breast Cancer Summer Research Program

June 1, 2004
Plaza A Conference Room
3rd Floor Founders

09:00 - 10:00 Mark Patrick and Betsy Patton
Laboratory Administrator and Program Coordinator

10:00 - 11:00 Susan Domchek, MD
Medical Oncology

11:00 - 11:30 Peggy Alfarano
HIPPA

11:30 - 12:00 Neha Vapiwala, MD
Lecture

12:00 - 01:00 Lunch

01:00 - 05:00 Eleanor Harris, MD
Radiation Oncology

June 2, 2004
Conference Room
Basement Founders

09:00 - 09:30 Linda Miller, BSN, RN
Director of Nursing Clinical Research

09:30 - 10:30 Ryan Smith, MD
Lecture

10:30 - 11:30 Ralph Ferro
Computer Training

11:30 - 12:00 Candace Correa, Medical Student
Lecture

12:00 - 01:00 Lunch

01:00 - 01:30 Betsy Patton
Database Coding

01:30 - 03:00 Wei-Ting Hwang, PhD
Biostatistics and Epidemiology

03:00 - 05:00 Gary Kao, MD, PhD
Scientific Research Methods
Breast Cancer Research in Radiation Oncology – 2003 Summer Training Program
Evaluation Form

Name: ________________________________ Date: ____________________

Faculty Mentor: _______________________

Project Title: _________________________

| Part A: For the following aspects of the summer research training program, please choose the best option describing the quality of each part of the program, if applicable to your project: |
|-----------------------------------------|-----------------|-----------------|-----------------|-----------------|
| 1. Breast cancer introductory lecture series | Excellent | Good | Satisfactory | Inadequate |
| 2. Departmental/Hospital orientation | Excellent | Good | Satisfactory | Inadequate |
| 3. Research methods and design | Excellent | Good | Satisfactory | Inadequate |
| 4. Clarity of research project | Excellent | Good | Satisfactory | Inadequate |
| 5. Mentoring and oversight | Excellent | Good | Satisfactory | Inadequate |
| 6. Access to mentor | Excellent | Good | Satisfactory | Inadequate |
| 7. Scope of research project appropriate | Excellent | Good | Satisfactory | Inadequate |
| 8. Shadowing a clinician | Excellent | Good | Satisfactory | Inadequate |
| 9. Support staff/ file room access | Excellent | Good | Satisfactory | Inadequate |
| 10. Laboratory facilities | Excellent | Good | Satisfactory | Inadequate |
| 11. Supplies | Excellent | Good | Satisfactory | Inadequate |
| 12. Office/computer facilities | Excellent | Good | Satisfactory | Inadequate |
| 13. Interaction with residents/students | Excellent | Good | Satisfactory | Inadequate |
| 14. Technical services | Excellent | Good | Satisfactory | Inadequate |
| 15. Interaction with department personnel | Excellent | Good | Satisfactory | Inadequate |

Part B: Please assess the following goals of the research training program, and whether they were accomplished during the program:

1. Did the program provide an opportunity to learn more about breast cancer research?

2. Was the project you were assigned or designed of the appropriate scope and level of difficulty?
3. Was the project of sufficient interest to you?

4. Did the program increase your interest in medical research?

5. Did the program increase your interest in breast cancer research?

6. Did the program change or affect your educational or intellectual goals? If so, how?

7. What do you think were the goals of your research program, and were they met?

8. Would you recommend this program to a peer?

Part C: Your comments and suggestions will be very helpful in improving the quality of the research training program. Please discuss any of your ideas for improvements or suggestions for changes.
Investigation of Cosmesis and Complications in Patients with DCIS
Speaker: Andrea DeNunzio

The Survival of Patients with Past Histories of Malignancies Prior to Early Stage Breast Cancer
Speaker: Chandresh Ladva

Late Cardiac Effects of Breast Irradiation: An Analysis of Electrocardiograms
Speaker: Jessica Liao

Death and Degradation: Discovering the Molecular Determinants of Taxol Sensitivity
Speaker: K. Ranh Voong

Effects of Initial Nodal Status, Patterns of Distant Metastases, and Her2/neu Status on Outcome in Metastatic Breast Cancer
Speaker: Kristin Meliambro

Density Determines Rapid Killing of Breast Cancer Cells by Taxol
Speaker: Anil Magge
Investigation of Cosmesis and Complications in Patients with DCIS

Andrea DeNunzio

Co-Mentor: Lawrence J. Solin, MD
Co-Mentor: Neha Vapiwala, MD

Overview

- Retrospective Study Designed to:
  - Evaluate Factors Affecting Cosmesis
  - Determine Complications Associated with Treatment
  - Evaluate Factors Affecting Occurrence of Complications

Background

- DCIS Diagnosed in Symptomatic Patients
- Advent of Mammography
- Diagnosis of DCIS in Asymptomatic Patients
- 15-20% of All New Breast Cancers Are DCIS

Methods: Population Selection

- Patients with DCIS Only
- Patients Treated with Breast Conservation Therapy Only
- Patients with No Chemotherapy or Hormone Therapy
- 95 Patients for Study

Methods: Selection of Potential Factors

- Previously Studied Factors:
  - Dose (Total, Whole Breast, and Tumor Bed Boost)
  - Fractionation
  - Volume of Excision
  - Breast and Cup Size
  - Weight
  - Age
  - Race
  - Axillary Dissection
Methods: Data Collection

Potential Factors:
- Age
- Race
- Body Mass Index (BMI)
- Bra Size and Cup Size
- Tangential Chest Wall Separation
- Volume of Resected Tissue
- Scar Length
- Number of Surgeries
- Dose Inhomogeneity
- Use of Wedges
- Axillary Dissection
- Sentinel Node Biopsy
- Presence of Moist Desquamation

Methods: Data Collection

- Record and Verify Previously Reported:
  - Cosmetic Scores Assigned During Follow-Up Examinations
  - Complications Experienced By Each Patient

Methods: Data Analysis

Creation of Cosmesis Timeline:
- Use Only Month and Year to Determine Time Interval
- Designate Each Month as a Number (Ex. June = 6)
- Subtract the Year and Month of Completion of Radiation Therapy from the Year and Month of Cosmetic Evaluation, respectively
- Use Magnitude and Sign of the Month Difference to Fine-Tune the Year of Cosmetic Evaluation

Methods: Data Analysis

Cosmesis:
- Range: 1-16 Years
- Median Time: 6 Years
- Over 5 Years: n=73
- Over 10 Years: n=21

Methods: Data Analysis

Creation of Complications Timeline
- Creation Similar to Cosmesis Timeline
- Determination of Relevant Factors:
  - Whether or Not the Patient Experienced a Given Complication
  - Duration of Complication

Methods: Data Analysis

Complications:
- Breast Edema
- Arm Edema
- Decreased Range of Motion
- Cellulitis
Future Analysis

- Finish Data Collection
- Pathology Reports
- Patient Films
- Finish Timelines
- Data Sort to Determine Relevant Links
- Meet With Statistician

Acknowledgements

Special Thanks To:

Dr. Harris
Betsy Patton
Dr. Vagiwala
Dr. Solin
P-Crew
In the beginning...

- Cancer plagued women...

and then it did again

**You had what now?:**
The Survival of Patients with Past Histories of Malignancies Prior to Early Stage Breast Cancer

From the fingertips of Chandresh Ladva

---

**A look into prior malignancies**

- Very little published
- French study with extrapulmonary malignancies prior to lung cancer
  - Massard G, et. al. (2000)

---

**Methods**

- Database screen
  - female
  - DCIS, Stage I, or II
  - prior malignancy
  - no contralateral breast cancer
- Matched
  - stage
  - age +/- 2 yrs.
  - date of diagnosis
  - +/- 5 yrs.
- Statistical analysis

---

**Data collected**

- Survival
- Family History
- Breast and Prior Malignancy Treatments

---

**Statistical Analysis**

- Continuous variables
  - age
  - total RT dosage
  - Student's t-test
- Kaplan-Meier curves
  - survival
- Categorical variables
  - family history
  - treatment methods
  - $\chi^2$ test for Independence

---

ahem...not quite done yet
Patient Characteristics

- Family History: Supraclav-- p = 1,000
- BC-- p = .3510; OC-- p = .0325
- Pos. Ax.-- p = .0028
- IMN-- p = .0065

Cases with detail

<table>
<thead>
<tr>
<th>Interval Between Cancers (Months)</th>
<th>Mean</th>
<th>Median</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of Prior Malignancy</td>
<td>58.7</td>
<td>7.9</td>
<td>106.5</td>
</tr>
</tbody>
</table>

Cases by Predominant PMs

Survival

<table>
<thead>
<tr>
<th>Survival Status</th>
<th>+PM (n=)</th>
<th>-PM (n=)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alive, NED</td>
<td>69</td>
<td>51</td>
</tr>
<tr>
<td>Alive with Disease</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dead, NED</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dead, Disease</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dead, Unknown Cause</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Follow-up Time in Months

<table>
<thead>
<tr>
<th>Mean</th>
<th>+PM</th>
<th>-PM</th>
<th>p = 0.5218</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>64</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>67.14975698</td>
<td>60.606375626</td>
<td></td>
</tr>
</tbody>
</table>
Late Cardiac Effects of Breast Irradiation: An Analysis of Electrocardiograms

Jessica Liao
Co-mentor: Dr. Eleanor Harris
Co-mentor: Candace Correa
Department of Radiation Oncology
Hospital of the University of Pennsylvania
8/13/04

Estimated New Cases of Cancer in Females in the US, 2003

Why Study Cardiac Diseases?
- Accidental heart irradiation
- Many methods to analyze cardiac diseases
- EKG is gold standard for sinus rhythm

Advantages and Disadvantages of using EKGs
- Advantages:
  - Easy to obtain
  - Results not subjective
- Disadvantages:
  - Inaccurate identification of MIs

Electrical Currents cause the Waves and Complexes seen on an EKG

Incidence of Heart Disease and Functional Significance of Changes in the Electrocardiogram 10 years After Radiotherapy for Breast Cancer. Sternder, et. al. (1986)
- 197 patients evaluated for heart disease
- Conclusions:
  - Increase in cardiac abnormalities after radiation, specifically ST and T-wave changes
  - The incidence of serious cardiac complications is low
ST and T-Wave Changes are Possible Indicators of Many Cardiac Diseases

- Myocardial Ischemia
- Myocardial Infarction
- Ventricular Hypertrophy

Our Study vs. Strender’s Study

- Chemotherapy Drugs
- Sample Size
- Before/After plus Left/Right

Research Method

- Screen Patients:
  - Had breast cancer radiation therapy
  - Has EKG history
- Collect Data:
  - EKG abnormalities
  - Chemotherapy
  - Radiation Doses and Fields
- Statistical Analysis:
  - X² test
  - t-test assuming unequal variance

Patient Characteristics for All Patients

| TABLE 1: PATIENT CHARACTERISTICS - ALL PATIENTS |  |  |
|-----------------|----------|----------|----------|----------|
| VARIABLES       | ALL PATIENTS | ALL PATIENTS | P-Value   |
| Age in Patients | 71.5      | 71.5      | 0.24     |
| Median Age      | 74.5      | 74.5      | 0.24     |
| Chemotherapy    | 124       | 217       | 0.00     |
| Chemotherapy    | 217       | 124       | 0.00     |
| Radiation       | 99        | 112       | 0.26     |
| Radiation       | 112       | 99        | 0.26     |
| Mean Radiation  | 4.29      | 3.86      | 0.50     |
| Mean Radiation  | 4.29      | 3.86      | 0.50     |

Patient Characteristics for Patients with Pre-XRT EKGs

| TABLE 2: PATIENT CHARACTERISTICS - PATIENTS WITH PRE-XRT EKG |  |  |
|-----------------|----------|----------|----------|
| VARIABLES       | LEFT HEART | RIGHT HEART | P-Value   |
| Age in Patients | 68        | 73        | 0.13     |
| Median Age      | 76        | 73        | 0.13     |
| Chemotherapy    | 115       | 115       | 0.30     |
| Chemotherapy    | 115       | 115       | 0.30     |
| Radiation       | 10        | 10        | 0.43     |
| Radiation       | 10        | 10        | 0.43     |
| Mean Radiation  | 4.24      | 4.24      | 0.38     |
| Mean Radiation  | 4.24      | 4.24      | 0.38     |

Research Groups
Patient Characteristics for Patients with Post-XRT EKGs

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>PRE-XRT</th>
<th>POST-XRT</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>53.7</td>
<td>55.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Gender</td>
<td>54%</td>
<td>52%</td>
<td>0.8</td>
</tr>
<tr>
<td>Hypertension</td>
<td>37%</td>
<td>42%</td>
<td>0.4</td>
</tr>
<tr>
<td>Diabetes</td>
<td>19%</td>
<td>20%</td>
<td>0.9</td>
</tr>
<tr>
<td>Smoking</td>
<td>24%</td>
<td>26%</td>
<td>0.6</td>
</tr>
<tr>
<td>History of CVD</td>
<td>6%</td>
<td>8%</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Patient Char. for Group Containing Both Pre and Post-XRT EKGs Validate Groups with Only Pre or Post-XRT EKGs

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>PRE-XRT</th>
<th>POST-XRT</th>
<th>P-Value</th>
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<td>0.5</td>
</tr>
</tbody>
</table>

Significant Outcomes

- 38 different cardiac diseases
  - Pre-xrt abnormalities - 54%
  - Post-xrt abnormalities - 61%
  - P-value < 0.04

Significant Outcomes for Patients with Both Pre and Post-XRT EKGs

- Compared with comparable right-sided patients, left-sided breast cancer patients experienced a statistically significant increase (p<0.05) in ST abnormalities after breast irradiation
  - No difference in specific location
  - No difference in myocardial infarctions
Discussion

- The percentage of EKG abnormalities noted before and after radiation were much higher than previously reported by the Strender group.
- Results corroborate their findings of a higher incidence of ST abnormalities after radiation.
- Difference of cardiac diseases due to breast irradiation of L vs. R

Future Work

- Are similar results in the increased incidence of cardiac diseases shown using other tests?
- Could prescription drugs be affecting our results?
- Since the likelihood of developing ischemia is higher, is this radiation to the left breast actually decreasing their overall survival?

References


Acknowledgements

- Elizabeth Harris, MD
- Carolina Cano
- Rebeca Pedro
Accomplishments

- Discovered Preliminary Evidence that:
  - Taxol may cause the accelerated degradation of checkpoint proteins
  - Mechanism may be the activation of proteins involved in Programmed Cell Death
- Became skilled with diverse laboratory techniques
- Writing manuscript with Anil Magge

Methods

Protein Analysis Protocol
1. Plate out cells
2. Treat cells
3. Harvest for pellets
4. Lysate cells
5. Run samples on SDS-PAGE gel
6. Transfer protein to membrane
7. Probe for proteins of interest
8. Expose and develop film

Western Blot Procedure

- Mitotic Checkpoint proteins (Bub1, CENP-E, BubR1, etc.)
- When inactivated or destroyed, leads to mitotic catastrophe

Death and Degradation: Discovering the Molecular Determinants of Taxol Sensitivity

K. Ranh Voong
Radiation Oncology Department
University of Pennsylvania
August 13, 2004

What are the Mitotic Checkpoint Proteins?

Western Blot Procedure

Taxol leads to degradation of Mitotic Checkpoint Proteins

- Bub1
- CENP-E
- Rad1
**What are Caspases?**

- Proenzyme
- Activated Enzyme

* Are a family of Proteases that are inactivated by cleavage (Proenzyme → Activated)
* Cleave/activate other proteins, ultimately bringing about cell death

**Targeted Mutations of Every Potential Caspase Cleavage Site in BubR1**

<table>
<thead>
<tr>
<th>Wildtype</th>
<th>D579E</th>
<th>D607E</th>
<th>D76E</th>
<th>W1079E</th>
<th>D579E W1079E</th>
</tr>
</thead>
</table>

- Caspases recognize 'Taxol' cleavage sites (D607E)

**BubR1 Instability Decreased with Specific Mutation of Caspase Cleavage Site**

- Wildtype
- D607E
- D607E W1079E

**Conclusions**

- Key Mitotic Checkpoint proteins are actually Unstable
- Taxol leads to accelerated degradation of BubR1 by activating Caspases
- Aspartic Acid 607 ("D607E") defines the prime Caspase cleavage site in BubR1
Future Implications

- Functional consequence of expressing BubR1 mutants
- Make cells more resistant to Taxol?
- Find specific Caspase involved in Taxol mediated killing
- Confirm that Caspases cleave other mitotic checkpoint Proteins

Acknowledgments

- Dr. Eleanor Harris
- Dr. Gary Kao
- Anil Mogge
- The Kao Laboratory:
  - Dr. Mijin Kim
  - Wes Ball & Katie Murphy
  - Melissa Dowling
  - Dr. Fang Liu
  - Shary Parker
  - Betsy Patton

Taxol Unmasks the Instability of Mitotic Checkpoint Proteins

<table>
<thead>
<tr>
<th>Nuclotide TAXOL</th>
<th>Wyoming</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Length</td>
<td></td>
</tr>
<tr>
<td>Degraded</td>
<td></td>
</tr>
</tbody>
</table>

In addition to BubR1, other proteins such as CDC42 and Actin may be targeted by Taxol.
Metastatic Breast Cancer

- Most common sites of metastasis, in order of frequency:
  - Bone: ~25% of breast cancers spread first to bone
  - Lung: ~60-70% of women who die from breast cancer had it spread to lungs
  - Liver: ~67% of women with metastatic breast cancer eventually have spread to liver
  - Less common: Brain, Bone Marrow, Ovaries, Eye, & Other areas

Effects of Initial Nodal Status, Patterns of Distant Metastases, and Her2/neu Status on Outcome in Metastatic Breast Cancer: The University of Pennsylvania Experience

Kristin Meliambro
August 13, 2004

Treatment for Distant Mets

- Majority of treatments focus on alleviating symptoms (palliative treatment), improving quality of life, prolongation of life
- Surgery: i.e. isolated lungs mets, pathologic fractures
- Radiation Therapy: i.e. painful bony mets, unresectable CNS mets (brain, meningeal, spinal cord), following surgery

Research Methods

- Total Number of Patients: 204 (possibly 230)
  - Intraductal/Infiltrating Ductal & Infiltrating Ductal: 181 patients
    - 105 node negative; 76 node positive
  - Infiltrating Lobular: 12 patients
    - 8 node negative; 4 node positive
  - Infiltrating Ductal/Lobular: 4 patients
    - 1 node negative; 3 node positive
  - Medullary: 3 patients
    - 2 node negative; 1 node positive
  - Colloid: 4 patients (all node negative)
  - Squamous Cell: 1 patient (node negative)
**Breakdown of Patients**

Node Negative v. Node Positive

**Study Questions**

- Looking for relationships between:
  - median survival time for distant mets and initial nodal status
  - number and types of metastatic sites and initial nodal status
  - median survival time and combination of metastatic sites (e.g., bone/brain v. bone/lungs v. lungs/liver, etc)

**Research Methods**

- Reviewed ~200 charts for following data per patient:
  - median survival time (months)
  - dates of each diagnosis of distant mets
  - number of distant mets sites at each diagnosis
  - total number of distant mets sites
  - chemotherapy per incident of distant mets
  - Her2/neu status

**Her2/neu status & Distant Mets**

- HER2/neu is overexpressed in 20-30% of Br. Cas
- tumor cells overexpressing HER2/neu may have up to 2 million copies of receptor on surface (compared to 20,000-50,000 copies in normal breast epithelial cells)
- HER2/neu overexpression correlates with more aggressive behavior, shortened disease free survival, and overall survival rates
- marker of response to chemotherapy and HT
- Question: Relationship between HER2/neu status and Distant Metastasis of Breast Cancer?

*http://www.amedicine.com/direct/topic2092.htm*

**Preliminary Results**

**Total Breakdown of Patients**

- 31% 1 distant met site only
- 21% 1 initial site, multiple later
- 48% 2 or more initial sites
**Preliminary Results**

**Single Site Distant Mets**
- Bone only: 4.1%
- Lung only: 9.2%
- Liver only: 0.2%
- Other: 55.1%

**Multiple Sites Distant Mets**
- Bone/Lung: 15.1%
- Bone/Liver: 8.6%
- Bone/Other: 3.7%
- Brain/Lung: 6.6%
- Brain/Other: 5.7%

**Observations**
- **Median Survival Time v. Sites of Distant Mets**
  - Longer median survival time for node negative patients for all multiple site mets combinations except:
    - Bone/brain – equal median survival time
    - Bone/lung: longer median survival time for node positive patients
    - Greatest difference in median survival time between node negative and node positive patients with bone/liver mets (31 months), followed by brain/lung mets (26 months)
**Future Study Questions**

- Is there a difference in median survival time between node negative and node positive patients with:
  - single site distant mets?
  - multiple site distant mets?

- For patients with multiple distant mets, is outcome better or worse depending on sequence of diagnosis, i.e. simultaneous diagnosis of > 2 sites v. sequential diagnosis of > 2 sites?

- If patients with multiple distant mets, is how does Her2/neu status affect median survival time?
- How might Her2/neu status affect future treatment recommendations?

**Future Study Questions: Her2/neu**

- Is there a difference in the rate of development of distant mets between Her2/neu positive & Her2/neu negative patients for:
  - single site distant mets?
  - multiple site distant mets?

- How does Her2/neu status affect median survival time?

**Acknowledgements**

Special Thanks to:
- Dr. Harris
- Dr. Vapiwala
- Dr. Solin
Density Determines Rapid Killing of Breast Cancer Cells by Taxol

Anil Magge
August 13, 2004
Department of Radiation Oncology
University of Pennsylvania

Accomplishments
- Became skilled with diverse laboratory techniques
- Identified a novel factor - CELL DENSITY - that determines if cancer cells can be rapidly killed by Taxol
- Discovered that specific sequencing with Radiation Therapy may reverse the resistance of densely growing cells to Taxol
- Writing manuscript with Ranh Voong

Cell Density Project

Background
- microtubules = "skeleton" of cell
  - dynamic constantly forming and disassembling
  - forms mitotic spindles
- disrupt microtubule dynamics → cell death
- TAXOL prevents disassembly
- Vinca-type, Nocodazole prevent formation
- cell death mechanism unclear

Cell Density Project

FACS and the Cell Cycle
- FACS (Flow Accurate Cyto genetic Sorting) measures the amount of cells in certain parts of the cell cycle by analyzing the DNA content
  - Gi - "G" phase before synthesis
  - S - phase (DNA replication)
  - G2 - "Next G" phase before M phase
  - M - cell division
- Cell Density Project

Cell Density Project
Cell Density Project

SKBR3 Breast Cancer cells
Time after Treat
Low Density
Moderate Density
High Density

Cell Density Project

OVCAR Ovarian Cancer cells
Time after Treat
Low Density
Moderate Density
High Density

Cell Density Project

ADRI Ovarian Cancer cells
Time after Treat
Low Density
Moderate Density
High Density

Cell Density Project
Conclusions

- Sparsely growing breast cancer cells are more easily killed by Taxol than densely growing cells.
- This in fact may be a general phenomenon; also seen with OVCAR, A2780, PC3, ...
- This may explain why LARGER tumors may respond poorer to chemotherapy.
Conclusion

- Sparsely growing breast cancer cells are more easily killed by Taxol than Densely growing cells

How can we sensitize Densely Growing cells to Taxol?

ANSWER:

- With specific order of cancer treatment combination
  - TAXOL BEFORE IR pulse leads to more cell death than the converse

Cell Density Project

Conclusions II

- Sparsely growing breast cancer cells are more easily killed by Taxol than Densely growing cells
- However the resistance of cells grown at high density can be partially reversed by treating with Taxol first then following it with Radiation

Cell Density Project

Acknowledgments

- Dr. Eleanor Harris
- Dr. Gary Kao
- Ranh Voong
- The Kao Laboratory:
  - Melissa Dowling
  - Dr. Mijin Kim
  - Wes Beff
  - Shary Parker
  - Dr. Fang Liu
  - Betsy Patton
Future Directions

- Show these effects in animal models of breast cancer
- Explore combinations of different chemotherapies with Taxol

Methods and Materials

Protocol for Taxol-IR Sequence Experiment
1. Plate out cells densely
2. Treat with Taxol or radiation
3. Incubate for 12 hours. Wash cells. Treat with radiation or Taxol.
4. Incubate 12 hours. Harvest Cells.
5. Wash cells that will be harvested 12 hours (24).
6. Harvest remaining samples
7. Process and run Flow Assisted Cytometry (FACS)
What about the future?

Possible inquiries and improvements
- Expanding study
- Larger population
- Longer follow-up
  - Simplicity allows easier long-term data collection for patients in database
- Comparison of cause of prior cancers and relationship with BC
  - Other cancers likely to couple with BC
  - Genetic, hormonal, chemogenic, etc.

Hasta luego...

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.